Behavioural Adaptation of Aquatic Cave Crustaceans: A Case Study in water lice *Asellus aquaticus*

Hajriz Berisha

Department of Systematic Zoology and Ecology
Biological Institute, Eötvös Loránd University
Budapest, Hungary

Ph. D. Thesis

Submitted to the

Ph. D. Program of Environmental Biology, Doctoral School of Environmental Sciences
at the Eötvös Loránd University, Budapest
Director: Tamás Turányi, DSc
Program Leader: Erika Tóth, DSc

Supervisor:
Prof. Gábor Herczeg
Department of Systematic Zoology and Ecology
Biological Institute
Eötvös Loránd University, Budapest

Budapest, August 2024

DOI: 10.15476/ELTE.2024.198

CONTENTS

1. INTRODUCTION

1.1. Overall description of the cave ecosystems

The first scientific exploration of caves began in Europe during the 17th and 18th centuries. Later, around 1890 in Europe and 1900 in North America, the correct understanding of cave geology started to gain attention. After 1900 in Europe, early biological studies focused on faunal surveys and species descriptions (Poulson & White, 1969). During these studies in cave biology, scientists observed that animals permanently residing in caves often shared similar features, particularly in their morphological characteristics (Howarth & Moldovan, 2018).

However, studies in the 20th century recognized that when populations of animal species colonize and adapt to the darkness of subterranean habitats, they must undergo not only morphological changes but also physiological and behavioral adaptations. These traits are described as "troglomorphic" (Pérez-Moreno et al., 2018), and animals exhibiting these traits are referred to as "troglobionts" (Soares & Niemiller, 2018). The morphological, physiological, and behavioral modifications of cave animals often converge across different taxa. The absence of light is a key selective environmental factor in caves, leading to predictable and convergent adaptations. These adaptations range from the more apparent changes in casual visitors to the extreme modifications seen in various cave species, such as the loss of eyes and pigmentation, the development of enhanced skin sensory organs, and the elongation of appendages. While the convergent morphology of cave animals is well-documented, there is a relative scarcity of studies that address the functions, behaviors, and fitness of these adaptations (Howarth, 1983; Hüppop, 2000; Derkarabetian et al., 2010; Culver et al., 2010; Trontelj et al., 2012; Soares & Niemiller, 2018).

Caves and other subterranean habitats offer a complex and promising ecosystem for in-depth studies of behavioral adaptations (Simonsen & Hesselberg, 2021). Adaptations conserved and evolved through natural selection play a crucial role in an organism's life, enhancing survival and fitness while enabling individuals to overcome various environmental challenges as they develop and grow (Adkins, 2017). Both biotic and abiotic characteristics of subterranean habitats—such as the absence of light, simplified communities, and reduced environmental variation—affect nearly every aspect of an organism’s existence (Simonsen & Hesselberg, 2021). Cave organisms have also developed specialized sensory organs and behaviors necessary for reproduction and foraging in these unique environments (Parzefall, 1986; Adkins, 2017; Simonsen & Hesselberg, 2021).

The term “cave” universally refers to natural underground openings formed by various processes in different rock types, with most being solution or karst caves created by the dissolution of carbonate rock by water (Gunn, 2004; Larned, 2012). Subterranean ecosystems in karstic areas, which include caves, fissures, and cracks, are geologically stable environments that can remain relatively unchanged for millions of years (Gibert & Deharveng, 2002).

Due to the absence of light, most subterranean habitats are resource-limited, with organic matter from the surface being the main energy source, as chemosynthesis contributes minimally (Soares & Niemiller, 2018). Food availability in caves is influenced by the cave type, surface conditions, and geographical location, with detritus and other organic materials being the primary resources, often introduced via flooding (Simon, 2019; White, Culver, & Pipan, 2019). Energy limitations in cave ecosystems are determined not just by carbon, but also by the availability of inorganic elements like iron, nitrogen, and sulfur, essential for chemolithoautotrophic processes (Engel, 2007). Karst groundwater typically contains ions such as Ca^{2+}, Mg^{2+}, HCO_3^-, Na^+, and SO_4^{2-}, reflecting the unique geochemical environment of these subterranean systems (Žák, Onac, & Perşoiu, 2008).

1.2. Factors that shape the cave communities

Darkness.

Caves are characterized by environmental conditions that are fundamentally distinct from those on the surface, such as constant darkness, reduced variability in abiotic factors like moisture and temperature, and generally insufficient nutrient supply (Hüppop, 2000; Lunghi & Manenti, 2020; Engel, 2019). Darkness is a defining feature of cave ecosystems, profoundly influencing the organisms that inhabit them. In the absence of light, energy in cave food webs primarily comes from microbial biomass produced by chemoautotrophs and from dead organic matter, such as detritus—mainly leaves and wood—that enters from the surface through water flow (Simon, 2019).

Carbon content.

The primary carbon inputs into karst basins are particulate organic carbon (POC) and dissolved organic carbon (DOC), which enter through sinks and shafts as well as through diffuse flow from the epikarst and soils (Simon et al., 2007, 2010; Spotl et al., 2011). Natural organic matter in aquifer systems is generally measured as total organic carbon. However, the concentration of total organic carbon in aquifer sediments differs from that in other sediments, such as marine or lacustrine, and typically decreases with depth through the soil zone, being even lower in aquifer sediments (Riddell et al., 2023).

Temperature.

The temperature in subsurface habitats, such as caves, is generally stable, though it can vary depending on location and time. Cave streams and pools that are fed by epikarst water—the interface zone between the surface and caves (Simon, 2019)—tend to have more stable temperatures and chemistry compared to cave streams that are connected to the surface through large openings (Hawes, 1939; Culver & Pipan, 2019).

Oxygen content.

Aquatic cave environments are generally hypoxic and poorly oxygenated, with highly variable oxygen levels over short distances due to groundwater flow and subsurface oxygen consumption, leading to fluctuating oxygen concentrations and intermittent hypoxia or near-anoxia (Malard & Hervant, 1999; Hervant & Malard, 2012). The survival of aquatic organisms in interstitial or karstic environments depends on their specific biochemical, behavioral, and physiological adaptations, including specialized respiratory mechanisms and other essential adjustments (Hervant et al., 1998).

Isolation (virtual island).

Caves function as "virtual" islands in a fundamental sense, being isolated habitats where cave-adapted organisms are separated from surface environments and exposed to the associated risks such as predators, sunlight, and environmental fluctuations (Mammola, 2019; Culver & Pipan, 2019). However, this analogy has limitations because karst landscapes contain interconnected passages, fractures, and small solution tubes that allow subsurface connectivity between caves. A more appropriate analogy might be the pools formed by dripping water from the epikarst, where the epikarst acts as the mainland and the drip pools represent the islands (Culver & Pipan, 2019).

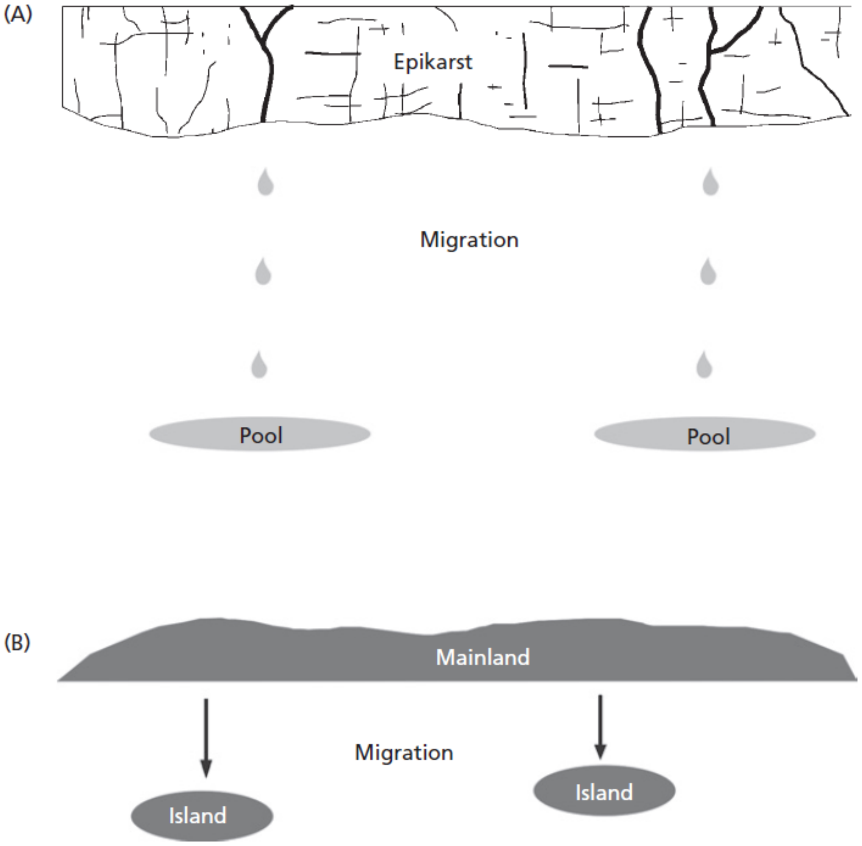

Figure 1. The relationship between epikarst, ceiling drips, pools (A), and the analogy between continents and islands (see Culver and Pipan 2019). Migrating species from epikarst dripping to the drip pools are described in Pipan et al. (2010).

Cave environments, while characterized by a poor nutrient supply, provide essential ecological opportunities such as food, protection from predators, and suitable conditions for hibernation, allowing various taxa to undergo extensive adaptive radiations and resulting in the development of diverse populations and species (Romero, 2012). The composition and structure of groundwater communities are influenced by multiple factors, including the size of pore spaces in sediments, broader geographical factors, the hydraulic conductivity of aquifers, and local-scale hydrological exchanges (Thulin & Innova, 2008). Although light is not a crucial factor for the successful colonization of subterranean habitats, species can thrive underground if they have access to appropriate energy sources, food, and mates; however, the size of organisms and the degree of contact between surface and subterranean environments limit the extent of colonization, with smaller organisms typically entering through soil or cracks and larger ones requiring more substantial connections (Trajano, 2012). The variation in subterranean biodiversity across regions is driven by a complex interplay of ecological, evolutionary, historical, and geographical factors, which provides key insights into the broader mechanisms influencing overall biodiversity (Gibert & Deharveng, 2002).

1.3. Subterranean organisms and their classification

Scientific research has predominantly focused on terrestrial, marine, and freshwater animals, with relatively little attention given to subterranean species, especially those in caves, such as insects, isopods, fish, and spiders, over the past 200 years (Slaney & Weinstein, 2008). Cave ecosystems are less densely populated than surface environments due to their stable conditions and the need for predator avoidance, but groundwater systems host a diverse array of fauna; approximately 7,000 species of stygofauna have been documented, though this number is likely an underestimate given the current limitations in groundwater research (Mejía-Ortíz & Hartnoll, 2006). Estimates by Culver and Holsinger (1992) suggest that there may be between 50,000 and 100,000 obligate subterranean species globally.

Recent reviews on the classification of subterranean organisms reveal that terminology and classification principles are complex and not straightforward, reflecting the intricate relationships between these organisms and their underground habitats (Sket, 2008). Different classification systems have been proposed over time. Schiner (1854) categorized cave-dwelling animals into three groups based on their ecological behavior: (1) surface-dwelling animals that occasionally inhabit caves; (2) troglophiles, which live in areas with partial light and can also be found on the surface; and (3) troglobites, which are exclusively adapted to cave environments and rarely emerge, except in extreme conditions like floods (Howarth & Moldovan, 2018; Culver & Pipan, 2019). Racovitza (1907) later refined this classification with more precise definitions: (1) trogloxenes, which visit caves but do not reproduce there; (2) troglophiles, which inhabit and reproduce in caves but can also be found outside; and (3) troglobites, which are strictly cave-dwelling, residing primarily in the deepest parts of caves and exhibiting significant morphological adaptations to life in complete darkness (Trajano, 2012; Howarth & Moldovan, 2018). Gibert et al. (1994) proposed an alternative terminology for groundwater fauna, distinguishing three categories: (1) stygoxenes, which replaces trogloxenes and refers to species that visit but do not reproduce in groundwater habitats; (2) stygophiles, which replaces troglophiles and denotes species that live in groundwater environments but can also be found in surface habitats; and (3) stygobionts, which replaces troglobites and describes species that are exclusively adapted to groundwater environments, with adaptations to life in complete darkness.

The term stygon was introduced by Husmann (1996) to define the community of organisms inhabiting groundwater, and he coined the term stygobiology for the scientific study of groundwater life (Husmann 1996, cited in Moldovan 2018). The earliest known cave-dwelling animals were aquatic species, with the first recorded discovery of such animals occurring in China in 1436, when the local doctor Mao Lan identified a troglophilic fish, now known as *Sinocyclocheilus grahami*, in South Yunnan (Ma & Zhao, 2012). In 1689, naturalist Janez Vajkard Valvasor discovered the blind amphibian salamander *Proteus anguinus* in the Carniola region of Slovenia, which was later described scientifically by Austrian naturalist Joseph Nicola Laurenti in 1767 (Valvasor, 1689; Laurenti, 1767, cited in Romero, 2009; Sket et al., 2004). *Proteus anguinus* is the only stygobiotic salamander in Europe and reaches over 25 cm in length, making it the largest known stygobiont (Gibert & Culver, 2005).

Figure 2. Cave-salamander, Proteus anguinus (photo credit: Gergely Balázs, a member of the research group at the Institute of Biology, Eötvös Loránd University, Budapest).

Groundwater species richness is significantly greater in Europe, with approximately 2,000 species, compared to Asia with 561 species, North America with 500 species, Africa with 335 species, and Australia with 500 species (Gibert & Culver, 2005). Groundwater ecosystems support a wide range of biodiversity, encompassing unicellular organisms such as protists (González-López et al., 2013) and multicellular organisms including sponges (Sket & Velikonja, 1986), hydrozoans (Zagmajster et al., 2011), flatworms (Leal-Zanchet et al., 2014), rotifers (Simões et al., 2013), mud dragons (Sørensen et al., 2000), roundworms (Reiss et al., 1999), ribbon worms (Moore & Gibson, 2006), water bears (Jørgensen et al., 2014), segmented worms (Gonzalez et al., 2018), molluscs (Culver, 2012), crustaceans (Galassi, 2001), water mites (Gerecke et al., 2018), aquatic beetles (Leys et al., 2004), fishes (Romero, 2001), and amphibians (Sket, 1997).

Crustaceans, including copepods, ostracods, amphipods, isopods, mysids, and decapods, are among the most characteristic inhabitants of aquatic cave environments (Mejía-Ortíz & Hartnoll, 2006). The class Crustacea includes approximately 35,000 described species, the majority of which are aquatic (Biggs et al., 2008). These invertebrate arthropods occupy diverse habitats worldwide, ranging from marine and freshwater to terrestrial and subterranean environments (Marshall et al., 1999; Romero, 2009).

1.4. Adaptation of animals in subterranean environments (caves)

Adaptation is a result of natural selection, a fundamental evolutionary process through which organisms adjust their traits to better fit their environments (Mitton, 2002; Lenski, 2017; Trontelj, 2019). According to Darwin’s theory, adaptation occurs gradually through small, advantageous changes and, when combined with concepts of mutation and inheritance, explains how organisms become well-suited to their surroundings (Lande, 2009; Lenski, 2017). In cave environments, adaptation has often involved instances of convergent evolution, resulting in a range of physiological (e.g., circadian rhythms in cave-dwelling Astyanax mexicanus, see McGaugh et al., 2014), morphological (e.g., specialized locomotion in cave-dwelling arthropods, see Krajl-Fišer et al., 2020), and behavioral traits (see Berisha et al., 2023). These adaptations have led cave-dwelling animals to exhibit shared troglomorphic characteristics (Protas & Jeffery, 2012; Floréz et al., 2021).

1.4.1. Behavioural adaptations

Behaviors are among the most flexible traits in the animal kingdom, and understanding them requires examining interactions with the environment throughout development (Wcislo, 2003; Beekman, 2019). Behavioral differences are often observed over time and across various contexts, where animals exhibit consistent patterns that distinguish them from other individuals within the same population. This phenomenon, known as "animal personality," categorizes individuals based on their stable behavioral tendencies, such as being consistently more aggressive, exploratory, or bold than others. The term "personality" refers to these underlying behavioral tendencies that vary among individuals but remain stable within each individual over time, affecting how they behave in different contexts. These consistent individual differences in behavior are often heritable and related to fitness (Beekman, 2019; Bergmüller, 2010; Bell et al., 2009; David et al., 2011; Stams & Groothuis, 2013).

Overall, individual plasticity contributes significantly to phenotypic variation in natural populations (Sultan & Spencer, 2002). The development of phenotypes occurs over time, during which the environment may change unpredictably and the organism may face selective pressures. Consequently, only the environmental conditions preceding significant selection episodes are crucial for the evolution of plasticity (Moran, 1992; Tufto, 2000; Lande, 2009).

Relationships between personality and plasticity, known as reaction norms, between individuals and variations in their environment create irreversible variations in the traits of individuals (Piersma & Drent, 2003). These variations are described as polyphenism, an animal’s ability to produce two or more discrete phenotypes from the same DNA to accommodate seasonal changes in the environment, and are present among arthropods and other invertebrate species (Piersma & Drent, 2003; Vogt, 2023).

In my thesis, I examined personality traits such as activity (the general level of movement of an individual), exploration (the individual’s response to novel situations), and shyness-boldness (the individual’s reaction to risky situations) (Réale et al., 2007). These behavioural traits, including aggressiveness (defined as harmful behaviour directed towards other animals to gain access to food, shelter, mates, and territory, as described by Benus et al., 1991) and sheltering (hiding under cover, Horváth, et al., 2021), have garnered extensive scientific attention in the study of animal behaviour (Réale et al., 2007; Martin et al., 2007; Aryomo & Watt, 2012).

Studies on animal personality suggest that most behavioural variations arise from intraindividual differences (Bell et al., 2009; Jolles et al., 2019). From an adaptive perspective, individuals within the same population can modify their behaviour based on current environmental conditions, leading to consistent individual differences over time and across various contexts (Jolles et al., 2019). Such variations are observed across numerous species (Dall & McNamara, 2004).

Behavioural traits, like other phenotypic traits, result from the complex interplay between an individual's genetic inheritance and environmental influences, with behavioural plasticity being a primary mechanism through which organisms adapt to environmental changes. Both phenotypic and behavioural plasticity can be ecologically advantageous by allowing a genotype to endure a wider range of environmental conditions (Ghalambor et al., 2010). Plasticity, as a trait with genetic variation, can evolve in response to natural selection (Ghalambor et al., 2015; Gibert et al., 2019). In the colonisation of new environments, behavioural plasticity is crucial for adaptation and is a key factor influencing survival (Westrick et al., 2019; Gunn, 2004). Understanding behavioural changes in response to environmental shifts is crucial for comprehending how animals adapt to hypogean environments, as these adaptations impact various aspects of their behaviour, including activity, feeding, reproduction, social interactions, photophobic responses, and circadian rhythms (Gunn, 2004). Given the absence of photosynthetic organisms in caves, primary productivity is lacking, and nutrient availability is usually limited (Bilandžija et al., 2020).

2. The aim of the research

In my thesis research, I utilized the freshwater crustacean isopod *Asellus aquaticus* as a model organism, which is recognised for its capacity to effectively colonise cave environments and exhibit troglomorphic adaptations (Pérez-Moreno et al., 2017; Herczeg et al., 2022). In addition to typical troglomorphic features such as eye reduction and depigmentation, cave-adapted populations of *A. aquaticus* demonstrate distinct changes in morphology, physiology, and behaviour, resulting in the formation of separate ecomorphs (Vitagliano et al., 1991; Protas et al., 2011; Pérez-Moreno et al., 2018; Zidar et al., 2018; Fišer et al., 2019; Verovnik & Konec, 2019; Balázs et al., 2021).

The aim of my thesis research was to investigate population variation in three behavioural traits—movement activity, exploration, and risk-taking—and to compare these traits between cave and surface populations of *Asellus aquaticus*. I applied various experimental manipulations to assess each behavioural trait.

The cave population studied, which is genetically isolated from its nearest surface counterpart for approximately 60,000 years based on molecular data (Pérez-Moreno et al., 2017), is located in Budapest, Hungary. This population resides in the Molnár János Cave (referred to as MJC cave hereafter), a hydrothermal cave known for being predator-free (Gergely, 2019). Surface populations were selected randomly for comparison.

1. I first investigated the movement activity of one cave population and three surface populations of *Asellus aquaticus* in a controlled laboratory environment. Individuals from each population were tested under both light and dark conditions (see the experimental setup in Chapter I) to observe their behavioural responses to different light regimes. I predicted that the cave-adapted population would exhibit heightened sensitivity to light, given that light is an unfamiliar stimulus for them, compared to the surface populations, under a 16-hour light and 8-hour dark cycle. As cave-adapted populations are free from predation due to the absence of predators, unlike surface populations which encounter numerous and diverse predators, I predicted that the increase in movement activity would be more pronounced in the cave-adapted population.

   Furthermore, since males of *Asellus aquaticus* are known to be more active in their search for mating compared to females, particularly in novel environments (Bertin et al., 2002; Karlsson et al., 2010), I predicted that males would show higher movement activity in their search for females. Thus, I hypothesised that movement activity in cave-adapted males would be greater than in cave-adapted females. I also predicted that mate-searching behaviour would be more pronounced in males (Bertin et al., 2002).

2. Next, I examined the exploratory behaviour of one cave population and four surface populations of *Asellus aquaticus* in a controlled laboratory environment. All populations were tested under both light and dark conditions (see the experimental setup in Chapter II) to assess their responses to these lighting conditions. Given that caves are ecologically isolated environments with low variability, I predicted that exploratory behaviour would be reduced in cave-adapted populations compared to surface populations. I hypothesised that recent cave colonists (surface-type) would be more exploratory compared to their surface conspecifics from the source population, and I predicted that individuals from populations entering a completely new habitat would exhibit the highest levels of exploratory behaviour compared to the average levels observed in their source populations.
3. Lastly, I investigated the risk-taking behaviour of one cave population and three surface populations of *Asellus aquaticus* in a controlled laboratory environment. All populations were tested under light and dark conditions (see the experimental setup in Chapter III for details) to observe their plastic responses to light. Given that cave-adapted populations lack predators, I predicted that their risk-taking behaviour would be more pronounced compared to surface populations, which face numerous and varied predators, as well as environmental and human interactions. I tested whether bold individuals would take greater risks (e.g., escape behaviour) in response to an unsuccessful predation threat compared to surface populations. Additionally, I assessed whether individuals would exhibit freezing behaviour or take fewer risks to escape (Fraser et al., 2001; Réale & Festa-Bianchet, 2003; Brown et al., 2007).

3. STUDY ORGANISM

3.1. Characteristics of the animals studied.

My thesis uses the freshwater isopod crustacean *Asellus aquaticus* (see Figure 3) as the research model. The order Isopoda encompasses over 11,000 species, which occupy a diverse range of aquatic and terrestrial environments (Hobbs, 2005).

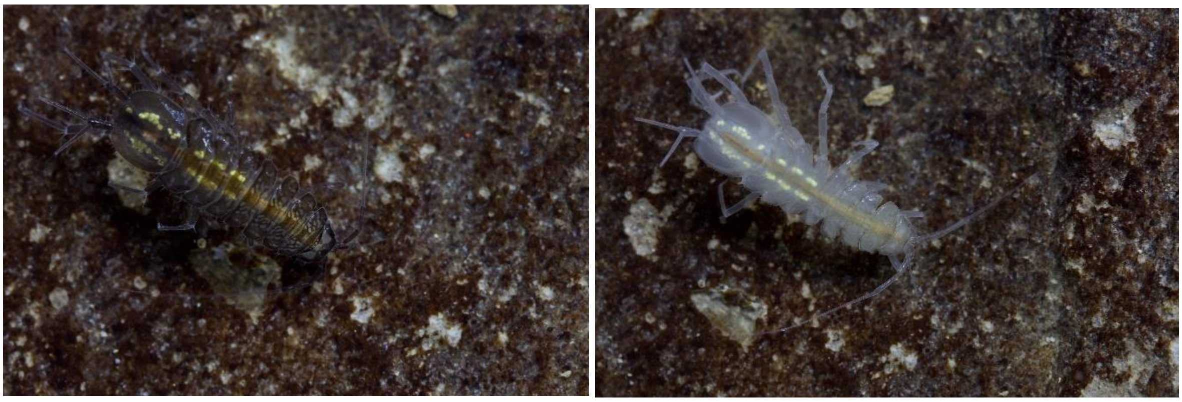

Figure 3 and Figure 4. Surface (left side) and cave-adapted (right side) Asellus aquaticus (photo credit: Gergely Balázs, a member of the research group at th, Institute of Biology, Eötvös Loránd University, Budapest).

3.1.1. Taxonomy:

(Based on Boyko et al., 2023)

Kingdom: *Animalia*
Phylum: *Arthropoda*
Class: *Malacostraca*
Order: *Isopoda*
Family: *Asellidae*
Gens: *Asellus*
Species: *Asellus aquaticus* Linneus 1758

3.1.2. Distribution

Asellus aquaticus is a highly eurytopic and eurythermal species, found in a variety of freshwater habitats ranging from coastal zones of lakes, small water reservoirs, and rivers to slightly brackish surface waters and some subterranean freshwaters (Oleszkiewicz et al., 2001; Prevorčnik et al., 2004; Verovnik et al., 2005; Prevorčnik et al., 2009). This species is widely distributed across Europe and North America. Although it is absent from some Mediterranean regions, *A. aquaticus* has successfully dispersed throughout Europe and has become established across the continent (Maltby, 1991; Turk et al., 1996; O'Callaghan et al., 2019). It is believed to have originated in the western part of the Pannonian Basin during the late

Miocene, with its initial distribution in temperate regions of Asia (Sworobowicz et al., 2015). *A. aquaticus* has also colonised numerous subterranean (cave) waters in Europe, where its cave-dwelling form displays morphological and other traits characteristic of troglobitic animals (see Figure 3) (Turk et al., 1996; Protas & Patel, 2009). Alongside its wide environmental distribution, phenotypic variation across environments and habitats in *A. aquaticus* indicates its high capacity for phenotypic change via phenotypic plasticity and genetic adaptation (Williams, 1962; Aston & Milner, 1980; Murphy & Learner, 1982; Tufto, 2000; Sultan & Spencer, 2002; Hargeby et al., 2004; Leimar et al., 2006; Hargeby et al., 2007; Lande, 2009; Richardson et al., 2014; Lürig et al., 2019).

Asellus aquaticus exhibits tolerance to organic pollution but is sensitive to prolonged exposure to high temperatures, which can adversely affect its survival, growth, and reproduction rates. While it shows tolerance to a variety of toxic heavy metals, *A. aquaticus* is particularly sensitive to ions of chromium (Cr), iron (Fe), lead (Pb), nickel (Ni), zinc (Zn), cadmium (Cd), mercury (Hg), and copper (Cu). The presence of these heavy metals in its aquatic habitats is typically linked to runoff from urban watercourses (Migliore & Guidici, 1990; Mullis et al., 1994; Pavel et al., 2021). Additionally, *A. aquaticus* serves as a valuable organism for studying the bioavailability of sediment trace metals such as palladium (Pd), platinum (Pt), and rhodium (Rh) (Moldovan et al., 2001). Furthermore, *A. aquaticus* displays pronounced fluorescence, a phenomenon where absorbed photons are re-emitted at a longer wavelength. Specifically, it exhibits fluorescence of green to yellow light ($550 \text{ nm} < \lambda < 750 \text{ nm}$) when observed under ultraviolet light ($300 \text{ nm} < \lambda < 400 \text{ nm}$) (Chambers, 1977; Zimmer et al., 2002). *A. aquaticus* is associated with b-mesosaprobic waters of the paleoarctic, an area characterized by low oxygen consumption and significant mineralization of organic materials with end products such as nitrates (Pavel et al., 2021)

3.1.3. Feeding

Asellus aquaticus is known to primarily feed on decaying vegetation, microscopic algae, fungi, and small invertebrates (Marcus & Willoughby, 1978; Grieve & Lau, 2018; Herczeg et al., 2020; Pavel et al., 2021; Horváth et al., 2022). In freshwater ecosystems, *A. aquaticus* encounters leaf litter, from which it can digest cellulose and lignin (plant biomass comprising cellulose, hemicelluloses, and lignin; see Brautest & Ellingsen, 2011), phenolics (a diverse group of compounds with a benzene ring and a hydroxyl group; see Ayad & Akkal, 2019), and other recalcitrant components (synthetic compounds that are either slowly biodegradable or non-biodegradable, ranging from simple halogenated hydrocarbons to complex polymers; see

Faber, 1979) from terrestrial plants (Zimmer & Bartholmé, 2003). The successful colonisation of *A. aquaticus* is attributed to the acquisition of gut microbial communities that produce enzymes necessary for the digestion of plant detritus (Zimmer et al., 2001). Additionally, ***A. aquaticus*** harbours bacterial endosymbionts in two digestive structures, the hepatopancreas and the hindgut, where endogenous digestive enzymes are secreted (Zimmer & Bartholmé, 2003; Wang et al., 2007; Bredon et al., 2019).

3.1.4. Morphology

Asellus aquaticus adults have a body plan consisting of a dorsoventrally flattened structure divided into three main regions: the cephalon (comprising the head and the first thoracic segment, which bears the maxillipeds), the pereion (comprising seven thoracic segments, each with a pair of walking legs, or pereopods), and the pleotelson (which includes the uropod, endopod, and exopod) (see Figures 5 and 6 for details) (Verovnik et al., 2009; Vick & Blum, 2010). The body length of *A. aquaticus* typically ranges from 12 mm to 20 mm (Chambers, 1977).

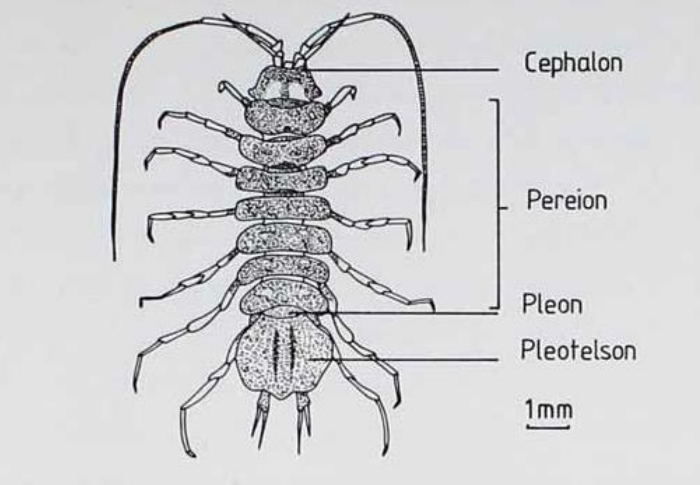

Figure 5. Dorsal view of (adult) Asellus aquaticus (photo source: see Millman, 1984)

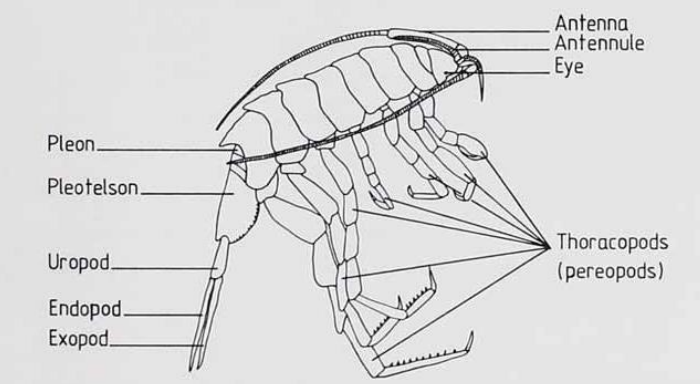

Figure 6. Morphological features of adult Asellus aquaticus (photo source: see Millman, 1984).

3.1.4.1. Differences between surface and cave individuals

Studies on the surface-cave divergence of *Asellus aquaticus* have provided extensive and detailed genetic and developmental analyses for this species, leading to significant efforts to establish it as a model organism for studying evolution in cave environments (Protas et al., 2011; Konec et al., 2015; Mojaddidi et al., 2018). Compared to its surface ancestors, cave populations of *A. aquaticus* (see Figure 3) exhibit several distinctive features, including a reduction in optical units, depigmentation, more elaborate sensory structures, and the elongation of certain appendages. Morphological differences, such as variations in eye size, pigmentation, and the number of antennal articles, emerge during embryonic development and are indicative of differential gene expression. These genes are likely involved in processes such as phototransduction, photoreception, and eye development (Turk et al., 1996; Prevorčnik et al., 2004; Protas et al., 2011; Yoshizawa et al., 2012; Soares & Niemiller, 2013; Mojaddidi et al., 2018; Konec et al., 2015).

3.1.5. Reproduction

Asellus aquaticus is a gonochoric species with eight homomorphic chromosome pairs (2n = 16) in both sexes (Montalenti & Rocchi, 1964; Salemaa, 1979; Bertin et al., 2002; Valentino et al., 2009). Breeding seasons for *A. aquaticus* commence in early spring and autumn (Oleszkiewicz et al., 2001). The species typically produces three generations per year: one in spring or early summer, another in late autumn or early winter, and a third in the following spring (Chambers, 1977; Oleszkiewicz et al., 2001). In *Asellus aquaticus*, copulation is preceded by a period known as mate guarding, during which a male guards a female by carrying her in precopula until insemination (see Figure 5 for further details on the life cycle and reproduction of *A. aquaticus*) (Adams et al., 1985). This behaviour represents a competitive strategy employed by males when fertilisation opportunities are limited (Bertin et al., 2002). The morphological and sexual dimorphisms associated with this mating behaviour are well-documented in *A. aquaticus* (Bertin et al., 2002; Biro et al., 2021).

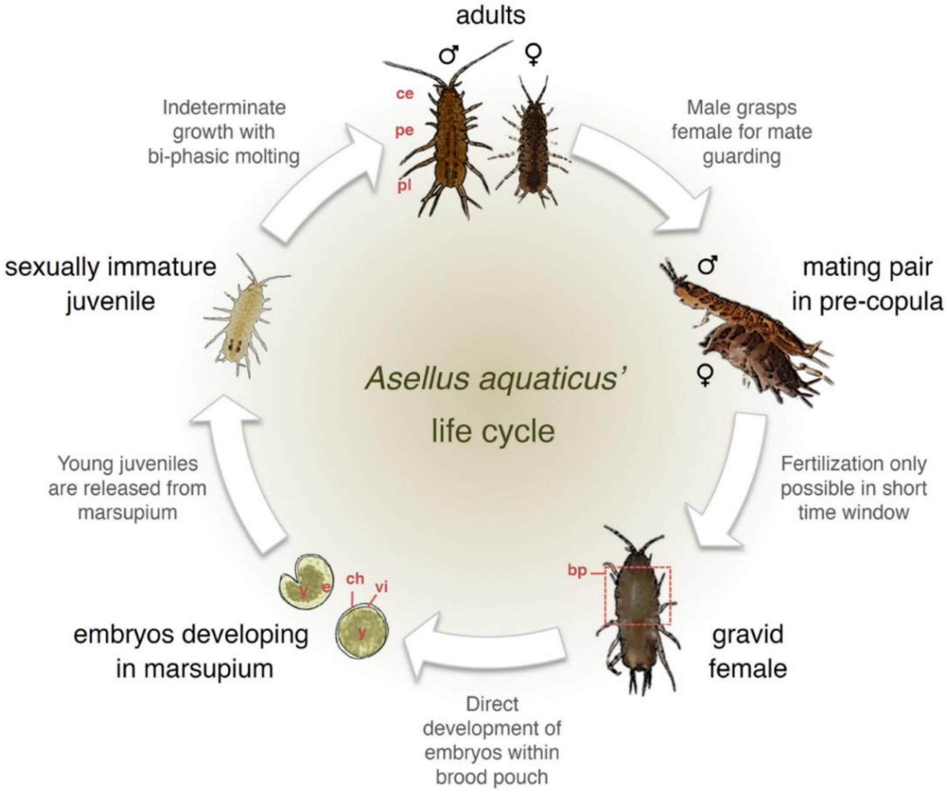

Figure 6. Life cycle and reproduction in Asellus aquaticus in a schematic view (image source: see Lafuente et al., 2021).

3.1.6. Behaviour

Asellus aquaticus serves as an excellent model for examining the relationship between environmental variability and diversification, particularly in terms of phenotypic plasticity. This species displays considerable variation within and between populations, across both continental and local scales, offering insights into the evolution of adaptive traits in response to diverse ecological pressures (Lafuente et al., 2021).

In cave environments, *A. aquaticus* populations exhibit distinct troglomorphic traits such as body depigmentation and loss of vision, adaptations that have evolved in response to the darkness and limited resources characteristic of subterranean habitats (Prevorčnik et al., 2004; Lafuente et al., 2021). These adaptations are believed to be critical for energy conservation in an environment where food scarcity is prevalent and where energy expenditure needs to be minimized to enhance survival (Hervant et al., 1997).

Behavioural adaptations in *A. aquaticus* significantly influence their survival strategies. These adaptations include movement, exploration, and risk-taking behaviors that are essential for navigating and exploiting new environments, allowing them to locate food sources and safer habitats effectively (Cote et al., 2010). For instance, dispersal and exploration behaviors are particularly well-studied, highlighting how these traits influence foraging success and mating opportunities in different ecological settings (Martins et al., 2007).

Defensive behaviors are crucial for predator avoidance, where *A. aquaticus* frequently utilizes vegetation and leaf litter for protection. These natural shelters provide not only safety from predators but also access to food resources, effectively integrating shelter and foraging needs (Horváth et al., 2021; Lafuente et al., 2021). The strategic use of shelters is aligned with the species' need to balance predator avoidance with resource acquisition, demonstrating an adaptive behavior that maximizes survival (Calizza et al., 2013).

The influence of predation on the behavior of *A. aquaticus* is considerable, where predation pressure dictates their activity levels. In environments with high predation risk, the species exhibits reduced movement to decrease predation risk, adapting their behavior to minimize exposure to predators while still fulfilling basic life functions like feeding and mating (Bell & Sih, 2007; Herczeg et al., 2009). Despite their evolutionary adaptations to cave life, *A. aquaticus* populations retain a preference for traditional surface food sources, indicating a conservative dietary strategy influenced by their ancestral dietary habits and the limited food availability in their cave habitats (Herczeg et al., 2020; Herczeg et al., 2022).

Sexual dimorphism is evident in *A. aquaticus*, influencing reproductive strategies and outcomes. Larger males or those with longer antennae have advantages in mate-finding due to their increased visibility and ability to dominate smaller rivals, showcasing how morphological and behavioral adaptations are shaped by reproductive and survival pressures (Ridley & Thomson, 1979; Bertin & Cézilly, 2003; Balázs et al., 2021). These morphological traits not only facilitate successful mating but also enhance the individuals' ability to evade predators, illustrating a multifaceted adaptation that serves multiple ecological functions (Harris et al., 2013).

Mate guarding behavior in *A. aquaticus* is significantly influenced by the presence of predators. During predation events, males may abandon mate-guarding to evade predators, thus reducing their predation risk but potentially impacting their reproductive success (Karlsson et al., 2010; Verrell, 1985). This behavior underscores the trade-offs that individuals must navigate between immediate survival and reproductive success, a common theme in the behavioral ecology of species facing high predation pressures (Dingemanse et al., 2003; Cote et al., 2010).

Furthermore, factors such as population density and sex ratio profoundly impact the mating behavior of *A. aquaticus* adults. Higher population densities can increase competition for mates, thereby influencing the mating strategies of males and females differently (Karlsson et al., 2010). These dynamics are crucial for understanding how *A. aquaticus* populations adapt their reproductive behaviors to local ecological conditions, highlighting the complex interactions between demographic structures and behavioral strategies (Gomes et al., 2019).

Asellus aquaticus provides a rich case study for understanding how ecological and evolutionary pressures such as predation, resource availability, and environmental variability shape the behavioral ecology of species. Their adaptive behaviors, particularly in response to predator presence and resource distribution, underline the complex interplay between evolutionary adaptations and environmental challenges, offering valuable insights into the mechanisms driving behavioral diversification and ecological success across diverse habitats (Schuett et al., 2018; Dingemanse et al., 2007).

3.2. Study system

The model system includes a cave population residing in the hydrothermal Molnár János Cave in Budapest, Hungary, which is a predator-free environment, as well as three surface populations from the cave's vicinity. These surface populations inhabit areas with complex communities, comprising various predators and diverse food sources.

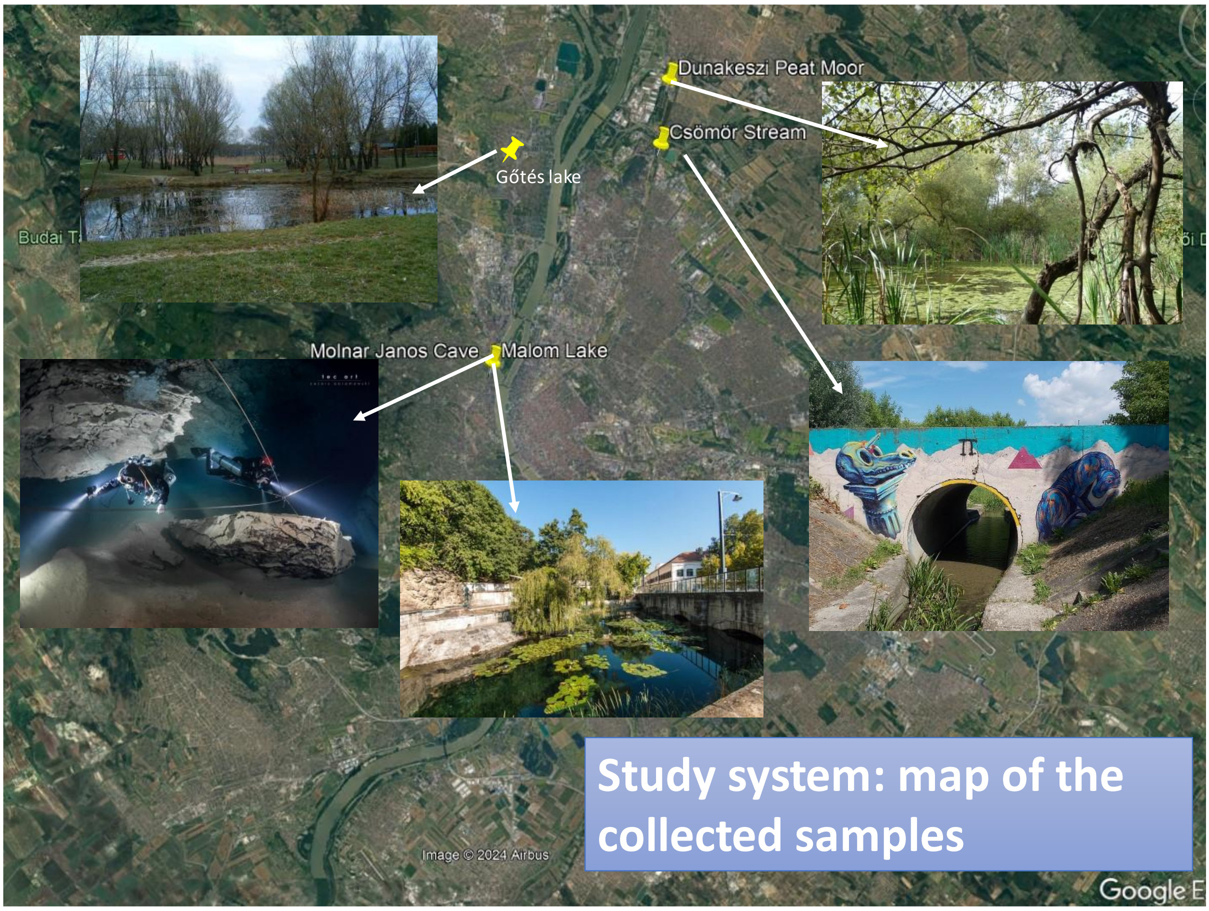

Figure 7. Map illustration of collected populations of *Asellus aquaticus* (image sources: Google).

The corridors of the Molnár János Cave (MJC) extend beneath a highly urbanised area, specifically Budapest, Hungary. The primary section of this cave system is submerged, reaching a maximum depth of 100 metres, with the known passages extending a total length of 5.5 km. The cave entrance is situated near the Danube River. Formed through thermal karst processes, the cave is generated by hot springs and lies below the karst water table, providing an exceptional opportunity to study solution processes both below and above this level. The cave is named after János Molnár, a pharmacist who first hypothesised its existence as part of the same water system as the nearby Malom Pond, which was dammed in the 13th century to supply water mills in the area. MJC is classified as a hypogene cave, meaning the water that formed it originates from underground rather than surface sources. The cave is filled with thermal water at temperatures of 23–24 °C. Chemical analyses reveal that the air within the MJC contains 6.5% CO_2 (Erőss et al., 2006; Bodor et al., 2015; Surányi et al., 2010).

The Molnár János Cave (MJC) is a predator-free environment, relying solely on endogenous, chemoautotrophic, mat-forming bacteria for its ecosystem. The first surface population used in this study is from Malom Lake (ML; 47.518277°N, 19.035999°E), a small pond at the entrance of MJC, formed by the cave's outflow. The water temperature in ML matches that of the cave and remains constant throughout the year. However, ML is exposed to natural surface light and is considered a typical surface habitat, with the presence of fish predators such as guppies (Poecilia reticulata), which were introduced in the 20th century (Berczik, 1956) and now occur in extremely high densities due to the absence of native fish. Molecular data indicate that the cave-adapted population of *Asellus aquaticus* has been isolated from its closest geographically surface population for at least 60,000 years.

Other surface populations included in this study are from Dunakeszi Peat-moor (DM; 47.615613°N, 19.126392°E), Csömör Stream (CS; 47.593393°N, 19.121970°E), and Gőtes Lake (47.354357°N, 19.22980°E). These sites experience the natural surface light regime and temperature fluctuations typical of non-thermal freshwater environments in the region. DM and CS are inhabited by a variety of vertebrate predators (including unidentified fish, amphibians, water birds, and semi-aquatic mammals) and invertebrate predators (such as odonate and dytiscid larvae, and erpobdellid leeches). All tested populations of *A. aquaticus* are located in Budapest or its nearby surroundings.

CHAPTER I

Berisha H, Horváth G, Fišer Ž, Balázs G, Fišer C, Herczeg G 2023.

Sex-dependent increase of movement activity in the freshwater isopod *Asellus aquaticus* following adaptation to a predator-free cave habitat. Current Zoology 69:418-425

SCIENTIFIC BACKGROUND

The behavior and movement of initial colonists in novel environments are critical for their survival and the successful establishment of populations. When organisms venture into unfamiliar habitats, they must first adapt to their new surroundings. This acclimatization process is crucial as it facilitates efficient resource utilization and enhances predator avoidance, both of which are vital for the survival and eventual proliferation of the species in new ecological niches (Russell et al., 2009). Predation exerts a profound selective pressure, influencing survival directly and thus affecting the probability of future reproduction (deRivera et al., 2003).

Furthermore, the interaction between predation pressure and basic behavioral activities such as foraging and mate searching is intricate. Typically, increased movement activity enhances foraging efficiency and the success of finding mates, but it concurrently elevates the risk of encountering predators (Fišer et al., 2019). This dynamic illustrates a fundamental ecological trade-off where the benefits of increased activity must be balanced against the potential costs of predation. Interestingly, the implications of increased movement activity can differ significantly between sexes, influenced largely by sex-specific roles in foraging and reproduction (Gwynne, 1987; Sakkaluk & Belwood, 1984).

Research spanning various taxa has highlighted how sex-specific responses to predation risk can lead to distinct behavioral adaptations. For instance, studies on the Bahamas mosquitofish (*Gambusia hubbsi*) have shown that in environments with high predation risk, behavioral lateralization becomes more pronounced, especially among females who exhibit increased rates of foraging and food consumption. In contrast, males in these environments display a less pronounced response, suggesting differing evolutionary pressures between the sexes (Hulthén et al., 2021).

Similar patterns have been observed in guppies (*Poecilia reticulata*), where females have been shown to adopt more risk-taking behaviors when experienced with predators compared to their predator-naïve counterparts. This adaptation potentially increases their chances of survival in predator-rich environments. Males, however, tend to exhibit consistent behavior regardless of previous predator encounters, indicating a possible inherent risk aversion or different adaptive strategies that do not fluctuate with predation pressure (Hulthén et al., 2021; Pärssinen et al., 2021; Harris et al., 2010).

In environments such as caves, where predation pressure is typically low and food resources are scarce, the behavioral strategy shifts. Here, animals might exhibit increased movement activities to optimize foraging and mating opportunities despite the higher energy costs associated with such activities. This adaptation is particularly advantageous in resource-limited environments where the benefits of locating scarce food and mates outweigh the risks associated with higher activity levels (Wilkens et al., 2000; Salin et al., 2010).

In my first thesis study, the movement activity of *Asellus aquaticus* was examined under two distinct conditions: comparing movement between cave and surface populations and assessing differences in movement activity between the sexes within these populations. Given the minimal predation in cave environments, it was hypothesized that cave populations would exhibit higher movement activity compared to surface populations, which face significant and varied predation threats. It was also anticipated that males would demonstrate increased movement activity, particularly in their pursuit of mates, considering the critical role of movement in reproductive success. The assessment of movement activity was conducted both in the presence and absence of light to determine the influence of light on movement patterns and to explore whether behavioral adaptations to darkness in cave populations manifest differently compared to surface populations. This multifaceted approach provides a comprehensive understanding of the ecological dynamics and evolutionary implications of behavioral adaptations in *A. aquaticus* across different environments (Gomes et al., 2019; Herczeg et al., 2020).

METHODOLOGY

Collecting and housing the experimental animals

For the first study on movement activity, a total of 200 adult *Asellus aquaticus* (25 males and 25 females from each population) were collected from one cave population (Molnár János Cave (MJC)) and three surface populations (Malom Lake (ML), 47.518277° N, 19.035999° E; Dunakeszi Peat-moor (DM), 47.615613° N, 19.126392° E; and Csömör Stream (CS), 47.593393° N, 19.121970° E) between 16th and 17th August 2018. The collected individuals were transported to the aquacultural facilities at the Institute of Biology, Eötvös Loránd University. Upon arrival, the animals were housed in plastic Petri dishes (90 × 25 mm, diameter × height), with the bottoms of the dishes roughened using emery paper to facilitate normal locomotion. The Petri dishes were filled to half their height with water from the respective source habitats and were regularly topped up to counteract evaporation. Throughout the experiment, the Petri dishes were maintained within light-controlled "recording chambers" (see Figure 9). Food sources from the original habitats were used for each population: dead leaves for the surface individuals and bacterial food for the cave-adapted individuals.

The laboratory temperature was maintained at 23–24 °C, matching the water temperature of the source habitats. Surface populations were acclimated to a daily light cycle similar to that of their original environment (16 hours light and 8 hours dark), whereas cave-dwelling individuals were acclimated to total darkness in a separate recording chamber. This acclimation was crucial for familiarising the animals with the new artificial environment and minimising stress-related behaviours during subsequent observations. All handling of cave-dwelling animals was conducted under a red-light regime. During the initial days in the laboratory, some animals perished, leaving 164 individuals for testing: 10 males and 16 females from MJC; 23 males and 23 females from ML; 24 males and 23 females from DM; and 22 males and 23 females from CS.

Experimental Setup

Acclimation and behavioural tests were conducted in four custom-designed recording chambers, each with dimensions of 100 × 55 × 105 cm (length × width × height). The acclimation period lasted four days before testing commenced. Each chamber was equipped with two types of lighting: LEDs at the top to simulate a daylight cycle (colour temperature = 4500 K, colour rendering index >90), and infrared (IR) LEDs at the bottom (920 nm). An opaque plexiglass sheet covered the IR LEDs to diffuse the light and provide a stable surface for placing Petri dishes containing the animals. The IR light was continuously illuminated during all recordings. Each chamber contained 50 Petri dishes (see Figure 9).

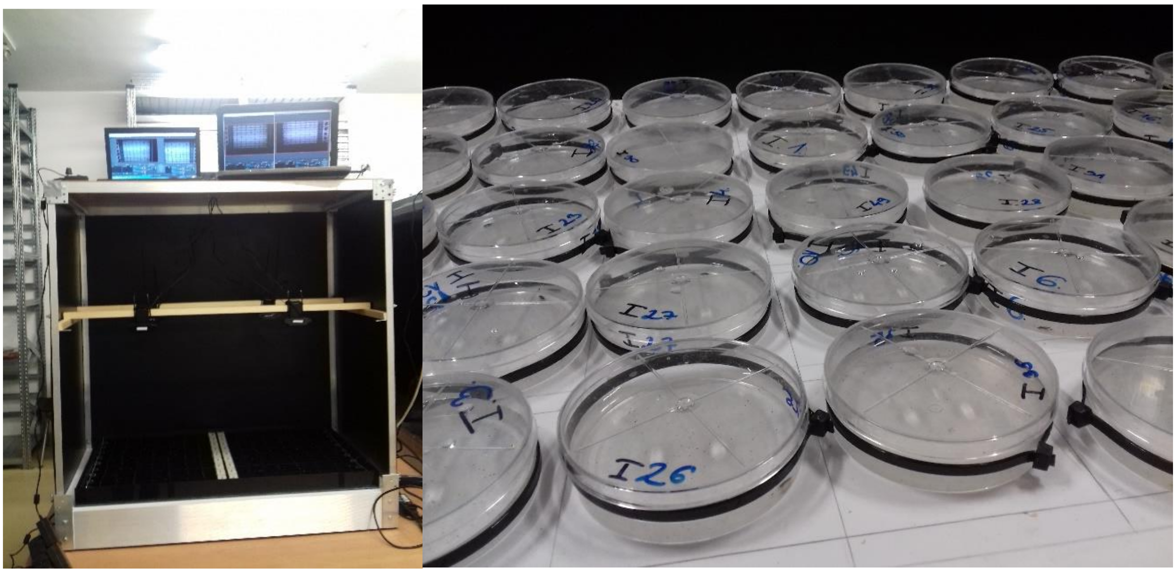

Figure 8. The custom-made recording chambers (note that their fronts could be closed to block out light completely). One the right side, the Petri dishes used for measuring movement activity are shown as being placed in the chambers.

The chambers were sealed with black plastic boards on the sides to prevent light from penetrating the interior. Each chamber was equipped with a webcam (Logitech C920 FullHD) to improve video quality under infrared light conditions. Video recordings were captured using OBS Studio software at five frames per second with HD resolution (1280 × 720 pixels).

Observations were conducted over two rounds on the 20th and 21st of August 2018. Each population was randomly divided into two groups with equal representation of sexes. One group was observed under daylight conditions on the first day and in darkness on the second day, while the other group experienced the reverse order. Animals from both cave and surface populations were randomly assigned within the same recording chamber for each light condition. Recordings commenced around 11 am and lasted for 60 minutes, with a 15-minute acclimation period prior to recording to allow animals to adjust to the experimental light conditions. Following recordings, the light was returned to the acclimation regime for all animals.

Although the dark treatment might seem less relevant for surface populations, recent research (Chapter 2, Horváth et al., 2023) suggests that *Asellus aquaticus* exhibits significant nocturnal activity, potentially surpassing diurnal activity. Given the incomplete understanding of how light or its absence affects isopod behaviour, a full-factorial experimental design was employed to account for these effects. Each video was analysed for 15 minutes, divided into three 5-

minute intervals: the start (0–5 min), middle (27.5–32.5 min), and end (55–60 min) of the 60-minute footage. Movement was tracked using the digitising tool DLTdv (Hedrick, 2008). Raw tracking data were processed into behavioural variables using a custom Excel macro. Behavioural data for each interval were calculated separately and then averaged. The following five variables were extracted for analysis: total distance moved, total time moving, number of movement bouts, and the mean and standard deviation of the distance moved per bout.

Statistical Analysis

Spearman rank correlations revealed strong relationships between the variables, as shown in Table 1. Consequently, a Principal Component Analysis (PCA) was conducted using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL) to identify independent principal components (PCs) that account for the majority of the observed variation. The Bartlett's test of sphericity was significant, confirming that the correlation matrices differed significantly from identity matrices. Following the Kaiser-Guttmann criterion, which suggests retaining only those PCs with eigenvalues greater than one, a single principal component was identified, explaining 64.8% of the total variation. High scores on this component were associated with individuals exhibiting both high movement activity and considerable variability in their activity levels (standard deviation), whereas low scores corresponded to less active individuals with lower variability in their activity levels.

To evaluate the effects of population, sex, and light regime on movement activity, a linear mixed model (LMM) was constructed using the lme4 package (Bates et al., 2014) in R version 4.1.2 (R Core Team, 2021). In this model, "movement activity" (i.e., PC1) served as the dependent variable, with "population," "sex," "light regime" (light vs. dark), and all possible 2- and 3-way interactions included as fixed factors. To control for habituation effects, the order of trials was included as a fixed effect but was not involved in any interactions. Individual identity was accounted for as random intercepts. The model's estimated marginal means were computed using the emmeans package (Lenth, 2019). Post hoc analyses were performed, and P-values were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) false discovery rate method to assess the significance of between-group differences.

RESULTS

Our analysis revealed significant effects of population, as well as a significant interaction between population, sex, and light regime, on movement activity (see Table 1). Post hoc comparisons indicated that individuals from the cave population exhibited significantly higher movement activity compared to those from the surface populations. Specifically, cave-dwelling individuals demonstrated a pronounced male-biased sexual dimorphism in movement activity under dark conditions, with males being notably more active than females.

Additionally, a significant habituation effect was observed across all populations, indicating that individuals generally exhibited reduced activity in the second round of observations compared to the first. This reduction in activity was consistent regardless of whether the individuals were from the cave or surface populations.

Furthermore, the analysis identified a significant random effect, highlighting notable differences in movement activity between individual animals (see Figure 10). This variation suggests that individual differences significantly contribute to the observed movement activity patterns.

Table 1. Results of the Linear Mixed Model on movement activity of *Asellus aquaticus* (statistically significant effects ($P < 0.05$) are bolded).

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

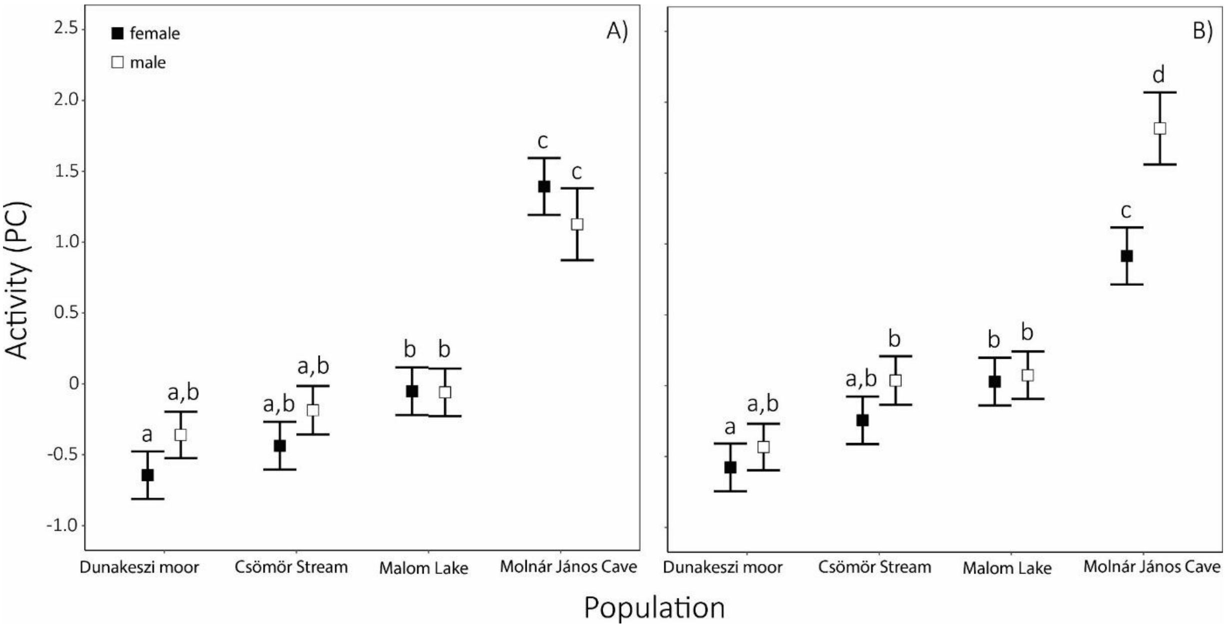

Figure 9. Movement activity of male and female *Asellus aquaticus* in (A) light and (B) darkness. Movement activity is the first principal component of a PCA on five behavioural variables measured from videos; higher values on the y-axis denote higher activity. Estimated marginal means ± standard errors 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).

DISCUSSION

The results of this study substantiate the initial predictions concerning movement activity in *Asellus aquaticus* populations. Specifically, the cave population from Molnár János Cave (MJC) exhibited significantly higher movement activity compared to the three surface populations: Malom Lake, Dunakeszi Peat-moor, and Csömör Stream. This finding supports our first hypothesis that cave-dwelling *A. aquaticus* show increased movement activity relative to their surface-dwelling counterparts. Additionally, the increase in movement activity was notably more pronounced in males than in females under natural conditions, such as darkness, thereby confirming our second hypothesis.

However, it is crucial to interpret these results with caution. The substantial individual differences observed in movement activity should not be hastily interpreted as indicative of inherent animal personality traits. Several factors constrain the generalisability of these findings. Firstly, only two measurements per individual were recorded, which might not fully capture the range of variability in movement activity. Secondly, the measurements were taken over a brief period, which may not accurately represent long-term behavioural patterns. Lastly, the artificial light conditions used during testing may not perfectly simulate the cave environment, potentially affecting the behaviour of cave-dwelling *A. aquaticus*. The behaviour observed under these conditions should, therefore, be interpreted with an understanding of these potential limitations.

In natural settings, the correlation between behavioural activity and predation risk is well-documented across various taxa. However, the effects of negligible predation risk on the evolution of sex-specific behavioural traits are less frequently studied. Research has shown that increased movement activity in surface-dwelling animals is often correlated with the presence of predators, whereas in subterranean habitats, such activity is typically associated with the search for food and mates (Kasumovic et al., 2007). Predation serves as a powerful selective force that shapes the evolution of defensive adaptations and behavioural traits such as activity levels, exploratory behaviour, and boldness (Tollrian & Harvell, 1999; Kortet et al., 2010). In the cave environment, where predation pressure is minimal, cave-adapted *A. aquaticus* may exhibit heightened movement activity as an adaptive strategy to address challenges related to food scarcity and mate-finding (Lima & Dill, 1990; Hervant et al., 1997; Kasumovic et al., 2007). This increased activity likely enhances their foraging efficiency and reproductive success in the absence of significant predatory threats.

Regarding the second hypothesis, the divergence in movement activity between cave and surface populations was more pronounced in males than in females. This male-biased sexual dimorphism in movement activity was evident under dark conditions, while no such dimorphism was observed in surface populations, including Malom Lake. Such sexual dimorphisms are often associated with sex-specific ecological roles and niche specialisation, which can influence traits such as body size, shape, and habitat utilisation (Thomson & Manning, 1981; Morris, 1984; Bertin & Cézilly, 2003; Balázs et al., 2021; Gherardi, 2004). The higher movement activity observed in male cave-dwelling *A. aquaticus* may be attributed to reduced predation pressures and lower population densities in the cave environment compared to surface habitats. Surface populations face varying predation pressures: guppies in Malom Lake, and fish and larval dragonflies in Csömör Stream and Dunakeszi Peat-moor (Horváth et al., 2021). These predatory pressures likely contribute to the observed differences in movement activity and the pronounced sexual dimorphism in the cave population.

Furthermore, the adaptation of cave-dwelling *A. aquaticus* to their unique environment likely includes behavioural adjustments to cope with the specific conditions of their habitat. The increased movement activity in cave individuals may reflect an evolutionary trade-off where the benefits of enhanced foraging and mating opportunities outweigh the costs associated with higher energy expenditure in an environment with limited food resources. This adaptive behaviour may also be crucial for maintaining genetic diversity and ensuring reproductive success within the cave environment.

The observed differences in movement activity and sexual dimorphism underscore the importance of considering both environmental and evolutionary contexts when studying behavioural adaptations. Future research should aim to explore these behavioural patterns further, particularly under more natural light conditions, and investigate how these adaptations contribute to the overall fitness and survival of *A. aquaticus* in varying environments.

CHAPTER II

Horváth G, Kerekes K, Nyitrai V, Balázs G, Berisha H, Herczeg G 2023. Exploratory behaviour divergence between surface populations, cave colonists, and a cave population of water louse, *Asellus aquaticus*. Behavioral Ecology and Sociobiology 77:15

SCIENTIFIC BACKGROUND

Animals exhibit a range of behavioural responses when confronted with novel environments, driven by both exploratory and avoidance mechanisms (Sommer-Trembo et al., 2022). Exploration, a key aspect of behavioural ecology, involves an individual interacting with new objects or environments to gather information essential for survival. This behaviour is particularly crucial for dispersing animals, as it aids in locating resources and identifying potential threats (Oosthuizen, 2020; Velasco et al., 2022; Blecher & Oosthuizen, 2023).

Exploratory behavior in animals is influenced by a complex mix of environmental, physiological, and social factors, with external elements like resource availability and environmental complexity playing crucial roles in shaping how animals interact with their surroundings. (Oosthuizen, 2020). Internally, an individual's exploratory tendencies are influenced by factors such as hormonal levels, cognitive capacity, and physical condition. For example, hormonal fluctuations can impact an animal's willingness to explore new environments, while cognitive abilities dictate how effectively it processes and responds to novel stimuli (Sommer-Trembo et al., 2022). Moreover, social dynamics, including competition with conspecifics, can also affect exploratory behaviour, as individuals may adjust their behaviour based on social interactions (Velasco et al., 2022).

Exploratory behaviour is not uniform across all contexts and is often adapted to specific environmental conditions. Factors such as light conditions, genetic background, age, sex, and social or reproductive states further influence exploratory tendencies (Oosthuizen, 2020; Blecher & Oosthuizen, 2023). For example, environmental complexity and light conditions can significantly impact an animal's exploration patterns, as these factors affect the sensory information available to the animal and its ability to navigate its environment (Trabalon, 2022).

Exploratory behavior in animals is constrained by a range of environmental, genetic, and developmental factors, with abiotic elements like habitat type and resource availability, as well as biotic factors such as predation risk and competition, significantly influencing their exploration patterns. (Trabalon, 2022). Genetic factors, including sexual dimorphism, can lead to differences in exploratory behaviour between males and females. For instance, some studies have shown that males and females may exhibit different exploratory tendencies based on their evolutionary and ecological roles (Tanaka, 2020; Oosthuizen, 2020). Maturation constraints also influence exploratory behaviour. The physiological state of an organism, including its age and developmental stage, can impact its exploratory tendencies. Younger or less mature individuals may exhibit different exploration patterns compared to older or more experienced ones (Rusell et al., 2010).

Research on exploratory behaviour spans a wide range of taxa, providing insights into how various animals adapt to their environments. Studies have examined exploratory behaviour in mammals, including pigs (Studnitz et al., 2007) and rodents (Tanaka, 2020; Oosthuizen, 2020; Blecher & Oosthuizen, 2023), as well as in birds (Velasco et al., 2022), fishes (Sommer-Trembo et al., 2022), and spiders (Trabalon, 2022). These studies highlight the diverse ways in which animals explore and adapt to their environments, reflecting the complex interplay of ecological, physiological, and social factors.

This study focuses on exploratory behaviour in surface and cave-adapted populations of *Asellus aquaticus* (Isopoda: Crustacea). By examining these populations, the study aims to determine whether adaptation to the unique and ecologically isolated cave habitat results in decreased explorativeness compared to their surface-dwelling counterparts. The research involves assessing dispersal-related exploration and dispersal speed in both the presence and absence of light. This approach will help elucidate how adaptation to different environmental conditions influences exploratory behaviour and whether recent colonists exhibit different exploration patterns compared to their source population.

METHODOLOGY

Collecting and housing the experimental animals

In this project, a comprehensive study was conducted involving multiple populations of *Asellus aquaticus* (Isopoda: Crustacea) to explore differences in behaviour and adaptation between surface and cave-dwelling individuals. Specifically, three surface populations were selected: Lake Gőtés (47.354357° N, 19.22980° E), Dunakeszi Peat-moor (47.615613° N, 19.126392° E), and Malom Lake (ML). Additionally, one cave population was sampled from Molnár János Cave (MJC). To examine recent adaptations, surface-type individuals from Malom Lake, which were collected approximately 250 meters from the entrance of Molnár János Cave, were included as recent cave colonists.

Sampling took place over three days, from 16th to 18th May 2019. For the surface populations, individuals were collected using hand sorting, a method that involves manually sifting through their habitat to capture the target species. In contrast, the cave population required a more specialized approach. Due to the challenging conditions within the cave, a modified Sket bottle was employed to collect specimens, and cave diving was necessary to reach the sampling sites. Only individuals with a size greater than 4 mm were selected for the study, ensuring that all sampled individuals were adults and thus comparable in terms of developmental stage. Upon capture, all specimens were transported to the aquacultural facilities at the Institute of Biology, Eötvös Loránd University, for controlled laboratory observation. Each animal was housed individually in plastic Petri dishes with dimensions of 90 × 25 mm (diameter × height) to minimize stress and prevent interaction between individuals. The laboratory conditions were carefully regulated to mimic natural environmental parameters as closely as possible.

For the cave and colonist populations, a constant darkness environment was maintained to replicate their natural habitat conditions. In contrast, the surface populations were subjected to a controlled light cycle of 16 hours of light followed by 8 hours of darkness, reflecting the natural diurnal light regimes typical of their habitats. The laboratory temperature was kept at 23–24 °C, which closely matched the temperature of their natural water sources, ensuring that temperature fluctuations did not influence behavioural observations.

To support the health and well-being of the animals, different feeding regimens were implemented based on their natural diets. Surface-dwelling individuals were provided with dead leaves, which are a natural food source in their typical habitats. In contrast, cave-dwelling and colonist individuals were fed bacterial food, reflecting the detrital diet they are adapted to in the cave environment. The study involved testing the following numbers of adult individuals: 10 males and 8 females from Molnár János Cave (MJC); 8 males and 5 females from the colonists; 17 males and 13 females from Malom Lake (ML); 14 males and 13 females from Lake Gőtés (GT); and 14 males and 15 females from Dunakeszi Peat-moor (DM). This sample size was chosen to ensure a robust analysis of behavioural differences and adaptations across the different populations.

Experimental Setup

Tests were run between 21st May and 1st June on 12 consecutive days. Animals had to be acclimated in a period of three days, under their natural light regime, without any disturbance, where potential colonists were acclimated in the dark. The tests started at approximately 11.00 am daily. Individual behaviour was tested in the chambers described below. A cluster of 18 open-top mazes made of Plexiglas (see Figure 11) was used to test novel area exploration and dispersal speed.

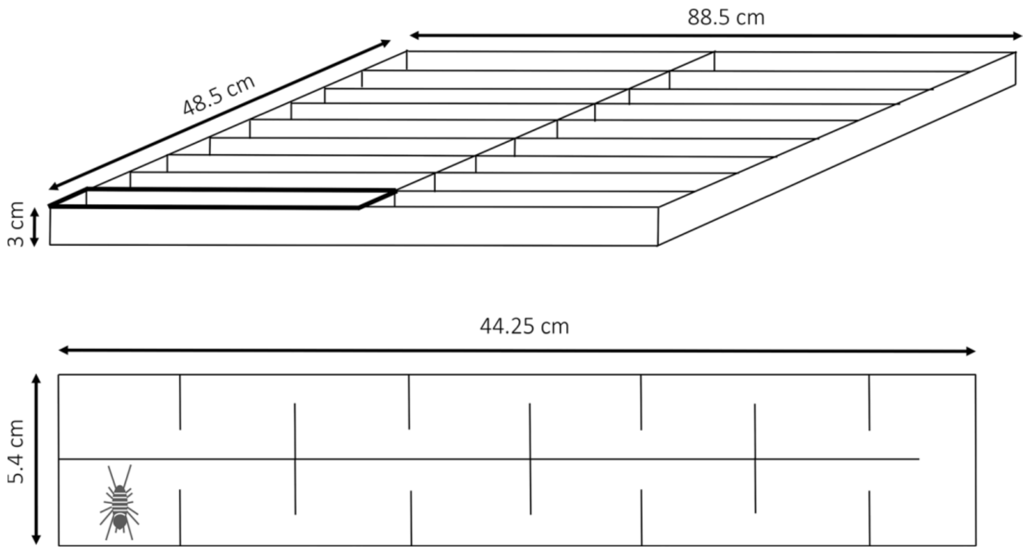

Figure 10. Schematic representation of one experimental arena with 18 mazes (top). Thick lines indicate a single arena maze with an individual in the starting position (bottom); note that the animal on the picture was magnified for better visibility (image source: Horváth et al., 2023)

Figure 11. Arena test – Exploration in a maze (image taken during research work)

Statistical Analysis

A generalised linear mixed model (short: GLMM) with negative binomial distribution and logit link function was used to analyse population, treatment and sex effects on maze area exploration. Here, 'population' 'treatment' (light vs dark), and sex and their interactions were added as fixed effects. Error distribution and link function applied in the GLMMs were chosen after inspection of Q-Q plots of the model residuals. To avoid over-parameterization, only the two-way interactions were included. The standardized (mean = 0, sd = 1) order of trials was added as a single fixed effect to control habituation to the test setup. Fixed effects were tested by Wald's chi-squared tests, and random effects by likelihood ratio tests. P - values for the probability ratio tests were calculated following Zuur et al. (2009).

The model's estimated marginal means were extracted using the emmeans package (Lenth 2019). Further, A report was built to assess the proportion of variance by given fixed factors (marginal R^2) and by both fixed and random factors (conditional R^2) present in the MuMIn package (Barton, 2009) based on the method of Nakagawa & Schielzeth (2010). Dispersal speed data were transformed into the square root to achieve average model residual distribution. The behavioural variables (exploration and dispersal) were analysed using a linear mixed model (LMM) built in the same way described for the GLMM above. The GLMM and the LMM (with the R packages lme4 and lmerTest, see Bates et al., 2015; Kusnetzova et al., 2017, respectively) were made in R 4.1.0 (R Developmental CoreTeam 2021). The 'rptR' (collection of functions that calculate point estimates, interval estimates and significance tests of the repeatability as well as variance components in mixed effects models, see Stoffel, et al., 2022) add-on package was used to calculate repeatability (a statistical test for animal personality's presence and 'strength', i.e., consistent among-individual differences over time or across ecological contexts). Enhanced agreement repeatability (hereafter: eaR) for novel area exploration and dispersal speed in the pooled sample (i.e., all populations combined) was calculated separately for light regimes. A GLMMs was built for the negative binomially distributed novel area exploration data, following Nakagawa and Schielzeth's (2010) methods, which utilise a multiplicative overdispersion GLMM with a logit-link and using penalised quasi-likelihood (PQL) estimation for repeatability on the original scale. The significance of eaR estimates (i.e., for random terms) was provided by randomisation tests, giving strong measures of statistical significance in the case of non-Gaussian data (Nakagawa and Schielzeth 2010). Quantification of uncertainty for the variance justified by fixed effects (as for other variance components) was provided by parametric bootstrapping. LMMs were run to estimate repeatability for dispersal speed. CIs were calculated by nonparametric bootstrapping, while significance for eaR estimates is provided by likelihood ratio test, both sampled at each 1000th iteration.

RESULTS

Recent colonists have performed higher dispersal activity than their source surface population. Further, the results from this study show that individuals from cave-adapted *A. aquaticus* are significantly less explorative and move slower than surface-type populations. Repeatability estimates for novel area exploration and dispersal speed are given (see Table 2 below). For novel area exploration, estimates in the pooled sample indicated significant, moderate repeatability in both treatments (light: eaR = 0.29 (95% CI = 0.13–0.35); dark: 0.22 (0.09–0.28)). Repeatability for dispersal speed in the pooled sample was present only in the presence of light, showing moderate strength (eaR = 0.21 (0.11–0.33)).

Population, treatment, and their interaction affected novel area exploration (see Table 2 below). All other populations in both treatments showed higher novel area exploration than cave individuals in the dark (see Figure 13 below). Surface populations were explorative more in the dark than in light, and the trend is weak in Dunakeszi Peat-moor. Treatment did not affect the potential colonists' exploration. Colonists explored the light more than surface populations, including their source population (Malom Lake), but their exploration in the dark was similar to the surface populations.

Table 2. Results of the GLMM on novel area exploration and the LMM on dispersal speed behavioural types of *Asellus aquaticus*. For the GLMM, type III Wald's chi-squared test was used, while in the LMM, the significance of the fixed effects was estimated based on Satterthwaite approximation. Non-significant individual × habituation interactions are shown here but removed later from the final models (Statistically significant effects p value <0.05).

	Novel area exploration		Dispersal speed	
	X^2 *(df)*	*P*	*F* (df_1, df_2)	*P*
Fixed effects				
Sex	3.46 (1)	0.07	1.96 (1, 116.36)	0.16
Population	**13.88 (4)**	**0.007**	**3.18 (4, 116.42)**	**0.016**
Treatment	34.13 (1)	<0.001	0.63 (1, 580.1)	0.43
Population x sex	2.00 (4)	0.74	0.39 (4, 116.42)	0.81
Population x treatment	**1155.03 (4)**	**<0.001**	**9.56 (4, 580.17)**	**<0.001**
Sex x treatment	**24.7 (1)**	**<0.001**	0.03 (1, 580.16)	0.87
Habituation	**70.11 (1)**	**<0.001**	**35.9 (1, 580.66)**	**<0.001**
Random effects				
Individual	**8275.23 (1)**	**<0.001**	**36.67 (1)**	**<0.001**
Individual x habituation	**2971 (1)**	**<0.001**	<0.001 (1)	>0.99

Statistically significant effects (P < 0.05) are bolded.

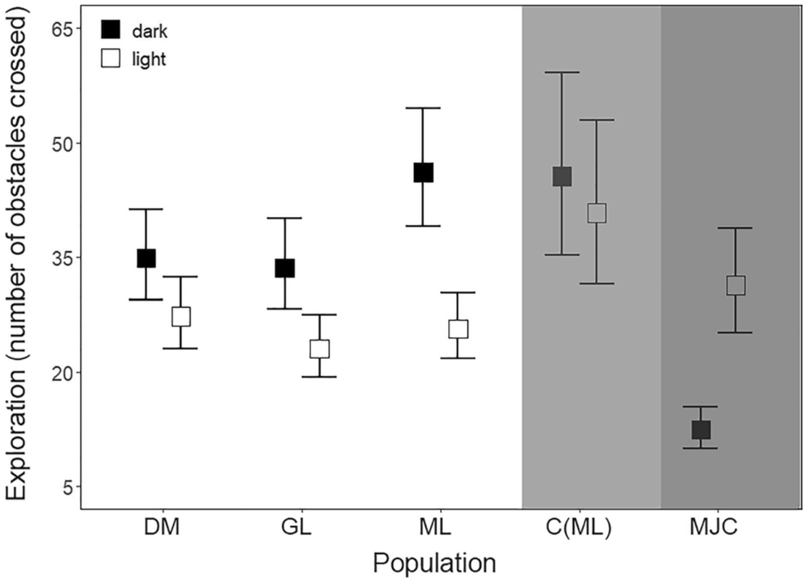

Figure 12. Exploration in the absence vs presence of light (significant population × environment interaction). White background indicates surface-type individuals (DM, GL, ML), light grey indicates surface-type individuals from the Malom Lake (CML) found in the cave, and dark grey indicates cave-adapted individuals (MJC). Back transformed estimated marginal means and 84% confidence interval (CI) are shown. Note that non-overlapping 84% Cis indicate significant differences at the P < 0.05 (Payton et al. 2003).

Furthermore, a significant sex × treatment interaction effect was found (see Figure 14): Males were more active than females in the dark but not in light (see Table 2). Habituation was also significant: individuals became less active over time. Finally, individual differences in novel area exploration and habituation were both significant. The fixed effects explained 36.9% of the total variance, and the full models explained 74.2%. A significant population and population × environment interaction effect on dispersal speed was found (see Table 2).

All populations in the dark and Dunakeszi Peat-moor (DM) and potential colonists in light dispersed faster than the cave population in the dark (see Figure 15). Cave *A. aquaticus* showed comparable dispersal speed to the surface populations in the — for them unnatural — light treatment. Potential colonists from Malom Lake dispersed faster than their source population (Malom Lake) in light and tended to do so in the dark (see Figure 15). Significant habituation in dispersal speed was found, with a decrease over time. Individual differences in dispersal speed were significant. The fixed effects explained 12.2% of the total variance, and the full models explained 27.2%.

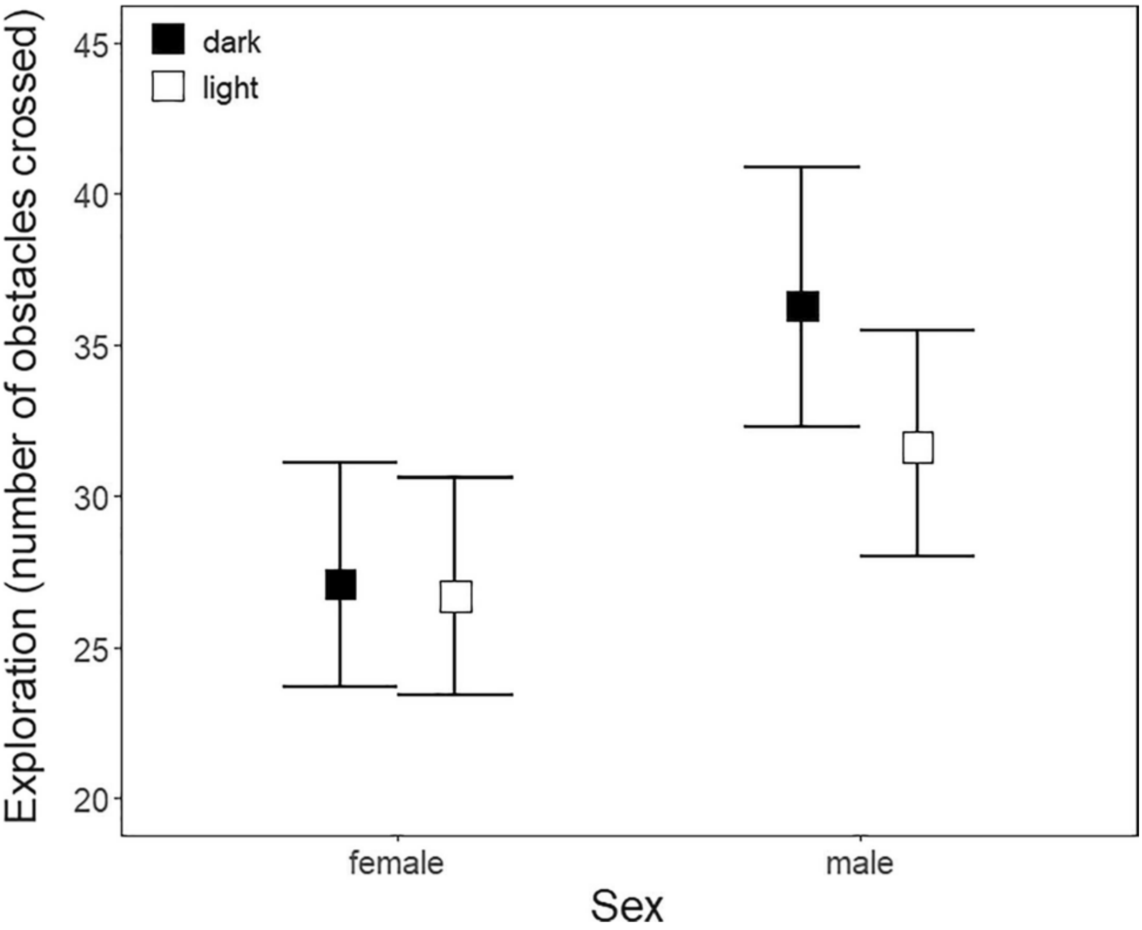

Figure 13. Exploration in the absence vs. presence of light in the female and male *Asellus aquaticus* (significant sex × environment interaction). Back transformed estimated marginal means and 84% confidence interval (CI) are shown. Note that non-overlapping 84% Cis indicate significant differences at the P < 0.05 (Payton et al. 2003).

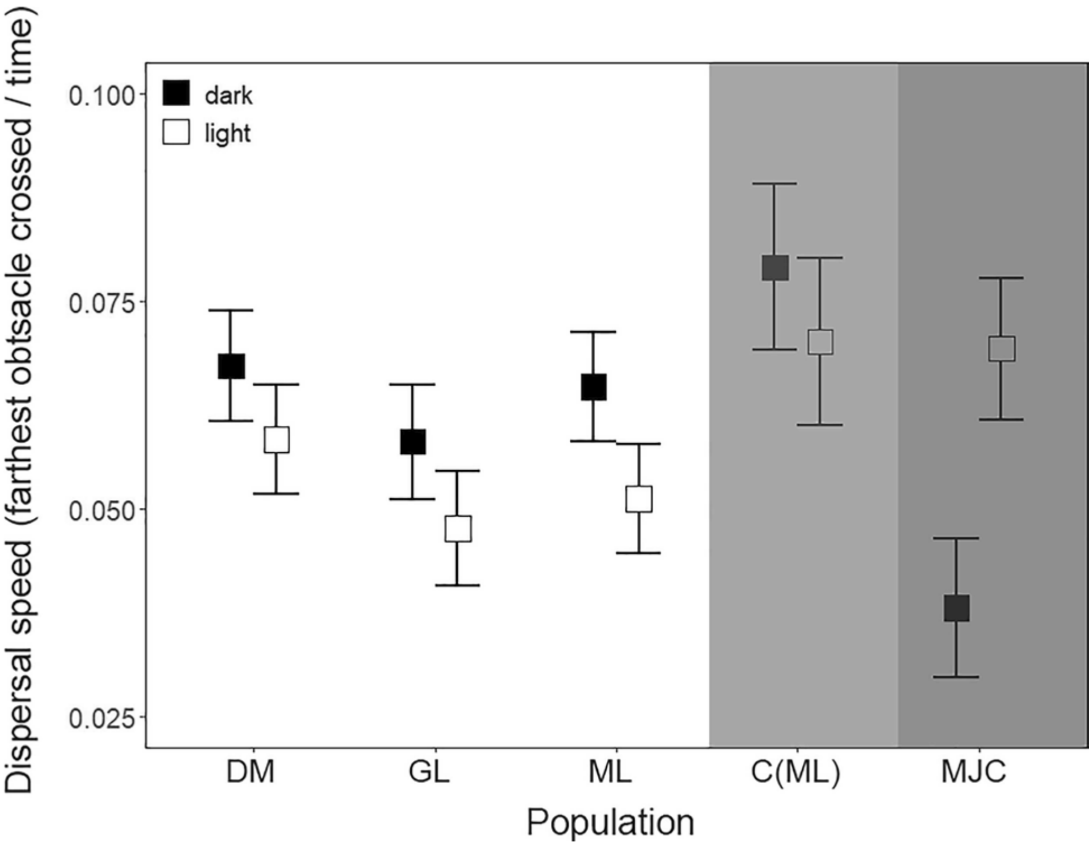

Figure 14. Dispersal speed in the absence vs presence of light in the four tested populations of *Asellus aquaticus* (significant population × environment interaction). White background indicates surface-type individuals (DM, GL and ML), while light grey indicates surface-type individuals from the Malom Lake (CML) found in the cave, and dark grey indicates cave-adapted individuals (MJC). Estimated marginal means and 84% confidence interval (CI) are shown. Note that non-overlapping 84% Cis indicate significant differences at the $P < 0.05$ (Payton et al. 2003).

DISCUSSION

The exploratory behaviours of *Asellus aquaticus* within the intricate cave labyrinths highlight the species' unique adaptations to extreme cave environments. This study was designed to explore how these behavioural adaptations—especially in isolated or "dispersal trap" habitats—affect the evolution of dispersal-related behaviours. The results confirm that surface populations of *A. aquaticus* exhibit higher exploratory tendencies compared to their cave-adapted counterparts, regardless of light conditions. Specifically, surface populations consistently demonstrated greater exploration behaviour than cave-adapted individuals when tested in the dark, underscoring the inherent differences between these populations.

Surface populations of *A. aquaticus* are likely more exploratory due to their need to constantly search for resources and avoid predators. This contrasts with cave-adapted populations, which have evolved to thrive in a stable, predator-free environment with less need for extensive exploration. This evolutionary adaptation allows cave-dwelling individuals to conserve energy and reduce unnecessary movement in their predictable environment.

Interestingly, while dispersal speed patterns were comparable for surface populations when tested in the dark, there was a minor divergence in behaviour between individuals from Gőtés Lake and Malom Lake when tested in light conditions. Recent cave colonists exhibited faster dispersal speeds than their surface source populations, suggesting that individuals with heightened exploratory tendencies are better suited for colonising new environments. This observation supports the hypothesis that exploratory behaviour plays a critical role in the successful establishment of individuals in novel habitats.

The unique cave environment appears to act as a dispersal trap. Although cave-adapted *A. aquaticus* displayed activity levels similar to surface populations under artificial light conditions, this increased activity is likely a result of photophobia. Despite their reduced eye structures, cave-dwelling individuals retain some ability to detect light, which can induce stress or heightened activity under unnatural lighting conditions (Pérez-Moreno et al., 2018; Fišer et al., 2016). In natural dark conditions, cave-adapted individuals showed decreased exploratory activity, reflecting their adaptation to a stable and risk-free environment where there is little need for additional exploration.

Surface populations, on the other hand, exhibited increased exploration of novel areas in the dark compared to light conditions. This finding is consistent with Andrikovics's (1981) observation that surface-dwelling *A. aquaticus* are more active at night, likely due to reduced predation risk in darkness. This nocturnal activity enhances their ability to locate resources and avoid predators, which may contribute to their success in colonising subterranean habitats.

Additionally, males of *A. aquaticus* were found to be more explorative than females in dark conditions. This behaviour is likely related to mate-searching activities or other reproductive behaviours (Kasumovic et al., 2007). The increased activity of males in darkness may be a strategy to increase mating opportunities or to compete more effectively for resources.

Dispersal is a critical behaviour influencing various ecological and evolutionary processes, including gene flow, species distribution, and environmental adaptation. Personality-dependent dispersal is a well-documented phenomenon, with bolder and more explorative individuals often dispersing further and colonising new habitats more effectively (Cote et al., 2010; Spiegel et al., 2017). The results of this study support the hypothesis that surface populations of *A. aquaticus* with higher exploratory tendencies are more likely to colonise new environments. This is further evidenced by the observation that potential colonists from Malom Lake demonstrated higher exploratory behaviour compared to other surface populations.

However, an alternative explanation for the increased behavioural activity of potential colonists is food scarcity rather than inherent exploratory tendencies. Observations indicate that these potential colonists preferred surface food over cave-specific resources (Herczeg et al., 2022), suggesting that they may be experiencing food deprivation in the cave. This scarcity could lead to increased foraging behaviour as individuals search for their preferred resources.

The study has several limitations, including the lack of data on how long potential colonists have been in the cave and the inability to distinguish between genetic and environmental effects on behaviour. While the observed behavioural differences are significant, it is unlikely that these are solely due to evolutionary adaptations. Instead, phenotypic plasticity—where individuals adjust their behaviour in response to immediate environmental conditions—provides a more plausible explanation for the observed behavioural variations. Future research should address these limitations by incorporating genetic analyses and exploring the long-term impacts of environmental conditions on behavioural traits. This approach would offer a more comprehensive understanding of how cave-adapted and surface populations of *A. aquaticus* adapt to their respective environments and how these adaptations influence their dispersal and colonisation strategies.

CHAPTER III

Berisha H, Horváth G, Herczeg G. Negligible divergence in risk-taking between surface *vs*. cave populations of the water louse, *Asellus aquaticus*. Manuscript.

SCIENTIFIC BACKGROUND

Risk-taking behavior refers to an individual's behavioral responses in threatening or risky situations (Krenhardt et al., 2021). Alterations in risk-taking behavior can occur due to a wide range of internal states and life history factors, such as nutritional state, survival probability, social dominance, body condition, and internal thermal state (Betini & Norris, 2012; Briffa et al., 2013; Eccard & Herde, 2013; Lima, 1998; Shuai, Zhang, & Zeng, 2016; Briffa et al., 2008; Carter, Goldizen, & Heinsohn, 2012; Dosmann & Mateo, 2014; Moschilla et al., 2018). The risk that animals are willing to take for a given resource depends on their energy budget and varies between individuals with different personality traits (Dammhahn & Almeling, 2012). The expression of personality traits has both ecological and evolutionary consequences, particularly when an individual's fitness is closely tied to the expression of a behavioral phenotype in a specific environmental context (Brown et al., 2005). Individual variability in behavior can often be predicted by examining demographic variables (e.g., size, age, or sex) or intrinsic factors, including reproductive status (Wilson et al., 1994). Individuals exhibit consistent behavioral differences across various circumstances or contexts, which are indicative of personality traits (Gosling, 2001). These behavioral differences have been observed in many animal groups (Boissy, 1995; Gosling, 2001). Personality traits, such as risk-taking, have been extensively studied (Adams et al., 1998; Wilson et al., 1993; 1994; Brown et al., 2005). Risk-taking behavior is thought to have an intrinsic component that leads to consistent individual differences in response levels (average behavioral expression) and response plasticity (behavioral changes when environmental conditions change) (Dammhahn & Almeling, 2012).

Individuals often differ in their risk-taking behavior, with some consistently bolder than their conspecifics (Sinn et al., 2010). In various species, bolder individuals are found to take more risks in the presence of predators (Jones & Godin, 2009; Smith & Blumstein, 2010; Brown et al., 2007; Dammhahn & Almeling, 2012), achieve greater foraging success (Dyer et al., 2009; Brown et al., 2007), explore novel environments more, and disperse further (Fraser et al., 2001; Réale & Festa-Bianchet, 2003; Brown et al., 2007) compared to their shyer counterparts, who tend to avoid unfamiliar situations and react by retreating or becoming quiet and vigilant

(Wilson et al., 1993; Réale et al., 2000). Fear responses, such as freezing, often accompany the behavior of shy individuals in novel situations (Brown et al., 2007).

Predator-induced phenotypic changes includes behaviour (from adjusting risk-taking to alterning habitat choice), physiology (from locomotor performance to toxin production), life history (from growth to fecundity) and morphology (from shape to defensive armour) (Tollrian & Harvell, 1999). It is also found to play an important role in species coexistence (Brown, et al., 1988). It particularly affects animals' risk-taking behaviour during foraging (when resources and predators coexist), parental care (during the breeding season when parents protect their nest), and mating (Oers et al., 2005; Moschilla et al., 2018; Krenhardt, et al., 2021).

Regarding exposure to predations, individuals within populations are known to have different personalities (Stamps, 2007) in response to predation, with bold individuals taking more risks, and with shy individuals taking fewer risks (Martins, et al., 2007). This kind of behavioural personality trait against predators is found to be present in different taxa, including mammals (grey lemurs, *Microcebus murinus, see*, Dammhahn & Almeling, 2012; kit fox *Vulpes macrotis*, Hall, et al., 2021) birds (Zebra finches *Taeniopygia guttata*, Martins, et al., 2007; Ariyomo & Watt, 2012*)*, fish (threespine stickleback, *Gasterosteus aculeatus*, Huntingford, 1976; Herczeg et al., 2009), and arthropods (field crickets, *Teleogryllus oceanicus*, Moschilla et al., 2018; hermit crabs, *Clibanarius vittatus*, Gorman, et al., 2018). In addition to studies observing predation's influence on risk-taking divergence within population, predation-mediated risk-taking divergence among populations of the same species is a widespread phenomenon across various animal taxa (Seghers, 1974; Giles & Huntingford, 1984; Dingemanse et al., 2007; Bell & Sih, 2007; Brydges et al., 2008; Herczeg et al., 2009). This variation in risk-taking behavior is influenced not only by predation pressure as a primary selective factor (Herczeg et al., 2009; Bell, 2005) but also by other factors such as habitat type, competition, and temperature fluctuations (Dingemanse et al., 2007; Bell & Sih, 2007; Herczeg et al., 2009).Predation pressure, a key evolutionary driver, varies significantly between surface and cave environments, with surface aquatic animals often adopting anti-predator behaviors such as minimizing surface time and seeking predator-free zones to mitigate predation risks (von Ende, 1979; Louw, et al., 2022). Compared to the surface, subterranean environments (i.e., caves) are considered to have less or negligible predation, however, behaviour assays on cave taxa are rare (Fišer, et al., 2019). In this study, the risk-taking behaviour of a cave-adapted population of *Asellus aquaticus* (Isopoda: Crustacea) is compared to surface populations'.

Asellus aquaticus is present in different freshwater habitats in Europe, including surface and cave freshwater habitats (Turk et al., 1996). *Asellus aquaticus* offers an excellent model for evolutionary studies (Pérez-Moreno, et al., 2017; Lafuente, et al., 2021; Lomheim, et al., 2023). *Asellus aquaticus* is found to experience negligible predation in the studied Molnár János Cave, and most of its behaviour activity is considered to be linked to foraging and reproduction, however, less or nothing is known about its behavioural response to the new environmental cue of predation. As predation is negligible in this cave, I predicted that cave-adapted *Asellus aquaticus* individuals show increased risk-taking behaviour compared to surface conspecifics. Here, I had no clear predictions regarding sexual differences.

METHODOLOGY

Collecting and housing the experimental animals

A sampling of populations from Molnár János Cave (MJC; 47.3148° N, 19.2992° E), Malom Lake (ML; 47.31709° N, 19.21232° E), and two different surface populations, Gőtés Lake (GL; 47.354357° N, 19.22980° E) and the Dunakeszi Peat-moor (DM; 47.615613° N, 19.126392° E) happened in the first week of October 2021. Samples were collected by hand sorting, except for the animals from the MJC, where cave diving was necessary. Gőtés Lake and Dunakeszi Peat-moor populations were chosen randomly to represent ‘typical’ surface populations. They experience natural surface light regimes and temperature fluctuations of normal, non-thermal freshwaters typical to the region. All animals were moved immediately after capture to the aquacultural facilities of the Institute of Biology at the Eötvös Loránd University (Budapest, Hungary). Animals were housed individually in 90 × 25 mm (diameter and height) plastic Petri dishes. Cave individuals were kept in constant darkness, while surface populations were in a 16-h light with 8-h dark daily light cycle. The temperature in the lab was set at the same conditions to the water source habitats (23–24 °C). Water from the natural habitats was used for keeping and testing throughout the laboratory. Individuals from surface populations were fed with decaying leaves from their habitat, while bacterial food collected from MJC was provided for cave-adapted individuals. A total of 82 individuals from all populations were tested: 8 males (M) / 8 females (F) from MJC; 9 M / 11 F from ML; 12 M / 11 F from GL; and 12 M / 11 F from DM.

Experimental Setup

The tests occurred for eight consecutive days between 4th and 11th October 2021. Animals were allowed to acclimate under their natural light regime without any disturbance. Individuals were acclimated in a period of two days before starting the tests. Individual behaviour was tested and video recorded in the chambers (a similar experimental setup was used in the first study, Chapter I). All individuals were tested twice in dark and light. Each population was randomly split into two groups, one was tested in the presence of light, while the other was in darkness. After 48 hours, treatments were changed. Each subgroup was alternately measured in light and dark. Two rounds of observations were done from 4th to 11th October 2021.

Recordings started at approximately 11 am daily. Petri dishes were filled with water from the habitat, and a pipette was used to transport individuals from their acclimatization-Petri dishes to the test-Petri dishes placed in chambers. The quick capture and release with the pipettes was used as mimicking an unsuccessful predatory attempt, resulting in release in a new, unfamiliar environment. Recordings started once the individual was released from pipettes. Each individual were tested in an interval of 5 minutes. Two individuals placed in two Petri dishes were placed in test chambers and were replaced by two other individuals after each 5 min. The following behaviours were extracted from the analyzing videos: latency to freeze/stop (sec); duration (sec) of the first stop; number of the stops during the observation period (count variable); and total duration (sec) of all stops during the observation period. Latency to first stop was used as a proxy for the immediate escape behaviour and duration of first stop as a measure of 'freezing' behaviour. The remaining two variables were envisoned to grasp relevant aspects of the general behavioural activity right after a mimicked predatory attack.

Statistical Analysis

To analyse population, treatment and sex effects on behavioural traits describing risk-taking, separate generalised linear mixed models (short as: GLMM) were used. This latter method was chosen as the transformation (log or square root) of count data is generally contraindicated (O'Hara & Kotze, 2010). Error distribution and link function applied in the GLMMs were chosen after inspection of Q-Q plots of the model residuals: we applied Poisson error distribution with log link in all models. All models were parameterized similarly: the behavioural trait in question was the response variable, while 'population' (the four sampled populations), 'treatment' (light *vs*. dark), sex and their two-way interactions were added as fixed effects. To avoid overparametrization, we did not include the three-way interaction. The standardised (mean = 0, sd = 1) order of trials (hereafter, 'time') was added as a single fixed effect to control habituation to the test setup. Individual identity as a random intercept was added. Random slopes (individual × time) as a random term were also added, but left in the final model only if it improved model fit. Fixed effects were tested by Wald's chi-squared tests, and random effects by likelihood ratio tests. *P*-values for the probability ratio tests were calculated following Zuur et al. (2009). Pairwise post hoc comparisons were made *via* the False Discovery Rate method (Benjamini & Hochberg 1995). The model's estimated marginal means were extracted using the emmeans package (Lenth 2019). The GLMMs were fitted with the R packages lme4 and lmerTest (Bates et al., 2015; Kusnetzova et al., 2017, respectively) in R 4.3.0 (R Developmental CoreTeam 2023).

RESULTS

Our initial Generalised Linear Mixed Model (GLMM) revealed significant interactions between population and treatment, as well as between treatment and sex, concerning the latency to the first stop (Table 3). Although several significant post hoc comparisons were identified, no consistent divergence was observed between surface and cave populations (Figure 16). Females exhibited a tendency towards increased latency to the first stop in dark conditions compared to light, a pattern that was not observed in males (Figure 17).

Table 3. LMMs on risk-taking variables of *Asellus aquaticus*

	Latency to first stop		**Duration of first stop**		**Number of stops**		**Duration of all stops**	
	χ^2 (df)	*P*	χ^2 (df)	*P*	χ^2 (df)	*P*	χ^2 (df)	*P*
Fixed effects								
Sex	0.76 (1)	0.38	0.22 (1)	0.64	0.32 (1)	0.57	0.18 (1)	0.68
Population	6.86 (3)	0.08	0.56 (3)	0.91	6.02 (3)	0.11	4.93 (3)	0.18
Treatment	**53.82 (1)**	**<0.001**	**21.62 (1)**	**<0.001**	0.07 (1)	0.79	**7.49 (1)**	**0.007**
Population × Sex	1.99 (3)	0.57	6.71 (3)	0.08	2.95 (3)	0.39	0.85 (3)	0.84
Population × Treatment	**295.72 (3)**	**<0.001**	**230.07 (3)**	**<0.001**	1.72 (3)	0.63	**28.98 (3)**	**<0.001**
Sex × Treatment	**56.22 (1)**	**<0.001**	**28.86 (1)**	**<0.001**	0.001 (1)	0.97	**9.36 (1)**	**0.002**
Habituation	0.01 (1)	0.92	**8.57 (1)**	**0.003**	3.78 (1)	0.052	**6.61 (1)**	**0.01**
Random effects								
Individual	**8001.94 (1)**	**<0.001**	**1122.25**	**<0.001**	**13.27 (1)**	**<0.001**	**5998.9 (1)**	**<0.001**
Individual × Habituation	**4295.9 (1)**	**<0.001**	**819.6**	**<0.001**	<0.001 (1)	>0.99	<0.001 (1)	>0.99

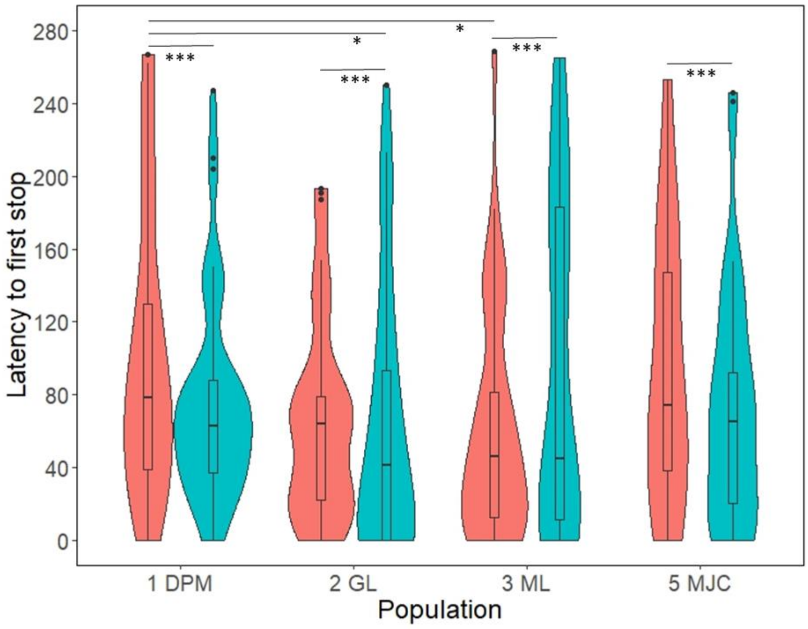

Figure 15. Differences in latency to first stop across different populations of *Asellus aquaticus* in dark (red) and light (blue). DPM = Dunakeszi Peat-moor, GL = Gőtés Lake, ML = Malom Lake, MJC = Molnár János Cave. Significant post hoc pairwise differences are also shown (False discovery rate; * denotes $p < 0.05$, while *** $p < 0.001$).

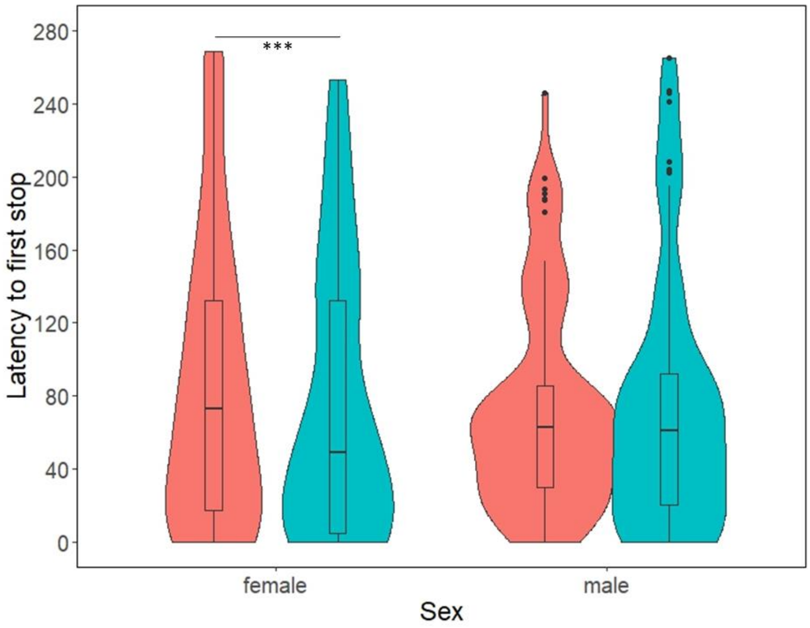

Figure 16. Differences in latency to first stop between female and male *Asellus aquaticus* in dark (red) and light (blue). Significant post hoc pairwise differences are also shown (False discovery rate; *** denotes $p < 0.001$).

The duration of the first stop was influenced by significant interactions between population and treatment, as well as between treatment and sex (Table 3). However, no consistent pattern emerged between surface and cave populations (Figure 18). Males tended to exhibit a longer duration of the first stop in light conditions compared to dark, while females showed no such difference. Nevertheless, this pattern was relatively minor (Figure 19).

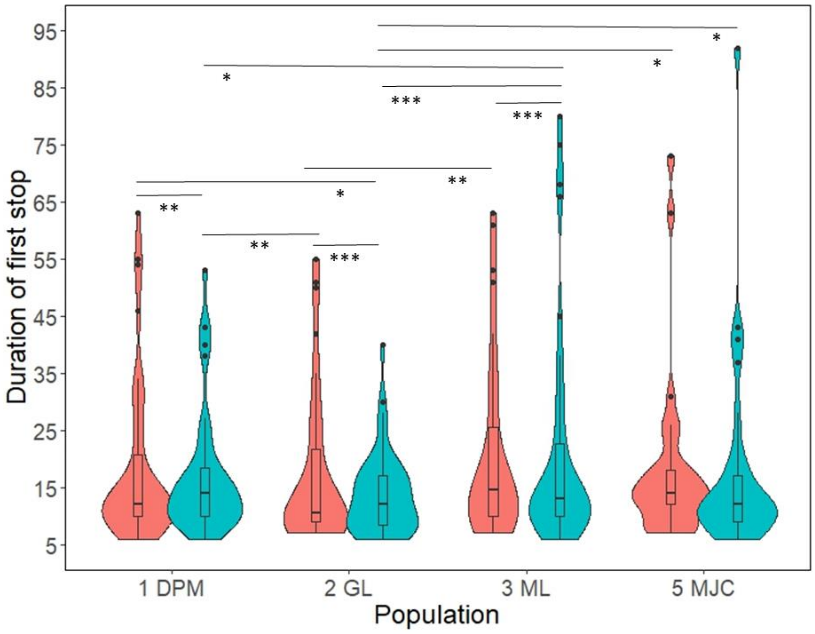

Figure 17. Differences in the duration of the first stop across different populations of *Asellus aquaticus* in dark (red) and light (blue). DPM = Dunakeszi Peat-moor, GL = Gőtés Lake, ML = Malom Lake, MJC = Molnár János Cave. Significant post hoc pairwise differences are also shown (False discovery rate; * denotes p < 0.05, ** p < 0.01, and *** p < 0.001).

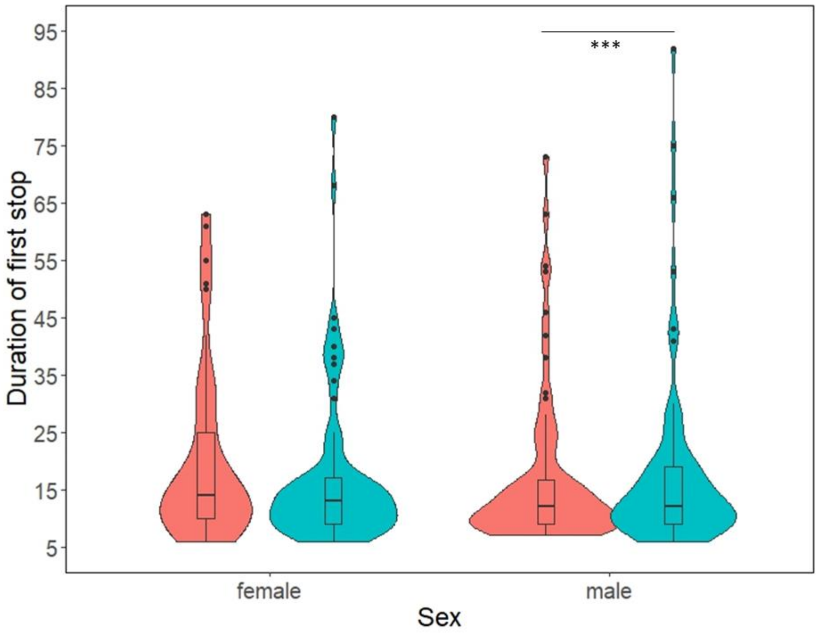

Figure 18. Differences in the duration of the first stop between female and male *Asellus aquaticus* in dark (red) and light (blue). Significant post hoc pairwise differences are also shown (False discovery rate; *** denotes p < 0.001).

The Generalised Linear Mixed Model (GLMM) analysis conducted on the number of stops made by individuals did not reveal any significant effects associated with population, treatment, sex, or their interactions (Table 3). This suggests that, across the different populations and conditions tested, there was no discernible pattern in the frequency of stops that could be attributed to these variables. However, a more detailed examination of the total duration of stops using a separate GLMM did uncover significant interactions between population and treatment, as well as between treatment and sex (Table 3). Despite these statistically significant interactions, no clear or consistent pattern emerged when comparing surface populations to cave-adapted populations (Figure 20).

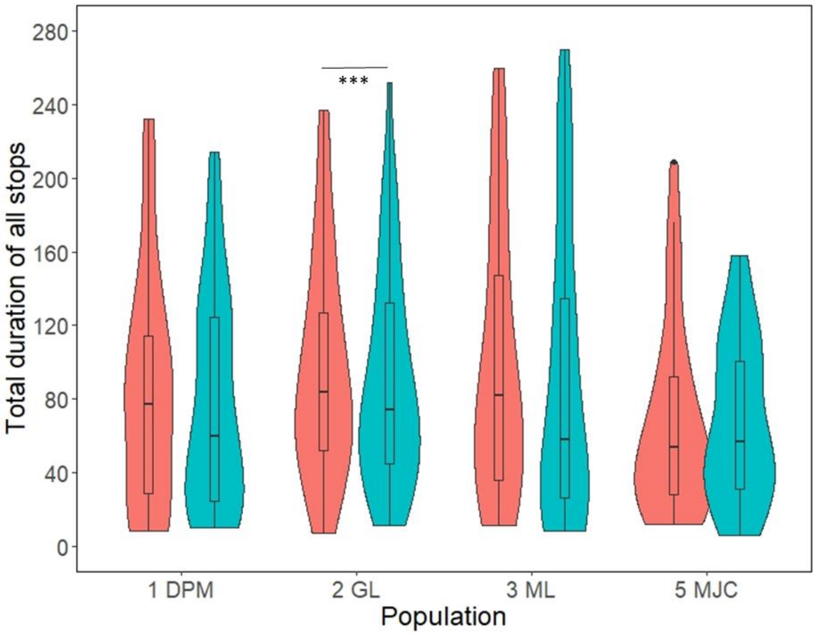

Figure 19. Differences in total duration of all stops across different populations of *Asellus aquaticus* in dark (red) and light (blue). DPM = Dunakeszi Peat-moor, GL = Gőtés Lake, ML = Malom Lake, MJC = Molnár János Cave. Significant post hoc pairwise differences are also shown (False discovery rate; *** denotes p < 0.001).

In particular, the data indicated that males tended to have shorter total stop durations when exposed to light conditions compared to when they were in the dark. This difference suggests a possible light-induced reduction in stopping behaviour for males, which could be indicative of increased activity or a heightened response to the light environment. Conversely, females did not exhibit a significant difference in total stop duration between light and dark conditions, suggesting that their stopping behaviour was less influenced by the environmental lighting conditions (Figure 21). This divergence in behavioural responses between males and females highlights potential sex-specific adaptations or behavioural strategies that could be at play, though the absence of a consistent surface versus cave pattern indicates that these effects are not directly related to the habitat of origin.

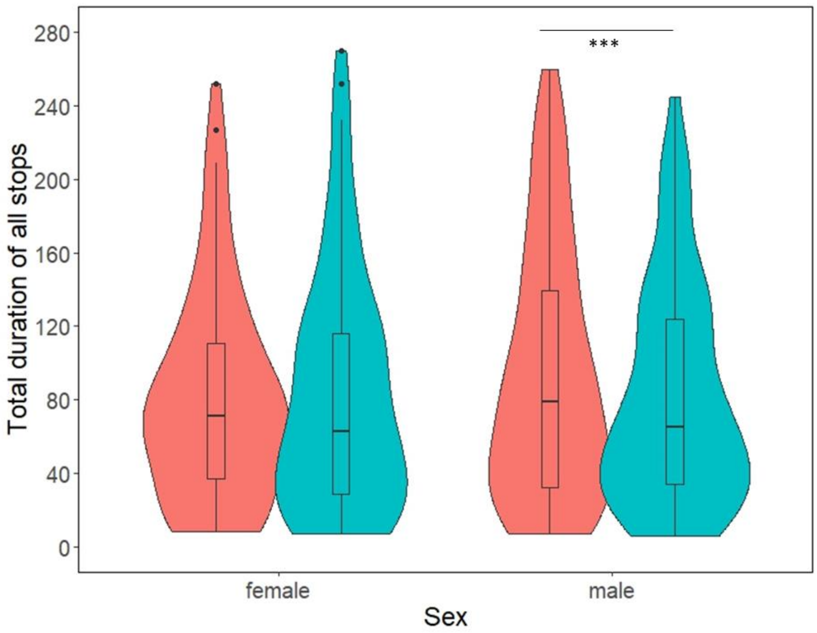

Figure 20. Differences in the duration of the first stop between female and male *Asellus aquaticus* in dark (red) and light (blue). Significant post hoc pairwise differences are also shown (False discovery rate; *** denotes $p < 0.001$).

Despite the significant population × treatment or treatment × sex effects, there are no systematic differences between the surface- and cave-adapted populations.

DISCUSSION

Accordig to general theory, negligible predation should select for increased boldness, including risk-taking behaviour, given its low costs. This hypothesis was tested in several systems with supporting results (Herczeg et al., 2009; Dingemanse, et al., 2007), but not yet in the cave (negligible predation risk) *vs.* surface (high and diverse predation risk) scenario. Our results from this study showed that the cave-adapted *Asellus aquaticus* population did not differ significantly from the surface poulations, neither in the light, nor in the dark regimes in four behavioural measures estimating risk-taking following a mimicked unsuccessful predatory attack. In general, there were population × treatment interactions, however, these were based on population variations unexplained by habitat type.

Compared to surface populations of *Asellus aquaticus* (Gőtés Lake, Dunakeszi Peat-moor, and Malom Lake), which face diverse and significant predation pressures, the cave environment of Molnár János provides negligible predation pressure. Behavioral studies on cave-adapted populations have been scarce (Fišer et al., 2019) but have started to accumulate recently (Herczeg et al., 2020; Horváth et al., 2021; Berisha et al., 2022; Horváth et al., 2023). This is especially relevant for risk-taking behavior, where our study did not support the predictions. This result is somewhat surprising, as predation-mediated risk-taking divergence among populations of the same species is commonly observed across various animal taxa (Seghers, 1974; Giles & Huntingford, 1984; Dingemanse et al., 2007; Bell & Sih, 2007; Brydges et al., 2008; Herczeg et al., 2009). Other factors may be driving the observed variations rather than the cave-surface differences. Behavioral traits and populations' life histories are influenced by ecological conditions, including resource distribution and predation risks (Gomes et al., 2019). Therefore, the population variations detected in our study may be attributed to other environmental factors beyond the marked cave-surface differences or could represent random, non-adaptive deviations.

We observed weak sex × treatment interactions, indicating that males spent more total time immobile in the dark compared to the light, while females exhibited an increased escape response in the dark. Although these patterns were statistically significant, their biological significance appeared limited due to the relatively small magnitude of the observed differences. Sexual differences in the behavior of *A. aquaticus* are well-documented (Manning, 1980; Bertin & Cézilly, 2003; Ridley & Thompson, 2010; Berisha et al., 2022), including findings detailed in the previous chapters. However, explaining the minor differences observed in the present study would be speculative, so I prefer to refrain from such explanations.

Taken together, our findings revealed no divergence in risk-taking behavior between the cave-adapted *A. aquaticus* population (which experiences negligible predation) and some surface-adapted populations (which face diverse and high predation). The cave-adapted population has been isolated from the nearest surface population for over 100,000 years (Pérez-Moreno et al., 2017), exhibiting various morphological and behavioral adaptations (Berisha et al., 2022; Horváth et al., 2023, Chapters 1-2). Therefore, the lack of divergence in risk-taking behavior is unlikely due to insufficient time or a lack of genetic basis for adaptation. The methods and variables used in this study are well-established (Urzcán et al., 2015, 2018), making it unlikely that the lack of divergence is an artifact of the experimental approach. To further investigate the reasons behind the absence of divergence in risk-taking behavior—contrary to other behaviors tested in the *A. aquaticus* system (Fišer et al., 2019; Herczeg et al., 2020; Horváth et al., 2021; Berisha et al., 2022; Horváth et al., 2023)—I recommend repeating the experiment with other closely or distantly related species and employing different methodologies within the same system.

CONCLUSIONS

The results from my doctoral studies suggest that the behavioral variation seen is the studied surface –cave *Asellus aquaticus* system is driven by different selective pressures. From the point of environmental selection, the negligible predation pressure and the seasonal and annual stability of the environment seems to be the key factors. However, patterns often differed between sexes, emphasizing that phenotypic variation in the wild is driven by natural selection operating *via* relative fitness, which is affected by environmental, sexual and fecundity selection in a habitat- and sex-specific way. This emphasises the importance of including both sexes in evolutionary population comparisons.

Variation in movement activity in a familiar environment followed my predictions, and could be explained by habitat-dependent predation pressure and sex-dependent benefits of the inreased activity under negligible predation risk. However, other factors, such as food availability and population density, might also contribute to this pattern. Further studies are necessary to determine the typical trajectory of *Asellus aquaticus* movement activity, particularly whether it is influenced by specific combinations of biotic and abiotic factors or results from other stochastic evolutionary events.

Patterns in exploration behaviour also followed my predictions, painting a picture where only the most explorative individuals from a surface population will disperse into the cave, but after successful adaptation to the cave environment, explorativeness will drop. This is because only the boldest part of a population might enter into a markedly different habitat, especially when the transition zone is as narrow as it is between caves and surface habitats. Hence, the founders of the cave populations are explorers. Later, as evolutionary adaptation to the unique cave habitat starts to take place over generations, explorativeness will provide no benefits within the homogenous and stable cave environments, while leaving the caves for cave-adapted individuals (e.g. having their eyes reduced and lost their pigments) is not really an option due to their highly likely inferiority in competitive or predatory situations. Therefore, I conclude that cave act as “dispersal traps”.

We also found that surface individuals moved more in an unfamiliar environment in dark than in light conditions. This is intuitively surprising in a species that is considered diurnal. However, earlier observations already indicated this possibility in other populations, hence, this pattern might be general in the species. Having higher exploratory drive in dark can be explained by the lowered predatory pressure of visually hunting predators and can be seen as a potential preadaptation to the cave environment, explaining while *Asellus aquaticus* is one of the rare species that could successfully colonise caves in several independent occasions.

My predictions regarding risk-taking behavior were not supported. The observed variations between populations may be influenced by factors other than cave-surface differences. However, even the significant findings did not reveal strong biological patterns.Whether this is a true biological phenomenon, or differences could be detected by applying different assays and tests is remain to be seen in furthr studies.

It is important to note that my studies focused on a single, highly specialized cave population. As a result, the findings cannot be generalized over caves but should instead be viewed as an example of how a species can colonize and adapt to a specific, unique cave environment—one that offers negligible predation risk and large quantities of food.

SUMMARY

Colonization of new environments has driven species to undergo morphological, physiological, and behavioral adaptations. While numerous studies have examined the adaptation of species' populations to new habitats, research on populations inhabiting subterranean environments—particularly those capable of colonizing caves—remains limited and often lacks clear definition, especially in terms of behavior. The Molnár János Cave offers an excellent study system to explore the colonization process of *Asellus aquaticus* (Isopoda: Crustacea), enabling the investigation of the evolution of troglomorphic features (such as reduced eyes and lost pigmentation) as well as behavioral traits in a cave environment. This thesis presents three studies focusing on three personality traits of high ecological and evolutionary relevance using the *Asellus aquaticus* species complex: movement activity (movements in a familiar, perceived-safe environment), exploration (movements in a new, potentially dangerous environment), and risk-taking behavior (movements following a simulated predatory attack in a new environment). This species is known for successfully colonizing caves in Europe on multiple independent occasions. In my thesis, I used the troglomorphic population from Molnár János Cave (Budapest, Hungary) as my model population and compared it to surface populations found nearby. Behavioral assays were conducted repeatedly under both light and dark conditions because: i) light is unnatural for the cave-adapted population, necessitating measurements in the dark; ii) surface *Asellus aquaticus* is considered a diurnal species, thus requiring measurements in the light; and iii) limited information exists on the behavior of surface-adapted individuals, aside from an old observation suggesting they are more active in the dark, making the comparison of behavior under different light conditions scientifically relevant for the surface populations.

The negligible predation in Molnár János Cave is expected to reduce the costs associated with movement activity for mate-searching and foraging in cave-adapted individuals. To test this hypothesis, I studied the movement activity of the populations under both light and dark conditions to determine whether movement activity increases in the cave population. Since males are expected to benefit more from increased movement activity due to its role in mate searching, I hypothesized that this increase would be greater in cave males than in females. To this end, I compared adult individuals of both sexes. This study included three surface populations, one of which (Malom Lake) shares the same water characteristics as the cave-adapted population due to its direct connection with the cave. The findings showed that cave-adapted individuals exhibited increased movement activity in the darkness compared to surface

conspecifics, supporting my first hypothesis. Additionally, the study found a greater increase in the movement activity of cave-adapted males compared to females, supporting my second hypothesis.

In addition to cave-adapted individuals, recent colonists from Malom Lake are also present in Molnár János Cave. This allows me to test potential divergence in explorativeness (a good proxy for dispersal drive and ability) at two levels. Given that adaptations to the cave environment would make individuals highly vulnerable in surface environments (characterized by light and high predation), I hypothesized that caves act as dispersal traps, resulting in reduced explorativeness. Furthermore, because colonizing markedly new habitats is challenging, I hypothesized that new colonists would exhibit higher explorativeness compared to the average observed in their source population. Both hypotheses were supported by the data: cave-adapted individuals tested in the dark were more explorative than surface-adapted individuals, and recent colonists were more explorative than individuals from Malom Lake. Additionally, I found that surface-adapted individuals are more explorative in the dark than in the light, suggesting that the studied species may be somewhat pre-adapted to the cave environment.

In the third study, I focused on risk-taking, a personality trait most directly associated with variation in predation risk. I hypothesized that cave-adapted *A. aquaticus* would exhibit greater risk-taking behavior compared to surface conspecifics. However, this hypothesis was not supported by the data. The observed significant variation in risk-taking among populations could not be explained by differences between cave and surface environments.

Acknowledgements

First of all, I am grateful to my supervisor, Prof. Gábor Herczeg, for his patience, guidance, and invaluable advice throughout this work since I have become a member of his team. I am also thankful to my project team members, particularly Dr. Gergely Horváth, for his help with experiments and for introducing me into advanced statistics and Dr. Gergely Balázs, for his help with field collections and experimental work. Also, I am grateful to Dr. Bálint Üveges and Dr. Tibor Kovács for their valuable suggestions during the initial thesis revision.

Last but not least, I am grateful to my family and friends for their continuous support, which kept me going.

My work was supported by the Stipendium Hungaricum Scholarship and the Hungarian National Research, Development and Innovation Office (#SNN-125627).

References

Adams, C. E., Huntingford, F. A., Turnbull, J. & Beattie, C., 1998. Alternative competitive strategies and the cost of food acquisition in juvenile Atlantic salmon (Salmo salar). *Aquaculture,* pp. 17-26.

Adams, J., Greenwood, P. & Yonow, T., 1985. Loading Constraints and Sexual Size Dimorphism in *Asellus aquaticus*. *Behaviour,* 92(3), pp. 227-287.

Adkins, B., 2017. *Animal Behaviour.* New York: Library Press.

Aiello, L. C. & Wheeler, P., 1995. The Expensive-Tissue Hypothesis: The Brain and the Digestive System in Human and Primate Evolution. *Current Anthropology,* 36(2), pp. 199-221.

Aijuan, L., Hartkainen, H. & Buser, C. C., 2022. Individual level microbial communities in the digestive system of the freshwater isopod *Asellus aquaticus*: Complex, robust and prospective. *Environmental Microiology Reports,* pp. 1-9.

Anselme, P., 2012. Loss in risk-taking: Absence of optimal gain or reduction in one's own resources?. *Behavioural Brain Research,* Volume 229, pp. 433-446.

Ariyomo, T. O. & Watt, P. J., 2012. The effect of variation in bodlness and aggresiveness on the reproductive success of zebrafish. *Animal Behaviour,* pp. 41-46.

Aston, R. J. & Milner, G. P., 1980. A comparison of *Asellus aquaticus* (L) above and below power station in organically polluted reaches of the River Trent. *Freshwater Biology,* 10(1), pp. 1-14.

Aubert, F. et al., 2014. Cooperate or compete? Influence of sex and body size on sheltering behaviour in the wall lizard Podalcis muralis. *Behaviour,* Volume 151, pp. 1903-1920.

Ayad, R. & Akkal, S., 2019. Phytochemistry and biological activities of algerian Centaurea and related genera. In: *Studies in Natural Products Chemistry.* s.l.:Elsevier, pp. 357-414.

Balázs, G. et al., 2021. Parallel morphological evolution and habitat-dependent sexual dimorphism in cave vs. surface populations of the *Asellus aquaticus* (Crustacea: Isopoda: Asellidae) species complex. *Ecology and Evolution,* pp. 1-15.

Barton, K., 2009. *MuMin - multi-model inference,* s.l.: R Package Version.

Bates, D. M., Mächler, M., Bolker, B. & Walker, S., 2014. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software arXiv,* Volume 67, pp. 1-48.

Beekman, M., 2019. Animal Personalities and Behavioural Genetics. In: *Encyclopedia of Animal behaviour.* s.l.:Academic Press, pp. 337-339.

Bell, A. M., Hankison, S. J. & Laskowski, K. I., 2009. The Repeatability of Behaviour: a meta-analysis. *Animal Behaviour,* Volume 77, pp. 771-783.

Belles, X., 1991. Survival, Oportunism and Convenience in the Processes of Cave Colonization by Terrestrial Faunas. *Oecologia aquatica,* Volume 10, pp. 325-335.

Benus, R. F., Bohus, B., Koolhaas, J. M. & van Oortmerssen, G. A., 1991. Heritable variation for aggression as a reflection of individual coping styles. *Experientia,* 47(10), pp. 1008-1019.

Bergmüller, R., 2010. Animal Personality and Behavioural Syndrome. In: *Animal Behaviour: Evolution and Mechanisms.* Berlin, Heidelberg: Springer, pp. 587-621.

Berisha, H. et al., 2022. Sex-dependent increase of movement activity in the freshwater isopod *Asellus aquaticus* following adaptation to a predator-free cave habitat. *Current Zoology,* Volume XX, pp. 1-8.

Bertin, A. & Cézilly, F., 2003. Sexual selection, antennae length and the mating advantage of large males in *Asellus aquaticus*. *Journal of Evolutionary Biology,* 16(4), pp. 698-707.

Bertin, A., David, B., Cezilly, F. & Alibert, P., 2002. Quantification of sexual dimorphism in *Asellus aquaticus* (Crustacea: Isopoda) using outline approaches. *Biological Journal of Linnean Society,* 77(4), pp. 523-533.

Betini, G. S. & Norris, R., 2012. The relationship between personality and plasticity in tree swallow aggression and the consequences for reproductive success. *Animal Behaviour,* pp. 137-143.

Bhat, A., Greulich, M. M. & Martins, E. P., 2015. Behavioural Plasticity in Response to Environmental Manipulation among Zebrafish (Danio rerio) Populations. *PLoS ONE,* 10(4), pp. 1-13.

Biggs, A. et al., 2008. *Biology.* s.l.:McGraw-Hill Companies: Glencoe & National Geographic.

Bilandžija, H. et al., 2020. Phenotypic plasticity as a mechanism of cave colonization and adaptation. *eLife,* p. e51830.

Biro, A., Balázs, G., Fišer, Ž. & Gábor, H., 2021. *Gender inequality in the dark: are adaptations to the cave environment sex-specific?.* Le Bourget-du-Lac, 18th International Congress of Speleology.

Biro, A., Balázs, G., Fišer, Ž. & Herczeg, G., 2021. *Gender inequality in the dark: are adaptations to the cave environment sex-specific?.* Le Bourget du Lac, 18th International Congres of Speleology.

Blecher, A. & Oosthuizen, M. K., 2023. Social isolation does not alter exploratory behaviour, spatial learning an memory in captive Damaraland mole-rats (Fukomys damarensis). *Animals,* 13(3), p. 543.

Blum, M., 2010. The isopod *Asellus aquaticus*: A novel arthropod model organism to study evolution of segment identity and patterning. *Palaeodiversity,* Volume 3, pp. 89-97.

Boissy, A., 1995. Fear and Fearfulness in Animals. *The Quarterly Review of Biology,* pp. 165-191.

Bouchon, D., Zimmer, M. & Dittmer, J., 2016. The Terrestrial Isopod Microbiome: An All-in-One Toolbox for Animal–Microbe Interactions of Ecological Relevance. *Frontiers in Microbiology,* 7(1388), pp. 1-19.

Boyko, C. et al., 2023. *World Marine, Freshwater and Terrestrial Isopod Crustaceans database,* s.l.: Catalogue of Life Checklist.

Brautest, T. & Ellingsen, T. E., 2011. Industrial Biotechnology and Commodity Products. In: *Comprehnsive Biotechnology.* 2nd Edition ed. s.l.:Elsevier, pp. 541-554.

Bredon, M. et al., 2019. Lignocellulose degradation in isopods: new insights into the adaptation to terrestrial life. *BMC Genomics,* 20(1), pp. 1-14.

Briffa, M., Bridger, D. & Biro, P., 2013. How does temperature affect behaviour? Multilevel analysis of plasticity, personality and predictability in hermit crabs. *Animal Behaviour,* pp. 47-54.

Briffa, M., Rundle, S. D. & Fryer, A., 2008. Comparing the strength of behavioural plasticity and consistency across situations: Animal personalities in the hermit crab Pagurus bernhardus. *Proceedings of the Royal Society B,* pp. 1305-1311.

Brown, C., Burgess, F. & Braithwaite, V. A., 2007. Heritable and experiental effects on boldness in a tropical poeciliid. *Behavioural Ecology and Sociobiology,* pp. 237-243.

Brown, J. S., Kotler, B. P., Smith, R. J. & Wirtz, W. O., 1988. The effects of owl predation on the foraging behaviour of desert rodents. *Oecologia,* 76(3), pp. 408-415.

Brusca, R. & Iverson, E. W., 1985. A Guide to the Marine Isopod Crustacea of Pacific Costa Rica. *Revista de Biologia Tropical,* Volume 33, pp. 1-77.

Brwon, C., Jones, F. & Braithwaite, V. A., 2005. In situ examination of boldness-shyness traits in the tropical poeciliid, Brachyraphis episcopi. *Animal Behaviour,* pp. 1003-1009.

Brydges, N. M., Colegrave, N., Heathcote, R. J. P. & Braithwaite, V. A., 2008. Habitat stability and predation pressure affect temperament behaviours in populations of three-spined sticklebacks. *Journal of Animal Ecology,* 77(2), pp. 229-235.

Calizza, E., Rossi, L. & Costantini, M. L., 2013. Predators and Resources Influence Phosphorus Transfer along an Invertebrate Food Web through Changes in Prey Behaviour. *PLoS ONE,* 8(6), p. e65186.

Carter, A., Goldizen, A. & Heinsohn, R., 2012. Personality and plasticity: Temporal behavioural reaction norms in a lizard, the Namibian rock agama. *Animal Behaviour,* pp. 471-477.

Carter, A., Goldizen, A. & Heinsohn, R., 2012. Personality and plasticity: Temporal behavioural reaction norms in a lizard, the Namibian rock agama. *Animal Behaviour,* 84(2), pp. 471-477.

Chambers, M. R., 1977. A comparison of the population ecology of *Asellus aquaticus* (L.) and Asellus meridianus Rac. In die reed beds of the Tjeukermeer. *Hydrobiologia,* 53(2), pp. 147-154.

Charmantier, A. et al., 2008. Adaptive phenotypic plasticity in response to climate. *Science,* 320(5877), pp. 800-803.

Chen, Y. R., Yang, J. X. & Lan, J. H., 1997. One new species of blind cavefishfrom Guangxi with comments on its phylogentic status (Cypriniformes: Cyprinidae: Barbinae). *Acta Zoologica Sinica,* Volume 22, pp. 219-223.

Chevin, L.-M., Mace, G. M. & Lande, R., 2010. Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS BIOLOGY,* 8(4), pp. 1-8.

Cote, J. et al., 2010. Personality-dependent dispersal: Characterization, ontogeny and consequences for spatially structured populations. *Philosophical Transactions of The Royal Society,* 365(1560), pp. 4065-4076.

Crispo, E., 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *Journal of Evolutionary Biology,* pp. 1460-1469.

Culver, D. C., 2012. Mollusks. In: W. B. White & D. C. Culver, eds. *Encyclopedia of Caves.* 2nd ed. Amsterdam: Elsevier, pp. 512-517.

Culver, D. C. & Holsinger, J. R., 1992. How many species of troglobites are there?. *National Speleological Society Bulletin,* Volume 54, pp. 79-80.

Culver, D. C., Holsinger, J. R., Christman, M. C. & Pipan, T., 2010. Morphological Differences Among Eyeless Amphipods in the Genus Stygobromus Dwelling in Different Subterranean Habitats. *Journal off Crustacean Biology,* 30(1), pp. 68-74.

Culver, D. C. & Pipan, T., 2019. *The Biology of Caves and Other Subterranean Habitats.* 2nd ed. Oxford: Oxford University Press.

Dall, S. R. X. & Mcnamara, J. M., 2004. The behavioural ecology of personality: Consistent individual differences from an adaptive perspective. *Ecology Letters,* Volume 7, pp. 734-739.

Dammhahn, M. & Almeling, L., 2012. Is risk taking during foraging a personality trait? A field test for cross context consistency in boldness. *Animal Behaviour,* pp. 1131-1139.

David, M., Auclair, Y. & Cézilly, F., 2011. Personality predicts social dominance in female zebra finches, Taeniopygia guttata, in a feeding context. *Animal Behaviour,* Volume 81, pp. 219-224.

De Meester, L., 1993. Genotype, Fish-Mediated Chemical, and Phototactic Behavior in Daphnia Magna. *Ecology,* 74(5), pp. 1467-1474.

deRivera, C. E., Backwell, P. R., Christy, J. H. & Vehrencamp, S. L., 2003. Density affects female and male mate searching in the fiddler crab, Uca beebei. *Behavioural Ecology and Sociobiology,* Volume 53, pp. 72-83.

Derkarabetian, S., Steinmann, D. B. & Hedin, M., 2010. Repeated and Time-Correlated Morphological Convergence in Cave-Dwelling Harvestmen (Opiliones, Laniatores) from Montane Western North America. *PloS ONE,* 5(5), p. 10388.

Devigne, C., Broly, P. & Deneubourg, J.-L., 2011. Individual Preferences and Social Interactions Determine the Aggregation of Woodlice. *PLoS ONE,* 6(2), p. e17389.

DeWitt, T. J., Sih, A. & Wilson, D. S., 2011. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution,* 13(2), pp. 77-81.

Dingemanse, N. J. et al., 2003. Natal dispersal and personalities in Great Tits (Parus major). *Proceedings of the Royal Society B,* Volume 270, pp. 741-747.

Dingemanse, N. J. et al., 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology,* Volume 76, pp. 1128-1138.

Dosmann, A. & Mateo, J., 2014. Food, sex and predators: Animal personality persists with multidimensional plasticity across complex environments. *Animal Behaviour,* pp. 109-116.

Dugatkin, L. A., 1991. Tendency to inspect predators predicts mortality risk in the guppy (Poecilia reticulata). *Behavioural Ecology,* pp. 124-127.

Dyer, J. R. G., Croft, D. P., Morrell, L. J. & Krause, J., 2009. Shoal composition determines foraging success in the guppy. *Behavioural Ecology,* pp. 165-171.

Eccard, J. & Herde, A., 2013. Seasonal variation in the behaviour of a short-lived rodent. *BMC Ecology,* pp. 2-9.

Engel, A. S., 2007. Observations on the Biodiversity of Sulfidic Karst Habitats. *Journal of Cave and Karst Studies,* 69(1), pp. 187-206.

Faber, M. D., 1979. Microbial degradation of recalcitrant compounds and synthetic aromatic polymers. *Enzyme and Microbial Technology,* 1(4), pp. 226-232.

Fenolio, D., 2018. *The Olm (Proteus anguinus),* Slovenia: SCIENCE PHOTO LIBRARY.

Fišer, Ž., Prevorčnik, S., Lozej, N. & Trontelj, P., 2019. No need to hide in caves: shelter-seeking behaviour of surface and cave ecomorphs of *Asellus aquaticus* (Isopoda: Crustacea). *Zoology,* Volume 134, pp. 58-65.

Floréz, J. S., Cadena, C. D., Donascimiento, C. & Torres, M., 2021. Repeated colonization of caves leads to phenotypic convergence in catfishes (Siluriformes: Trichomycterus) at a small geographical scale. *Zoological Journal of the Linnean Society,* pp. 1-17.

Fraser, D. F. et al., 2001. Explaining Leptokurtic Movement Distributions: Intrapopulation Variation in Boldness and Exploration. *The American Naturalist,* pp. 124-135.

Galassi, D. M. P., 2001. Groundwater copepods: Diversity patterns over ecological and evolutionary scales. *Hydrobiologia,* 454(1), pp. 227-253.

George, R. Y., 1972. Biphasic moulting in Isopod Crustacea and the finding of an unusual mode of moulting in the Antarctic genus Glyptonotus. *Journal of Natural History,* 6(6), pp. 651-656.

Gerecke, R., Martin, P. & Gledhill, T., 2018. Water mites (Acari: Parasitengona: Hydrachnidia) as inhabitants of groundwater-influenced habitats–considerations following an update of Limnofauna Europaea. *Limnologica - Ecology and Management of Inland Waters,* 69(4), pp. 81-93.

Gergely, B., 2019. *Colonisation and adaptation of stygobiont species,* Budapest: ELTE Online Public Access Catalog.

Ghalambor, C. K., Angeloni, L. M. & Carroll, S. P., 2010. Behaviour as Phenotypic Plasticity. pp. 90-107.

Ghalambor, C. K., Martin, L. B. & Woods, A. H., 2015. Plasticity, Complexity, and the Individual. *Integrative Organismal Biology,* pp. 2-22.

Ghalambor, C. K., Mckay, J. K., Carroll, S. P. & Reznick, D. N., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology,* pp. 394-407.

Gherardi, F., 2004. Resource partitioning between sexes in the ‘‘unconventional’’ hermit crab, Calcinus tubularis. *Behavioural Ecology,* 15(5), pp. 742-747.

Gibert, J. & Culver, D. C., 2005. Diversity Patterns in Europe. In: D. C. Culver & W. B. White, eds. *Encyclopedia of Caves.* s.l.:Elsevier, pp. 196-201.

Gibert, J. & Deharveng, L., 2002. Subterranean Ecosystems: A Trunctated Functional Biodiversity. *Bioscence,* 52(6), pp. 473-481.

Gibert, P., Debat, V. & Ghalambor, C. K., 2019. Phenotypic plasticity, global change, and the speed of adaptive evolution. *Current Opinion in Insect Science,* Volume 35, pp. 34-40.

Giles, N. & Huntingford, F. A., 1984. Predation risk and inter-population variation in anti-predator behaviour in the three-spined stickleback Gasterosteus aculeatus L. *Animal Behaviour,* Volume 32, pp. 264-275.

Gomes, E., Desouhant, E. & Amat, I., 2019. Evidence for risk-taking behavioural types and potential effects on resource aquisition in a parasitoid wasp. *Animal Behaviour,* Volume 154, pp. 17-28.

Gonzalez, B. C., Worsaae, K., Fontaneto, D. & Martinez, A., 2018. Anophthalmia and elongation of body appendages in cave scale worms (Annelida: Aphroditiformia). *Zoologica Scripta,* 47(1), pp. 106-121.

González-López, L. et al., 2013. First data on testate amoebae in speleothems of caves in igneous rocks. *Cuadernos Laboratoiro Xeoloxico de Laxe,* Volume 37, pp. 37-56.

Gorman, D., Ragagnin, M. N., McCarthy, I. D. & Turra, A., 2018. Risk-taking and risk-avoiding behaviors by hermit crabs across multiple environmental contexts. *Journal of Experimental Marine Biology and Ecology,* pp. 25-29.

Gosling, S. D., 2001. From mice to men: What can we learn about personality from animal research?. *Psychological Bulletin,* pp. 45-86.

Gotthard, K. & Nylin, S., 1995. Adaptive Plasticity and Plasticity as an Adaptation: A Selective Review of Plasticity in Animal Morphology and Life History. *Oikos,* pp. 3-17.

Grieve, A. & Lau, D. C. P., 2018. Do autochthonous resources enhance trophic transfer of allochthonous organic matter to aquatic consumers, or vice versa?. *Ecosphere,* 9(6), pp. 1-16.

Griffiths, S., Armstrong, J. D. & Metcalfe, N. B., 2003. The cost of aggregation: juvenile salmon avoid sharing winter refuges with siblings. *Behavioural Ecology,* 14(5), pp. 602-606.

Gunn, J., 2004. *Encyclopedia of Caves and Karst Sciences.* New York: Fitzroy Dearborn An Imprint of the Taylor and Francis Group.

Gwynne, D. T., 1987. Sex-biased predation and the risky mate-locating behaviour of male tick-tock cicadas (Homoptera: Cicadidae). *Animal Behaviour,* 35(2), pp. 571-576.

Haddock, S. H. D. & Dunn, C. W., 2015. Fluorescent proteins function as a prey attractant: experimental evidence from the hydromedusa Olindias formosus and other marine organisms. *Biology Open,* 4(9), pp. 1094-1104.

Hahn, H. J., 2006. The GW-Fauna-Index: A first approach to a quantitative ecological assessment of groundwater habitats. *Limnologica,* 36(2), pp. 119-137.

Hall, L. K., Larsen, R. T., Knight, R. N. & McMillan, B. R., 2021. The influence of predators, competitors, and habitat on the use of water sources by a small desert carnivore. *Ecosphere,* 12(5), p. e03509.

Hargeby, A., Blindow, I. & Andersson, G., 2007. Long-term Patterns of Shifts between Clear and Turbid States in Lake Krankesjön and Lake Tåkern. *Ecosystems,* 10(1), pp. 29-36.

Hargeby, A., Johansson, J. & Ahnesjö, J., 2004. Habitat-specific pigmentation in a freshwater isopod: Adaptive evolution over a small spatiotemporal scale. *Evolution,* 58(1), pp. 81-94.

Harris, S. et al., 2011. Changes in behavioral trait integration following rapid ecotype divergence in an aquatic isopod. *Journal of Evolutionary Biology,* 24(9), pp. 1887-1896.

Harris, S., Green, K. K. & Petersson, L. B., 2013. Predator faunas past and present: Quantifying the influence of waterborne cues in divergent ecotypes of the isopod *Asellus aquaticus*. *Oecologia,* 173(3).

Harris, S., Ramnarine, I. W., Smith, H. G. & Pettersson, L. B., 2010. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy Poecilia reticulata. *OIKOS,* Volume 119, pp. 1711-1718.

Hassall, M. & Tuck, J. M., 2007. Sheltering behaviour of terrestrial isopods ins grasslands. *Invertebrate Biology,* 126(1), pp. 45-56.

Hawes, R. S., 1939. The Flood Factor in the Ecology of Caves. *Journal of Animal Ecology,* Volume 8, pp. 1-5.

Herczeg, G. et al., 2020. Is foraging innovation lost following colonization of a less variable environment? A case study in surface- vs. cave-dwelling *Asellus aquaticus*. *Ecology and Evolution,* pp. 1-9.

Herczeg, G., Nyitrai, V., Balázs, G. & Horváth, G., 2022. Food preference and food type innovation of surface-vs. cave-dwelling waterlouse (*Asellus aquaticus*) after 60 000 years of isolation. *Behavioural Ecology and Sociobiology,* 76(1), pp. 1-11.

Herczeg, G. & Välimäki, K., 2011. Intraspecific variation in behaviour: Effects of evolutionary history, ontogenetic experience and sex. *Journal of Evolutionary Biology,* 24(11), pp. 2434-2444.

Herrera, M., Castanheira, M. F., Conceição, L. E. C. & Martins, C., 2014. Linking risk taking and the behavioral and metabolic responses to confinement stress in gilthead seabream Sparus aurata. *Applied Animal Behaviour Science,* Volume 155, pp. 101-108.

Hervant, F. & Malard, F., 2012. Responses to Low Ocygen. In: *Encyclopedia of Caves.* s.l.:Elsevier, pp. 651-657.

Hervant, F. et al., 1997. Comparative Study on the Behavioral, Ventilatory, and Respiratory Responses of Hypogean and Epigean Crustaceans to Long-term Starvation and Subsequent Feeding. *Comparative Biochemistry and Physiology,* 118(4), pp. 1277-1283.

Hervant, F., Mathieu, J., Garin, D. & Freminet, A., 1996. Behavioral, Ventilatory, and Metabolic Responses of the Hypogean Amphipod Niphargus virei and the Epigean Isopod *Asellus aquaticus* to Severe Hypoxia and Subsequent Recovery. *Physiological Zoology,* pp. 1277-1300.

Hervant, F., Mathieu, J. & Messana, G., 1998. Oxygen Consumption and Ventilation in Declining Oxygen Tension and Posthypoxic Recovery in Epigean and Hypogean Crustaceans. *Journal of Crustacean Biology,* 18(4), pp. 717-728.

Heupel, M. R. & Simpfendorfer, C. A., 2005. Quantitative analysis of Aggregation behaviour in Juvenile Blacktip Sharks. *Marine Biology,* 147(5), pp. 1239-1249.

Hobbs, H. H., 2005. Crustacea. In: D. C. Culver & W. B. White, eds. *Encyclopedia of Caves.* s.l.:Elsevier Academic Press, pp. 142-153.

Horecka, K. & Neal, S., 2022. Critical Problems for Research in Animal Sheltering, a Conceptual Analysis. *Frontiers in Veterinary Science,* Volume 9, p. 804154.

Horváth, G. et al., 2023. Exploratory behaviour divergence between surface populations, cave colonists and and a cave population in the water louse, *Asellus aquaticus*. *Behaviour Ecology and Sociobiology,* 77(15), pp. 1-12.

Horváth, G., Sztruhala, S. S., Balázs, G. & Herczeg, G., 2021. Population divergence in aggregation and sheltering behaviour in surface versus cave-adapted *Asellus aquaticus* (Crustacea: Isopoda). *Biological Journal of the Linnean Society,* pp. 1-12.

Howarth, F. G., 1983. Ecology of Cave Arthropods. *Annual Review of Entomology,* Volume 28, pp. 365-389.

Howarth, F. G. & Moldovan, O. T., 2018. The Ecological Classification of Cave Animals and their Adaptations. In: O. T. Moldovan, Ľ. Kováč & S. Halse, eds. *Cave Ecology.* s.l.:Springer, Cham, pp. 41-67.

Howarth, F. G. & Moldovan, O. T., 2018. The Ecological Classification of Cave Animals and Their Adaptations. In: *Cave Ecology.* s.l.:Springer, Cham, pp. 41-67.

Huertos, M. L., 2020. Typologies of Aquatic Systems. In: M. L. Huertos, ed. *Ecology and Management of Inland Waters.* California: Elsevier, pp. 255-56.

Hulthén, K., Heinen-Kay, J. L., Schmidt, D. A. & Langerhand, B. R., 2021. Predation shapes behavioral lateralization: insights from an adaptive radiaton of livebearing fish. *Behavioural Ecology,* 32(6), pp. 1321-1329.

Huntingford, F. A., 1976. The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, Gasterosteus Aculeatus. *Animal Behaviour,* 24(2), pp. 245-260.

Hüppop, K., 2000. How do cave animals cope with food scarcity in caves?. In: *Subterranean Ecosystems.* s.l.:Elsevier Press, pp. 417-432.

Ioannou, C. C. & Dall, S. R. X., 2016. Individuals that are consistent in risk-taking benefit during collective foraging. *Scientific Reports.*

Jablonszky, M. et al., 2017. Escape ability and risk-taking behaviour in a Hungarian population of the collared flycatcher (Ficedula albicollis). *Behavioural Ecology and Socibiology,* 71(3).

Jolles, J. W., Briggs, H. D., Araya-Ajoy, Y. G. & Boogert, N. J., 2019. Personality, plasticity and predictability in sticklebacks: bold fish are less plastic and more predictable than shy fish. *Animal Behaviour,* Volume 154, pp. 193-202.

Jones, K. A. & Godin, J.-G. J., 2009. Are fast explorers slow reactors? Linking personal type and anti-predator behavior. *Proceedings of the Royal Society B,* pp. 625-632.

Jørgensen, A., Boesgaard, T. M., Møbjerg, N. & Kristensen, R. M., 2014. The tardigrade fauna of Australian marine caves: With descriptions of nine new species of Arthrotardigrada. *Zootaxa,* 3802(4), pp. 401-443.

Jormalainen, V., Toumi, J. & Yamamura, N., 1994. Intersexual conflict over precopula duration in mate guarding Crustacea. *Behavioural Processes,* 32(3), pp. 265-283.

Karlsson, K., Eroukhmanoff, F. & Swensson, E. I., 2010. Phenotypic Plasticity in Response to the Social Environment: Effects of Density and Sex Ratio on Mating Behaviour Following Ecotype Divergence. *PLoS ONE,* 5(9), p. e12755.

Karlsson, K., Eroukmanhoff, F., Härdling, R. & Svensson, E. I., 2010. Parallel divergence in mate guardig behaviour following colonization of a novel habitat. *Journal of Evolutionary Biology,* 23(12), pp. 2540-2549.

Kasumovic, M. M., Bruce, M. J., Herberstein, M. E. & Andrade, M. C. B., 2007. Risky mate search and mate preference in the golden orb-web spider (Nephila plumpies). *Behavioural Ecology,* 18(1), pp. 189-195.

Keskin, B. & Köse, E. Ö., 2015. Understanding Adaptations and Natural Selection: Common Misconceptions. *International Journal of Academic Research in Education,* 1(2), pp. 2149-2913.

Konec, M., Delic, T. & Trontelj, P., 2016. DNA barcoding sheds light on hidden subterranean boundary between Adriatic and Danubian drainage basins. *Ecohydrology.*

Konec, M. et al., 2015. Parallels between two geographically and ecologically disparate cave invasions by the same species, *Asellus aquaticus* (Isopoda, Crustacea). *Journal of Evolutionary Biology,* 28(4).

Koralesky, K. E., Rankin, J. M. & Fraser, D., 2022. The everyday work of One Welfare in animal sheltering and protection. *Humanities & Social Sciences Communicationa,* pp. 1-9.

Kortet, R., Hedrick, A. V. & Vainikka, A., 2010. Parasitism, predation and the evolution of animal personalities. *Ecology letters,* Volume 13, pp. 1449-1458.

Kotrschal, A. et al., 2012. Artificial Selection on Relative Brain Size in the Guppy Reveals Costs and Benefits of Evolving a Larger Brain. *Current Biology,* 23(2), pp. 167-171.

Krajl-Fišer, S. et al., 2020. The interplay between habitat use, morphology and locomotion in subterranean crustaceans of the genus Niphargus. *Zoology,* Volume 139, p. 125742.

Krenhardt, K. et al., 2021. Sex-dependent risk-taking behaviour towards different predatory stimuli in the collared flycatcher. *Behavioural Processes.*

Kusnetzova, A., Brockhoff, P. B. & Christensen, R. H. B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software,* 82(13), pp. 1-26.

Lafuente, E. et al., 2021. Building on 150 Years of Knowledge: The Freshwater Isopod *Asellus aquaticus* as an Integrative Eco-Evolutionary Model System. *frontiers in Ecology and Evolution,* Volume 9, pp. 1-22.

Lafuente, E. et al., 2021. Building on 150 Years of Knowledge: The Freshwater Isopod *Asellus aquaticus* as an Integrative Eco-Evolutionary Model System. *Frontiers in Ecology and Evolution,* Volume 1-23.

Lande, R., 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology,* 22(7), pp. 1435-1446.

Larned, S. T., 2012. Phreatic groundwater ecosystems: research frontiers for freshwater ecology. *Freshwater Biology,* Volume 57, pp. 885-906.

Larned, S. T., 2012. Phreatic groundwater ecosystems; research frontiers for freshwater ecology. *Freshwater Biology,* Volume 57, pp. 885-906.

Lawrence, M. J., Godin, J.-G. J. & Cooke, S. J., 2018. Does experimental cortisol elevation mediate risk-taking and antipredator behaviour in a wild teleost fish?. *Comparative Biochemistry and Physiology, Part A,* pp. 75-82.

Leal-Zanchet, A., Souza, S. & Ferreira, R., 2014. A new genus and species for the first recorded cave-dwelling Cavernicola (Platyhelminthes) from South America. *ZooKeys,* Volume 442, pp. 1-15.

Lefebure, T. et al., 2006. Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments. *Molecular Ecology,* Volume 15, pp. 1797-1806.

Leimar, O., Hammerstein, P. & Dooren, T. J. M. V., 2006. A New Perspective on Developmental Plasticity and the Principles of Adaptive Morph Determination. *The American Naturalist,* 167(3), pp. 367-376.

Lenski, R. E., 2017. What is adaptation by natural selection? Perspectives of an experimental microbiologist. *Plos GENET,* 13(4), pp. 1-12.

Lenth, R. V., 2016. Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software,* 69(1).

Leys, R., Wats, C. H. S., Cooper, J. J. B. & Humphreys, W. F., 2004. Evolution of subterranean diving beetles (Coleoptera: Dytiscidae Hydroporini, Bidessini) in the arid zone of Australia. *Evolution,* 57(12), pp. 2819-2834.

Lima, S. L., 1998. Stress and Decision Making under the Risk of Predation: Recent Developments from Behavioral, Reproductive, and Ecological Perspectives. *Advances in the Study of Behaviour,* pp. 215-290.

Lima, S. L. & Dill, L. M., 1990. Behavioural decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology,* Volume 68, pp. 619-640.

Lomheim, H. J. et al., 2023. Transcriptomic analysis of cave, surface, and hybrid samples of the isopod *Asellus aquaticus* and identification of chromosomal location of candidate genes for cave phenotype evolution. *EvoDevo,* 14(1), pp. 1-15.

Louw, C. J., Ferreira, S. M. & Marshal, J. P., 2022. Water dependence structures predation risk for large herbivores in insular protected areas. *Mammalian Biology,* 102(4), pp. 1-16.

Lürig, M. D. et al., 2019. The role of plasticity in the evolution of cryptic pigmentation in a freshwater isopod. *Journal of Animal Ecology,* Volume 88, pp. 612-623.

Lürig, M. & Matthews, B., 2021. Dietary-based developmental plasticity affects juvenile survival in an aquatic detritivore. *Proceedings of the Royal Society B,* Volume 288, pp. 1-10.

Maguire, W., 2023. *Asellus aquaticus (Linnaeus, 1758),* s.l.: British Myriapod and Isopod Group.

Malard, F. & Hervant, F., 1999. Oxygen supply and the adaptations of the animals in groundwater. *Freshwater Biology,* Volume 41, pp. 1-30.

Maltby, L., 1991. Pollution as a probe of life-history adaptation in *Asellus aquaticus* (Isopoda). *OIKOS,* Volume 61, pp. 11-18.

Ma, L. & Zhao, Y.-h., 2012. Cavefish of China. In: D. C. Culver & W. B. White, eds. *Encyclopedia of Caves.* s.l.:Elseiver, pp. 107-125.

Mammola, S., 2019. Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. *Ecography,* 42(7), pp. 1331-1351.

Manning, J. T., 1980. Sex Ratio and Optimal Male Time Investment Strategies in *Asellus aquaticus* (L.) and a. Meridianus Racovitza. *Behaviour,* 74(3-4), pp. 264-273.

Marcus, J. H. & Willoughby, L. G., 1978. FUNGI AS FOOD FOR THE AQUATIC INVERTEBRATE *ASELLUS AQUATICUS. Transactions of the British Mycological Society,* 70(1), pp. 143-146.

Marshall, J., Kent, J. & Cronin, T., 1999. Visual adaptations in crustaceans: Spectral sensitivity in diverse habitats. In: S. N. Archer, et al. eds. *Adaptive Mechanisms in the Ecology of Vision.* Dordrecht: Springer, pp. 285-327.

Martinez, G. & Defeo, O., 2006. Reproductive biology of the isopod Excirolana braziliensis at the southern edge of its geographical range. *Helgoläander Meeresuntersuchungen,* 60(4), pp. 273-280.

Martin, T. L. F. et al., 2007. Speed of exploration and risk-taking behaviour are linked to corticosterone titres in zebra finches. *Hormones and Behaviour,* Volume 52, pp. 445-453.

Mathot, K. J. et al., 2011. Disentangling the roles of frequency-vs. state-dependence in generating individual differences in behavioural plasticity. *Ecology Letters,* Volume 14, pp. 1254-1262.

McGaugh, S. E. et al., 2014. The cavefish genome reveals candidate genes for eye loss. *Nature Communications,* 5(1), p. 5307.

Mejía-Ortíz, L. M., 2019. Crustacea. In: *Encyclopedia of Caves.* s.l.:Elsevier, pp. 333-347.

Mejía-Ortíz, L. M. & Hartnoll, R. G., 2006. A New Use for Useless in Cave Crustaceans. *Crustaceana,* 79(5), pp. 593-600.

Merilä, J. & Hendry, A. P., 2013. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications,* pp. 1-14.

Merilä, J. & Hendry, A. P., 2014. Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications,* pp. 1-14.

Migliore, L. & Guidici, M. d. N., 1990. Toxicity of heavy metals to *Asellus aquaticus* (L) (Crustacea: Isopoda). *Hydrobiologia,* Volume 203, pp. 155-164.

Millman, A. E., 1984. *Responses to lead in the freshwater isopods Asellus aquaticus and A. meridianus.* Leicester: University of Leicester.

Mitchell, D. J. & Biro, P. A., 2017. Is behavioural plasticity consistent across different environmental gradients and through time?. *Proceedings of the Royal Society B,* Volume 284, pp. 1-8.

Mitchell, R., 1971. *TYPHLOCHACTIDAE,* s.l.: The Scorpion Files.

Mitton, J. B., 2002. Adaptation and Natural Selection: Overview. In: *Encyclopedia of Life Sciences (eLS).* s.l.:Encyclopedia of Life Sciences, pp. 1-6.

Mojaddidi, H., Fernandez, F. E., Erickson, P. A. & Protas, M. E., 2018. Embryonic origin and genetic basis of cave associated phenotypes in the isopod crustacean *Asellus aquaticus*. *Scientific Reports,* pp. 1-13.

Moldovan, M. et al., 2001. Bioaccumulation of palladium, platinum and rhodium from urban particulates and sediments by the freshwater isopod *Asellus aquaticus*. *Water Research,* 35(17), pp. 4175-4183.

Montalenti, G. & Rocchi, A., 1964. Note cariologiche sul genere Asellus. *Bolletino di Zoologia,* 31(2), pp. 341-349.

Moore, J. & Gibson, R., 2006. On a new genus of freshwater hoplonemertean from Campbell Island. *Freshwater Biology,* 2(3), pp. 187-202.

Moran, N. A., 1992. The Evolutionary Maintenance of Alternative Phenotypes. *The American Naturalist,* 139(5), pp. 971-989.

Morris, D. W., 1984. Sexual differences in habitat use by small mammals: evolutionary strategy or reproductive constraint?. *Oecologia,* Volume 65, pp. 51-57.

Moschilla, J. A., Tomkins, J. L. & Simmons, L. W., 2018. State-dependent changes in risk-taking behaviour as a result of age and residual reproductive value. *Animal Behaviour,* pp. 95-100.

Moschilla, J. A., Tomkins, J. L. & Simmons, L. W., 2018. State-dependent changes in risk-taking behaviour as a result of age and residual reproductive value. *Animal Behaviour,* pp. 95-100.

Mösslacher, F. & Châtelliers, C. d., 1996. Physiological and behavioural adaptations of an epigean and a hypogean dwelling population of *Asellus aquaticus* (L.) (Crustacea, Isopoda). *Archiv fur Hydrobiologie,* 138(2), pp. 187-198.

Mullis, R., Ellis, J. B., Revit, D. M. & Shutes, R. B. E., 1994. An Evaluation of the Toxic Influences on *Asellus aquaticus* (L) in an Urban Stream Environment. *Water Science and Technology,* 29(1-2), pp. 199-207.

Murphy, P. M. & Learner, M. A., 1982. The life history and production of *Asellus aquaticus* (Crustacea: Isopoda) in the River Ely, South Wales. *Freshwater Biology,* Volume 12, pp. 435-444.

Nakagawa, S. & Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological reviews of the Cambridge Philosophical Society,* 85(4), pp. 935-956.

Needham, A. E., 1942. Micro-Anatomical Studies on Asellus. *Journal of Cell Science,* pp. 49-72.

Needham, A. E., 2009. 24. On Relative Growth in *Asellus aquaticus*. *Journal of Zoology,* pp. 289-313.

O'Callaghan, I., Harrison, S., Fitzpatrick, D. & Sullivan, T., 2019. The freshwater isopod *Asellus aquaticus* as a model biomonitor of environmental pollution: A review. *Chemosphere,* Volume 235, pp. 498-509.

Oers, K. v., Klunder, M. & Drent, P. J., 2005. Context depencence of personalities: risk-taking behaviour in a social and a nonsocial situation. *Behavioural Ecology,* pp. 716-723.

O'Hara, R. B. & Kotze, D. J., 2010. Do not log-transform count data. *Methods in Ecology and Evolution,* 1(2), pp. 118-122.

Oleszkiewicz, A., d'Oedenberg, G. M. & Chapman, J., 2001. Experience in controlling *Asellus aquaticus* in water distribution systems. *Water Science and Technology: Water Supply,* 1(2), pp. 217-223.

Oosthuizen, M. K., 2020. Exploratory behaviour, memory and neurogenesis in the social Damaraland mole-rat (Fukomys damarensis). *Journal of Experimental Biology,* Volume 223, pp. 1-9.

Overington, S. E., Morand-Ferron, J., Boogert, N. J. & Lefebvre, L., 2009. Technical innovations drive the relationship between innovativeness and residual brain size in birds. *Animal Behaviour,* 78(4), pp. 1001-1010.

Pärssinen, V. et al., 2021. Variation in predation regime drives sex-specific differences in mosquitofish foraging behaviour. *OIKOS,* pp. 1-8.

Parzefall, J., 1986. Behavioural Ecology of Cave-Dwelling Fishes. In: T. J. Pitcher, ed. *The Behaviour of Teleost Fishes.* Boston: Springer, pp. 433-458.

Pavel, A. B. et al., 2021. The Spatio-Temporal Distribution of the Freshwater Crustacean *Asellus aquaticus* Linneaus, 1758, in the Danube Delta. *Section Ecology & Environmental Protection,* pp. 569-578.

Pavković, Ž., Potrebić, M., Kanazir, S. & Pešić, V., 2020. Motivation, risk-taking and sensation seeking behavior in propofol anesthesia exposed peripubertal rats. *Progress in Neuropsychopharmacology & Biological Psychiatry,* p. 109733.

Pérez-Moreno, J. L., Bálazs, G. & Bracken-Grissom, H. D., 2018. Transcriptomic insights into the loss of vision in Molnár János Cave's crustaceans. *Integrative and Comparative Biology,* 58(3).

Piersma, T. & Drent, J., 2003. Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution,* 18(5), pp. 228-233.

Pipan, T., Holt, N. & Culver, D. C., 2010. How to protect diverse, poorly known, inaccessible fauna: identification and protection of soruce and sink habitats in the epikarst. *Aquatic Conservation: Marine and Freshwater Ecystems,* Volume 20, pp. 748-755.

Pisula, W., 2004. Exploratory Behaviour - inquisitiveness in animals. In: M. Bekoff, ed. *Encyclopedia of Animal Behaviour.* s.l.:Greenwood Press, pp. 574-581.

Poulson, T. L. & White, W. B., 1969. The Cave Environment. *Science,* 165(3897), pp. 971-981.

Premate, E., Zagmajster, M. & Fišer, C., 2021. Inferring predator–prey interaction in the subterranean environment: a case study from Dinaric caves. *Scientific Reports,* 11(21682).

Prevorčnik, S., Blejec, A. & Sket, B., 2004. Racial differentiation in *Asellus aquaticus* (L.) (Crustacea: Isopoda: Asellidae). *Archiv fur Hydrobiologie,* 160(2), pp. 193-214.

Prevorčnik, S., Jugovic, J. & Sket, B., 2009. Geography of morphological differentiation in *Asellus aquaticus* (Crustacea: Isopoda: Asellidae). *Journal of Zoological Systematics and Evolutionary Research,* 47(2), pp. 124-131.

Protas, M. E., Trontelj, P. & Patel, N. H., 2011. Genetic basis of eye and pigment loss in the cave crustacean, *Asellus aquaticus*. *PNAS,* 108(14), pp. 5702-5707.

Protas, M. & Jeffery, W. R., 2012. Evolution and development in cave animals: form fish to crustaceans. *Wiley Interdiciplinary Reviews: Developmental Biology,* 1(6), pp. 823-845.

Protas, M. & Patel, N. H., 2009. The genetic basis of morphological change in the cave isopod, *Asellus aquaticus*. *Developmental Biology,* 331(2), p. 462.

R: A Language and Environment, 2020. *A Language And Environment For Statistical Computing,* Viena: R Foundation for Statistical Computing.

Raveh, S., Vogt, D., Montavon, C. & Kölliker, M., 2014. Sibling Aggregation Preference Depends on Activity Phase in the European Earwig (Forficula auricularia). *Ethology,* Volume 120, pp. 776-782.

Reader, S. M., 2003. Innovation and social learning: individual variation and brain evolution. *Animal Biology,* 53(2), pp. 147-158.

Reader, S. M. & Laland, K., 2002. Social Intelligence, Innovation, and Enhanced Brain Size in Primates. *Proceedings of the National Academy of Sciences,* 99(7), pp. 4436-4441.

Réale , D., Gallant, B. Y., Leblanc, M. & Festa-Bianchet, M., 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour,* Volume 60, pp. 589-597.

Réale, D. J. F. & Festa-Bianchet, M., 2003. Predator-induced selection on temperament in bighorn ewes. *Animal Behaviour,* pp. 463-470.

Réale, D. J. F. et al., 2009. Male personality, life-history strategies and reproductive success in a promiscuous mammal. *Journal of Evolutionary Biology,* pp. 1599-1607.

Re, C. et al., 2018. Common Genetic Basis of Eye and Pigment Loss in Two Distinct Cave Populations of the Isopod Crustacean *Asellus aquaticus*. *Integrative and Comparative Biology,* 58(3), pp. 421-430.

Reed, T. E. et al., 2010. Phenotypic Plasticity and Population Viability: The Importance of Environmental Predictability. *Proceedings of the Royal Society B,* 277(1699), pp. 3391-3340.

Reiss, W., Giere, O., Kohls, O. & Sarbu, S. M., 1999. Anoxic thermomineral cave waters and bacterial mats as habitat for freshwater nematodes. *Aquatic Microbial Ecology,* 18(2), pp. 157-164.

Richardson, B. R. et al., 2014. Adaptive responses reveal contemporary and future ecotypes in a desert shrub. *Ecological Applications,* 24(2), pp. 413-427.

Riddell, J., Downey, A. R., Vesper, D. J. & Padilla, I. Y., 2023. Total organic carbon concentrations in clastic cave sediments from Butler Cave, Virginia, USA: implications for contaminant fate and transport. *Environnmental Earth Sciences,* 82(9), pp. 2-16.

Ridley, M. & Thompson, D. J., 2010. Size and Mating in *Asellus aquaticus* (Crustacea: Isopoda). *Ethology,* 51(4), pp. 380-397.

Romero, A., 2001. An Introduction to the Special Volume on the Biology of Hypogean Fishes. *Environmental Biology of Fishes,* Volume 62, pp. 7-12.

Romero, A., 2009. *Cave Biology.* Edinburgh: Cambridge University Press.

Romero, A. J., 2012. Caves as Biological Spaces. *Polymath: An Interdiciplinary Arts and Sciences Journal,* 2(3), pp. 2-16.

RStudio Team, 2020. *RStudio: integrated development for R,* s.l.: s.n.

Rusell, J. C., McMorland, A. J. & MacKay, J. W. B., 2009. Exploratory behaviour of colonizing rats in novel environments. *Animal Behaviour,* Volume 79, pp. 159-164.

Sakkaluk, S. K. & Belwood, J. J., 1984. Gecko phonotaxis to cricket calling song: A case of satellite predation. *Animal Behaviour,* 32(3), pp. 659-662.

Salemaa, H., 1979. The Chromosomes of *Asellus aquaticus* (L.) - a Technique for Isopod Karyology. *Crustaceana,* 36(3), pp. 316-318.

Salin, K., Voitorun, Y., Mourin, J. & Hervant, F., 2010. Cave colonization without fasting capacities: An example with the fish Astyanax fasciatus mexicanus. *Comparative Biochemistry and Physiology,* Volume 156, pp. 451-457.

Salma, U. & Thomson, M., 2017. Social aggregation of the marine isopod Cirolana harfordi does not rely on the availability of light-reducing shelters. *Physiological Entomology,* Volume 10.1111/phen.12229.

Schuet, W. et al., 2018. Ground beetles in city forests: Does urbanization predict a personality trait?. *PeerJ,* 6(1), p. e4360.

Seghers, B. H., 1974. Schooling Behavior in the Guppy (Poecilia reticulata): An Evolutionary Response to Predation. *Evolution,* 28(3), pp. 486-489.

Sgrò, C. M., Terblanche, J. S. & Hoffmann, A. A., 2015. What Can Plasticity Contribute to Insect Responses to Climate Change?. *Annual Review of Entomology,* pp. 533-451.

Shuai, L.-Y., Zhang, Z. & Zeng, Z.-G., 2016. When should I be aggressive? A state-dependent foraging game between competitors. *Behavioural Ecology,* pp. 1-8.

Simões, L. B., Ferreira, T. & Bichuette, M. E., 2013. Aquatic biota of different karst habitats in epigean and subterranean systems of Central Brazil – visibility versus relevance of taxa. *Subterranean Biology,* Volume 11, pp. 55-74.

Simon, K., Pipan, T. & Culver, D. C., 2007. A conceptual model of the flow and distribution of organic carbon in caves. *Journal of Cave and Karst Studies,* 69(2), pp. 1-5.

Simon, K. S., 2019. Cave Ecosystems. In: *Encyclopedia of Caves.* Auckland: Elsevier Inc., pp. 223-226.

Simon, K. S., Pipan, T., Ohno, T. & Culver, D. C., 2010. Spatial and temporal patterns in abundance and character of dissolved organic matter in two karst aquifers. *Fundam. Appl. Limnol.,* 177(2), pp. 81-92.

Simonsen, D. & Hesselberg, T., 2021. Unique behavioural modifications in the web structure of the cave orb spider Meta menardi (Araneae, Tetragnathidae). *Scientific Reports,* 11(92).

Sinn, D. L., Moltschaniwskyj, N. A., Wapstra, E. & Dall, S. R. X., 2010. Are behavioural syndromes invariant? Spatiotemporal variation in shy/bold behaviour in squid. *Behavioural Ecology and Sociobiology,* pp. 693-702.

Sket, B., 1997. Distribution of Proteus (Amphibia: Urodela: Proteidae) and its possible explanation. *Journal of Biogeography,* Volume 24, pp. 263-280.

Sket, B., 2005. Dinaric Karst, Diversity in. In: D. C. Culver & W. B. White, eds. *Encyclopedia of Caves.* s.l.:Elsevier Academic Press, pp. 159-166.

Sket, B., 2008. Can we agree on an ecological classification of subterranean animals?. *Journal of Natural History,* Volume 42, pp. 1549-1563.

Sket, B., Paragamian, K. & Trontelj, P., 2004. A Census of the Obligate Subterranean Fauna of the Balkan Peninsula. In: H. I. Griffiths, B. Kryštufek & J. M. Reed, eds. *Balkan Biodiversity.* Dordrecht: Springer, pp. 309-322.

Sket, B. & Velikonja, M., 1986. Troglobitic freshwater sponges (Porifera, Spongillidae) found in Yugoslavia.. *Stygologia,* Volume 2, pp. 254-266.

Slaney, D. & Weinstein, P., 2008. Cave Habitat Colonization. In: J. L. Capinera, ed. *Encyclopedia of Entomology.* Dordrecht: Springer.

Smith, B. R. & Blumstein, D. T., 2010. Behavioral types as predictors of survival in Trinidadian guppies (Poecilia reticulata). *Behavioural Ecology,* pp. 919-926.

Soares, D. & Niemiller, M. L., 2013. Sensory Adaptations of Fishes to Subterranean Environments. *BioScience,* 63(4), pp. 274-283.

Soares, D. & Niemiller, M. L., 2018. Extreme adaptations in caves. *The Anatomical Record Advances in Integrative Anatomy and Evolutionary Biology,* 303(1).

Sol, D. et al., 2005. Big brains, enhanced cognition, and response of birds to novel environments. *Proceedings of the National Academy of Sciences,* 102(15), pp. 5460-5465.

Sol, D. & Lefebvre, L., 2000. Behavioural flexibility predicts invasion success in birds introduced to New Zealand. *Oikos,* 90(3), pp. 599-605.

Sommer-Trembo, C. et al., 2022. Does presence of conspecifics facilitate exploratory behaviour in a cichlid fish (Etroplus suratensis)?. *Behaviour,* 159(10), pp. 899-914.

Sommer-Trembo, C. et al., 2022. Does the presence of conspecifics facilitate exploratory behaviour in a cichlid fish (Etroplus suratensis)?. *Behaviour,* Volume 159, pp. 899-914.

Sørensen, M. V., Jørgensen, A. & Boesgaard, T. M., 2000. A new Echinoderes (Kinorhyncha: Cyclorhagida) from a submarine cave in New South Wales, Australia. *Cahires de Biologie Marine,* 41(2), pp. 167-179.

Spiegel, O., Leu, S., Bull, M. C. & Sih, A., 2017. What's your move? Movement as a link between personality and spatial dynamics in animal populations. *Ecology Letters,* 20(1), pp. 3-18.

Spotl, C. et al., 2011. Stable isotope imprint of hypogene speleogenesis: Lessons from Austrian caves. *Chemical Geology,* pp. 2-19.

Sprouse, P. & Gluesenkamp, A., 2010. *Scorpion Species Alacran tartarus,* Oaxaco, Mexico: National Science Foundation.

Stamps, J., 2007. Growth-mortality tradeoffs and "personality traits" in animals. *Ecology Letters,* 10(5), pp. 355-363.

Stams, J. & Groothuis, T. G. G., 301-325. The Development of Animal Personality: relevance, concepts and perspectives. *Biological Reviews,* Volume 85, p. 2010.

Steel, C. G. H., 2011. Stages of the intermoult cycle in the terrestrial isopod Oniscus asellus and their relation to biphasic cuticle secretion. *Canadian Journal of Zoology,* 60(3), pp. 429-437.

Steel, E. A., 2009. Some observations on the life history of *Asellus aquaticus* (L.) and Asellus meridianus Racovitza (Crustacea: Isopoda). *Journal of Zoology,* 137(1), pp. 71-87.

Stein, H. et al., 2012. Stygoregions - a promisin approach to a bioregional classification of groundwater systems. *Scientific Reports,* 2(673), pp. 1-9.

Stoffel, M., Nakagawa, S. & Schielzeth, H., 2022. *Package 'rptR'.* [Online] [Accessed 14 October 2019].

Stomp, M. et al., 2008. The Timescale of Phenotypic Plasticity and Its Impact on Competition in Fluctuating Environments. *The American Naturalist*, pp. 169-185.

Studnitz, M., Jensen, M. B. & Pederesen, L. J., 2007. Why do pigs root and in what will they root? A review on the exploratory behaviour of pigs in relation to environment enrichment. *Applied Animal Behaviour Science,* Volume 107, pp. 183-197.

Sultan, S. E. & Spencer, H. G., 2002. Metapopulation Structure Favors Plasticity over Local Adaptation. *The American Naturalist,* pp. 271-282.

Surányi, G., Dombradi, E. & Leél-Őssy, S., 2010. CONTRIBUTIONS OF GEOPHYSICAL TECHNIQUES TO THE EXPLORATION OF THE MOLNAR JANOS CAVE (BUDAPEST, HUNGARY). *ACTA CARSOLOGICA,* 39(3), pp. 565-576.

Swaisgood, R. R., Owings, D. H. & Rowe, M. P., 1999. Conflict and assessment in a predator-prey system: Ground squirrels versus rattlesnakes. *Animal Behaviour,* 57(5), pp. 1033-1044.

Sworobowicz, L. et al., 2015. Revisiting the phylogeography of *Asellus aquaticus* in Europe: insights into cryptic diversity and spatiotemporal diversification. *Freshwater Biology,* Volume 60, pp. 1824-1840.

Tanaka, T., 2020. Comparsion of sex differences in motor activities of exploratory and spontaneous behaviour of laboratory CD1 mice (Mus musculus). *Journal of Experimental and Applied Animal Sciences,* 3(2), pp. 115-127.

Thomas, G. W. C., Dohmen, E., Hughes, D. S. T. & Richards, S., 2020. Gene content evolution in the arthropods. *Genome Biology,* 21(1), pp. 1-14.

Thomson, D. J. & Manning, J. T., 1981. Mate Selection By Asellus (Crustacea: Isopoda). *Behaviour,* 78(3), pp. 178-186.

Thomson, R. L., Forsman, J. T. & Mönkkönen, M., 2011. Risk taking in natural predation risk gradients: support for risk allocation from breeding pied flycatchers. *Animal Behaviour,* 82(6), pp. 1443-1447.

Thulin, B. & Innova, G., 2008. *Ecology and living conditions of groundwater fauna,* Stockholm: Swedish Nuclear Fuel and Waste Management Co.

Thünken, T. et al., 2010. Impact of olfactory nos-host predator cues on aggregation behaviour and activity in Polymorphus minutus infected Gammarus pulex. *Hydrobiologia,* 654(1), pp. 137-145.

Tollrian, R. & Harvell, D. C., 1999. *The Ecology and Evolution of Inducible Defenses.* s.l.:Princeton University Press.

Tomova, L., Andrews, J. L. & Blakemore, S.-J., 2021. The importance of belonging and the avoidance of social risk taking in adolescence. *Developmental Review,* p. 100981.

Trabalon, M., 2022. Effects of Wolf Spiders' Captive Environment on their Locomotor and Exploratory Behaviours. *Insects,* 13(135), pp. 1-15.

Trajano, E., 2012. Ecological Classification of Subterranean Organisms. In: W. B. White & D. C. Culver, eds. *Encyclopedia of Caves.* s.l.:Elsevier, pp. 275-277.

Trajano, E., 2012. Evolution of Lineages. In: *Encyclopedia of Caves.* s.l.:Elsevier Academic Press, pp. 230-234.

Trontelj, P., 2019. Adaptation and natural selection in caves. In: *Encyclopedia of Caves.* s.l.:Academic Press, pp. 40-46.

Trontelj, P., Blejec, A. & Fišer, C., 2012. Ecomorphological convergence of cave communities. *Evolution,* 66(12), pp. 3852-3865.

Tufto, J., 2000. The Evolution of Plasticity and Nonplastic Spatial and Temporal Adaptations in the Presence of Imperfect Environmental Cues. *The American Naturalist,* 156(2), pp. 121-130.

Tufto, J., 2015. Genetic evolution, plasticity, and bet-hedging as adaptive responses to temporally autocorrelated fluctuating selection: A quantitative genetic model. *Evolution,* 69(8), pp. 2034-2041.

Turk-Prevorcnik, S. & Blejec, A., 1998. Aseullus aquaticus infernus, new subspecies (Isopoda: Asellota: Asellidae), from Romanian hypogean waters. *Journal of Crustacean Biology,* 18(4), pp. 763-773.

Turk, S., Sket, B. & Sarbu, S., 1996. Comparison between some epigean and hypogean populations of *Asellus aquaticus* (Crustacea: Isopoda: Asellidae). *Hydrobiologia,* Volume 337, pp. 161-170.

Unwin, E. E., 2008. Notes upon Reproduciton os *Asellus aquaticus*. *Zoological Journal of Linnean Society,* 34(228), pp. 335-343.

Unwin, E. E. & Steebing, T. R. R., 1919. Notes upon the Reproduction of *Asellus aquaticus.. Zoological Journal of the Linnean Society,* pp. 335-343.

Urszán, T. J., Hettyei , A., Garamszegi, L. Z. & Herczeg, G., 2015. Behavioural consistency and life history of Rana dalmatina tadpoles. *Oecologia,* Volume 178, pp. 129-140.

Urzcán, T. J. et al., 2015. No personality without experience? A test on Rana dalmatina tadpoles. *Ecology and Evolution,* pp. 5847-5856.

Urzcán, T. J. et al., 2018. Experience during development triggers between individual variation in behavioural plasticity. *Journal of Animal Ecology,* 87(5), pp. 1264-1273.

Valentino, F., Marco, A. D. & Rocchi, A., 2009. Nuclear DNA content of *Asellus aquaticus* and Proasellus coxalis. *Journal of Zoological Systematics and Evolutionary Research,* 21(4), pp. 262-265.

van Tienderen, P. H., 1997. Generalists, Specialists, and the Evolution of Phenotypic Plasticity in Sympatric Populations of Distinct Species. *Evolution,* 51(5), pp. 1372-1380.

Vandel, A., 1965. *Biospeleology: The Biology of Cavernicolus Animals.* First Edition ed. Touluse: Pergamon Press.

Velasco, A. C., Ferrer, E. & Sans, J. J., 2022. Exploratory behaviour and foraging strategies in Mediterranean blue tits. *Animal Biodiversity and Conservation,* 45(2), pp. 287-298.

Verovnik, R., Prevorcnik, S. & Jugovic, J., 2009. Description of a neotype for *Asellus aquaticus* Linne, 1758 (Crustacea: Isopoda: Asellidae), with description of a new subterranean Asellus species from Europe. *Zoologischer Anzeiger,* pp. 101-118.

Verovnik, R., Sket, B. & Trontelj, P., 2005. The colonization of Europe by the freshwater crustacean *Asellus aquaticus* (Crustacea: Isopoda) proceeded from ancient refugia and was directed by habitat connectivity. *Molecular Ecology,* Volume 14, pp. 4355-4369.

Verrell, P., 1985. Predation and the Evolution of Precopula in the Isopod *Asellus aquaticus*. *Behaviour,* 95(3-4), pp. 198-202.

Via, S. & Lande, R., 1985. GENOTYPE-ENVIRONMENT INTERACTION AND THE EVOLUTION OF PHENOTYPIC PLASTICITY. *Evolution,* 39(3), pp. 505-522.

Vick, P. & Blum, M., 2010. The isopod *Asellus aquaticus*: A novel arthropod model organism to study evolution of segment identity and patterning. *Palaeodiversity,* pp. 89-97.

Vincze, E. et al., 2021. Corrigendum to "Conistency and plasticity of risk-taking behaviour towards humans at the nest in urban and forest gret tits, Parus major". *Animal Behaviour,* pp. 161-172.

Vitagliano, G. et al., 1991. Importance of longevity, growth and diapause in the evolution of *Asellus aquaticus*. *Bolletino di Zoologia,* 58(2), pp. 113-117.

Vogt, G., 2023. Evolution, Functions and Dynamics of Epigenetic Mechanisms in Animals. In: *Handbook of Epigenetics.* s.l.:Academic Press, pp. 521-549.

von Ende, C. N., 1979. Fish predation, interspecific predation, and the distribution of two Chaoborus species. *Ecology,* 60(1), pp. 119-128.

Wang, Y., Brune, A. & Zimmer, M., 2007. Bacterial symbionts in the hepatopancreas of isopods: Diversity and environmental transmission. *FEMS Microbiology Ecology,* 61(1), pp. 141-152.

Watt, P. J., Nottingham, S. F. & Young, S., 1997. Toad tadpole aggregation behaviour: evidence for a predator avoidance function. *Animal Behaviour,* Volume 54, pp. 865-872.

Wcislo, W. T., 2003. Behaviour Environments and Evolutionary Changes. *Annual Review of Ecology and Systematics,* 20(1), pp. 137-169.

Weiss, A. & King, J. E., 2006. Personality and Subjective Well-Being in Orangutans (Pongo pygmaeus and Pongo abelii). *Journal of Personality and Social Psychology,* pp. 501-511.

Westrick, S. E. et al., 2019. Rapid evolution and behavioural plasticity following introduction to an environment with reduced predation risk. *Ethology,* 125(11), pp. 1-9.

Wetzel, R. G., 2001. *Limnology: Lake and River Ecosystems.* Third edition ed. s.l.:Elsevier.

White, W. B., Culver, D. C. & Pipan, T., 2019. *Encyclopedia of Caves.* s.l.:Elsevier Inc..

Wilkens, H., Culver, D. C. & Humphreys, W. F., 2000. *Ecosystems of the World 30: Subterranean Ecosystems.* s.l.:Elsevier.

Williams, W. D., 1962. Some Remarks On Phenotypic Variation and Genetic Isolation in Asellus (Isopoda, Asellota). *Crustaceana,* 3(4), pp. 279-284.

Wilson, D. S. & Clarck, A. B., 1993. Shy-bold continuum in pumpkinseed sunfish (Lepomis gibbosus): An ecological study of a psychological trait. *Journal of Comparative Psychology,* pp. 250-260.

Wilson, D. S., Clarck, A. B., Coleman, K. & Dearstyne, T., 1994. Shyness and boldness in humans and other animals. *TREE (Elsevier).*

Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T., 1994. Shyness and Boldness in Humans and Other Animals. *Trends in Ecology & Evolution,* pp. 442-446.

Wilson, D. S., Coleman, K., Clark, A. B. & Biederman, L., 1993. Shy)old continuum in pumpkinseed sunfish (Lepomis gibbosus): An ecological study of a psychological trait. *Journal of Comparative Psychology,* pp. 250-260.

Wilson, G. D. F., 1991. Functional Morphology and Evolution of Isopod Genitalia`. In: *Crustacean Sexual Biology.* New York: Columbia University Press, pp. 229-245.

Wolff, C., 2009. The embryonic development of the malacostracan crustacean Porcellio scaber (Isopoda, Oniscidea). *Development Genes and Evolution,* 219(11-12), pp. 545-564.

Wood-Gush, D. G. M. & Westergaard, K., 1989. Exploratory Behaviour and the Welfare of Intensively Kept Animals. *Journal of Agricultural Ethics,* Volume 2, pp. 161-169.

Yoshizawa, M., Yamamoto, Y., O'Quin, K. E. & Jeffery, W. R., 2012. Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. *BMC Biology,* 10(1).

Zagmajster, M., Porter, M. & Fong, D., 2011. Freshwater hydrozoans in caves with report on new records. *Speleobiological Notes,* Volume 3, pp. 4-10.

Žák, K., Onac, B. P. & Perşoiu, A., 2008. Cryogenic carbonates in cave environments: A review. *Quaternary International,* 187(1), pp. 84-96.

Zidar, P. et al., 2018. Energy reserves in the water louse *Asellus aquaticus* (Isopoda, Crustacea) from surface and cave populations: seasonal and spatial dynamics. *Fundamental and Applied Limnology,* pp. 1-13.

Zimmer, M. & Bartholmé, S., 2003. Bacterial endosymbionts in *Asellus aquaticus* (Isopoda) and Gammarus pulex (Amphipoda) and their contribution to digestion. *Limnology and Oceanography,* 48(6), pp. 2208-2213.

Zimmer, M. et al., 2001. Hepatopancreatic endosymbionts in coastal isopods (Crustacea: Isopoda), and their contribution to digestion. *Marine Biology,* 138(5), pp. 955-963.

Zimmer, M., Geisler, S., Walter, S. & Brendelberger, H., 2002. Fluorescence in *Asellus aquaticus* (Isopoda: Asellota): a first approach. *Evolutionary Ecology Research,* Volume 4, pp. 181-187.

Zuur, A. et al., 2009. *Mixed Effect Models and Extensions in Ecology With R.* New York: Springer Science+Business Media.