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Resistance to flooding of different species of terrestrial oribatid mites (Acari, Oribatida)

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Abstract

Resistance to flooding was tested in six terrestrial oribatid mite species with different ecological needs. The silvicolous *Galumna lanceata* and *Oppiella* (*Oppiella*) *marginedentata* showed low average survival times of seven and 14 days, the moss-dwelling *Zygoribatula exilis* tolerated a month of submergence on average, the eurytopic *Eueremaeus oblongus* and *Tectocepheus velatus sarekensis* survived an average period of 52 and 80 days and the euryoecious *Plathynothrus peltifer* showed the highest tolerance with 130 days median lethal time. Accordingly, overflow tolerances are clearly species dependent, but may also be correlated with the ecological needs of each species. Most of the inferred survival times clearly exceed the periods of naturally occurring flooding in typical terrestrial temperate habitats. Therefore, terrestrial oribatid mites may show a basic predisposition for overflow tolerances.

We also performed the opposite experiment, i.e. we exposed the aquatic *Hydrozetes lemnae* permanently to atmospheric air. More than half of the individuals survived 130 days but all tested specimens completely reduced activity which indicates that *H. lemnae* initiates a state of dormancy when being outside of water, probably to withstand longer lasting droughts in nature.

Keywords plastron | larviparity | survival time | aquatic | fresh water

1. Introduction

The majority of oribatid mites inhabit arboreal or soil-litter environments and are typical air-breathing terrestrial animals. Most of these environments are subject to rainfall and thus to short periods of inundation, whereas heavy or seasonal rainfall often results in long-term submergence of these habitats. How oribatid mites cope with these unfavourable conditions is largely unknown, but the few existing experimental studies revealed remarkable survival abilities in certain cases. Schuster (1979) showed that typical terrestrial species, e.g. *Nothrus silvestris* Nicolet, 1855 and *Steganacarus magnus* Nicolet, 1855, could survive periods of 74 to 226 days submerged in freshwater and Franklin et al. (2001) demonstrated that several species from Amazonian floodplains, as for example *Rostrozetes foveolatus* Sellnick, 1925 or *Rostrozetes rimachensis* Beck, 1965, are even able to survive maximum periods of 107 to 684 days under water.

There are four possible mechanisms allowing oribatid mites to survive periods of inundation; (1) retreat into air-filled pockets in the substrate, (2) tolerance of anoxia, which means the animals completely cease activity and stay in dormancy during these extreme conditions, (3) effective cuticular respiration directly from the water and (4) plastron respiration, i.e. a layer of air is kept on the body surface supplying the tracheal system with oxygen (Zinkler 1999). The Amazonian *R. foveolatus*, for example, can survive ten days without any oxygen and hence is tolerant to anoxia (Messner et al. 1992). Effective cuticular respiration was proven in *S. magnus*, where the bases of the legs are supposed the primary region of oxygen uptake (Baumgärtl et al.

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1994a,b). Plastron respiration was mostly demonstrated in aquatic (Krantz & Baker 1982) and marine intertidal oribatid mites (e.g. Pugh et al. 1987, Pfingstl & Krisper 2014) where the presence of air-retaining structures is clearly correlated with the permanently or periodically submersed mode of life (Messner et al. 1992). In typical terrestrial mites, plastrons were rarely reported and in the few cases air-retaining structures were either weakly developed (Messner et al. 1992) or had a low resistance to hydrostatic pressure and were easily wettable (Pugh et al. 1987).

Regardless of what mechanism is used, it is assumable that terrestrial oribatid mites dwelling in habitats prone to frequent inundation will show higher flooding tolerances than mites living in dryer environments. Messner et al. (1992) provided first evidence for such a correlation by demonstrating that populations of *Rostrozetes ovulum* (Berlese, 1908) from Amazonian floodplains showed higher resistance to flooding than upland populations. Schuster (1979), on the other hand, investigated the inundation resistances of littoral versus terrestrial soil mites and detected that both groups are more or less equally resistant to fresh water, but terrestrial mites showed significant lower survival in seawater.

Flooding resistances were mainly investigated in tropical floodplain species or in coastal associated taxa, but studies on typical terrestrial species are lacking so far and the ability and mechanisms used to survive periods of flooding remain unknown for most of these.

To demonstrate overflow tolerances in further terrestrial mites, we tested six oribatid mite species; the silvicolous (=inhabiting woodlands and all of its habitats, including soil, litter and trees) *Galumna lanceata* (Oudemans, 1900) and *Oppiella* (*Oppiella*) *marginedentata* Strenzke, 1951 (Weigmann 2006), the epilithic and epiphytic moss-dwelling *Zygoribatula exilis* Nicolet, 1855 (Wunderle et al. 1990, Weigmann 2006), the eurytopic *Eueremaeus oblongus* (C.L. Koch, 1835) (Mihelčič 1963) and *Tectocepheus velatus sarekensis* Trägardh, 1910 (Weigmann 2006) and the euryoecious *Platynothrus peltifer* (C.L. Koch, 1839) (Weigmann 2006).

As overflow tolerances may be a basic predisposition of terrestrial mites that may have allowed some taxa to colonize aquatic habitats, some of these aquatic taxa may still show ancestral terrestrial traits and may in return be able to survive long periods under terrestrial conditions. To test this, we also performed the opposite experiment by exposing the aquatic *Hydrozetes lemnae* Coggi, 1899 to atmospheric air.

2. Material and methods

Sample locations: (1) Graz, Eustacchio Gründe, Austria, a) moss from a dead trunk lying on the ground and moss (growing at a height of about 1.60 m above the forest floor) from a living tree trunk, 3 Oct. 2017, *Zygoribatula exilis*, *Oppiella* (*Oppiella*) *marginedentata*, *Galumna lanceata*; b) dry moss (approx. 40 cm above ground) from a stonewall adjacent to street, 3. Oct. 2017, *Tectocepheus v. sarekensis*, *Eueremaeus oblongus*. (2) Preding, South Styria, Austria, plant material and soil from a flooded damp meadow, 8 Oct. 2017, *Platynothrus peltifer*, *Hydrozetes lemnae*, leg. J. Baumann.

Sample extraction: Living mites were extracted with a Berlese-Tullgren apparatus for approximately 24 hours (a standard light bulb 40W was used as heat source) and collected in plastic vessels supplied with moist plaster. Afterwards, they were transferred with a fine brush into the experimental vessels. As heat extraction causes stress and may affect the animals, only the most active individuals were selected for the experiments to reduce the risk of using already physically weakened individuals.

Experimental setup (survival of flooded terrestrial mites): Experiments started directly after the extraction in October 2017. In total, 115 specimens were used for these experiments and for each species 18 to 20 adult individuals were tested. Single individuals were put into small cylindrical translucent plastic vials (height 2.5 cm, diameter 2.5 cm) filled with approx. 5 ml fresh water (tap water) and were exposed to the circadian day-night (light-dark) cycle. The water was not aeriated specifically before used in the tests, but boxes were opened every day for several minutes allowing short aeriation. Vials were lined with filter paper to provide grip for the mites and were closed with a cap (a small space of air remained under the cap) to prevent external contamination of the water. Each vial was opened and checked on a daily base under the dissecting microscope (Leica Wild M8). In few cases, the boxes could only be checked every second or third day. In case that dead animals were recorded during these checks, time of death was assumed the day after the last check in order not to artificially stretch survival times. Inactive animals were stimulated with a fine brush and observed over a five minutes interval before they were finally recorded as dead. In most cases dead animals could be easily identified by widely opened anal plates and extruded spermato- or ovipositors.

Experimental setup (survival of aquatic mites in atmospheric air): In total 26 specimens of *H. lemnae* were tested and single individuals were transferred from fresh water into cylindrical plastic vials (same size and type as in all experiments) supplied with moistened plaster without any water. Each vial was checked in the same manner as the above-mentioned experiments, but one or two drops of tap water were added to the plaster with each check to keep vessels moisturized and to prevent complete desiccation.

Median lethal time (LT_{50}) was defined as the time in which half of the animals have died (e.g. LT_{50} = 9 days → after 9 days 10 of 20 individuals have died). If more than one individual had died within two days, LT_{50} was defined as the day on which more than half of the individuals have died.

All experiments were performed at room temperature (approx. 24°C) and were ceased after a 165 days period if not already finished.

No food was provided for the submerged animals to avoid any contamination of the water, but in a few cases animals were observed to feed on the filter paper in the vial (for details see results part).

Species were determined using the identification key provided by Weigmann (2006).

3. Results

3.1 Survival of terrestrial oribatid mites submerged with fresh water

Galumna lanceata - survived a period of five to 31 days and reached the LT_{50} value already on day seven (Fig. 1). At the beginning of the experiment individuals were often found drifting on the water surface but were put back on the ground with a fine brush. All flooded individuals reduced activity completely, meaning they stopped walking and moving and only reacted when physically touched.

Oppiella (Oppiella) marginedentata - The first animals died on the third day and the last individual died on day 35. The median lethal time was reached on day 14. Activity was considerably reduced, they did not move most of the time and if they were observed moving they walked very slowly and ponderously.

Zygoribatula exilis - the first individuals of this species died on day 6 and the median lethal time was reached on day 34. The last two individuals further survived from day 47 to day 66 and therefore stretched the curve (Fig. 1). Activity was reduced underwater in the same way as in *O.* (*O.*) *marginedentata*. During the first two weeks individuals were sometimes found drifting on the surface of the water.

Tectocepheus v. sarekensis - the first individuals died on day 38. The species showed a LT_{50} value of 80 days and the last individual survived 120 days. Activity was reduced to a certain extent but not completely ceased, animals either sat quietly on the substrate or moved around normally.

Eueremaeus oblongus - minimum survival of this species was five days, maximum survival 129 days, median lethal time was reached after 52 days (Fig. 1). Activity was also slightly reduced, most of the animals walked around ponderously throughout the experiments.

Platynothrus peltifer - it took 44 days until the first animal died. This species showed the highest LT_{50} value

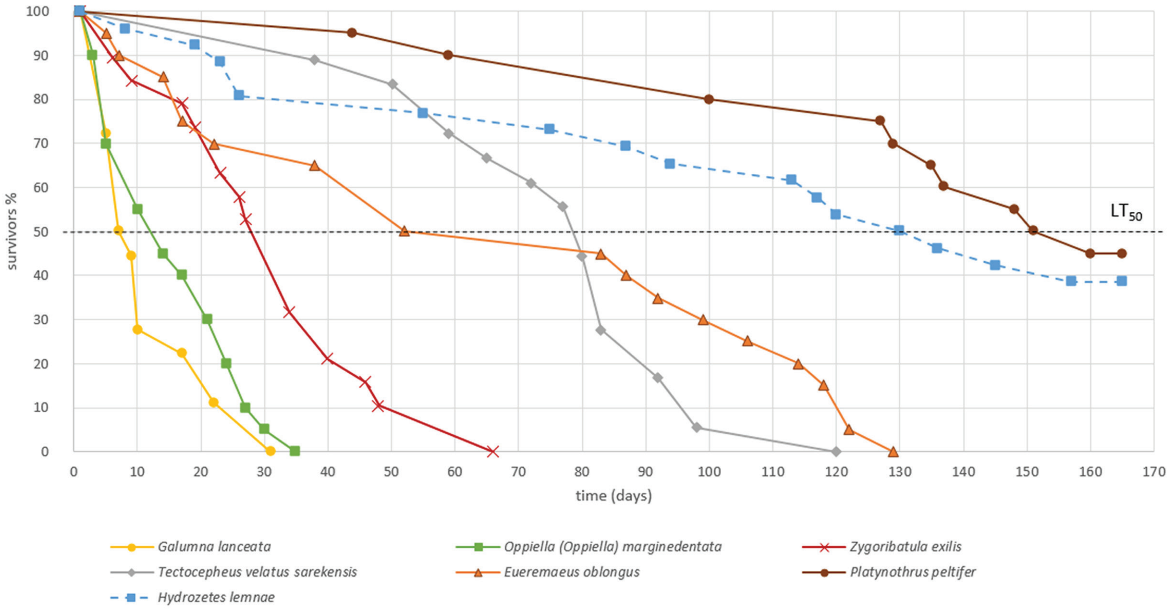

Figure 1. Survival times (LT_{50}= median lethal time) for six terrestrial oribatid mites flooded with freshwater. *Hydrozetes lemnae* (dashed line) was kept in air to perform the opposite experiment.

with 151 days. Nine of the 20 individuals even survived the investigation period of 165 days (Fig. 1). Animals were active, moving around and feeding on the filter paper. We were also able to observe parthenogenetic larviparous reproduction of *P. peltifer* under water as two juveniles were suddenly appearing after thirteen days in the experimental vessels and they survived 16 and 108 days. Additionally, we found two larvae in a mother's body in a microscopic slide.

3.2 Survival of aquatic oribatid mites kept in atmospheric air

Hydrozetes lemnae - the first individual died on the sixth day and the LT_{50} value was reached at 130 days. Ten of the 26 animals survived the investigation period of 165 days (Fig. 1).

When transferred to air the individuals nearly stopped their activity completely and only moved when they were touched with a fine brush. After 165 days, when the study was ceased, the surviving individuals were put back into fresh water, where they started to move and walk within only a few minutes.

4. Discussion

Resistances to inundation clearly vary between the tested species and this variation may indicate a correlation between overflow tolerance and the ecological requirements of each species. The two silvicolous species, *Galumna lanceata* and *Oppiella* (*O.*) *marginedentata* showed lowest median lethal times with seven and 14 days, their maximum survival was approximately a month. Both species usually occur in temperate coniferous or deciduous forests (e.g. Weigmann 2006), these habitats may be very moist but are seldom subject to complete flooding. Although *G. lanceata* was also reported from riparian forests, it was clearly classified as xerophilous species (Mihelčič 1967), which is well in accordance with its low survival in water. Franklin et al. (2001) tested an undetermined *Galumna* species and detected maximum survival of only six to seven days, which is similar to the present results. However, there are other *Galumna* species that may very well show high resistances to flooding; *Galumna elimata* (C.L. Koch, 1941) was demonstrated to possess a cerotegumental plastron structure underneath the pteromorphs (Alberti et al. 1981), *Galumna alata* (Hermann, 1804) was found submerged under stones in Antarctic and sub-Antarctic regions (Pugh & Dartnall 1994), *Galumna dimorpha* Krivoluckaja, 1952 is mostly occurring in floodplains (Weigmann 2006) and *Galumna obvia* (Berlese, 1915) was characterized as heliophilic and hygrophilic species inhabiting wet forest soils (Brandstetter & Krisper 2011). These examples indicate that overflow tolerance in *Galumna* is rather correlated with ecology than with systematic relationship.

Zygoribatula exilis occurs in dry epiphytic habitats (e.g. Weigmann & Kratz 1981) similar to *G. lanceata* and *O. (O.) marginedentata.* Therefore, it may be surprising that it survived inundation twice as long. However, in contrast to the latter species, which mostly occur in the litter on the forest floor, *Z. exilis* is virtually absent from this microhabitat and can mainly be found in mosses at trunk bases and on tree stumps (Wunderle et al. 1990). In this habitat phytotelmata may be formed after rainfall and persist over longer periods. Therefore, an elevated resistance to flooding may be advantageous for *Z. exilis*.

Tectocepheus v. sarekensis and *Eueremaeus oblongus* showed both remarkable long survival with more than 50 days median lethal time and more than four months maximum survival. *Tectocepheus v. sarekensis* is known to occur in a wide range of dry and wet habitats (e.g. Weigmann 2006) and the same applies to *E. oblongus*. The latter was reported to dwell in mosses on diverse substrates, e.g. rocks, trees and stumps, whereas it can occur in open landscapes (Mihelčič 1963) and in forests (Weigmann 2006). Both species are hence eurytopic and the high resistance to flooding may be one of the key abilities allowing them to colonize such a vast range of habitats.

Platynothrus peltifer showed by far the highest overflow tolerance with more than four months median lethal time. This species is euryoecious and hygrophilous as it may occur in wet forest (Franz 1954, Weigmann 2006), peatlands, springs and creeks (Schatz & Gerecke 1996). Schatz & Gerecke (1996) even suggested this species to be a constant part of submerged mite biocenoses. Therefore, the high overflow tolerance found in *P. peltifer* is supposedly correlated with its semiaquatic lifestyle. Schuster (1979) also tested individuals of *P. peltifer* from the North Sea coast and from inland and both showed nearly equal median survival times with 68 and 74 days. Though these times are high and confirm the elevated overflow tolerance of this species, the present study, with comparable experimental conditions, revealed significantly higher resistances for the same taxon. Messner et al. (1992) demonstrated similar intraspecific differences for *R. ovulum*, with floodplain populations showing higher tolerances than upland populations, and the same may apply to different populations of *P. peltifer*.

Apart from a possible ecological correlation, the question arises which mechanisms are used by the species to survive flooding. We were not able to observe obvious plastrons in any of the investigated species, but all of them reduced or ceased activity completely during submersion, except for *P. peltifer*. The latter species moved around, fed on the filter paper and even reproduced under water which indicates that either plastron or effective cuticular respiration is used. In *Galumna* cerotegument structures are only weakly developed (Messner et al. 1992) and hence probably not suitable for plastron respiration. Therefore, it is assumable that tolerance to anoxia allowed *G. lanceata* to survive seven days on average. The obviously longer survival times of all other species may be a result of a combination of resistance to anoxia and plastron or cuticular respiration, but this is presently just conjecture. Further comprehensive morphological and physiological studies are necessary to answer this question in detail.

When flooding events happen in the field, mites are surely not always forced to tolerate unfavourable conditions, they may also have the chance to evade inundation. Smrž (1996) stated that flooding selects two ethologigal groups, the ‘divers’ and the ‘swimmers’. The divers sink immediately after contact with water and exhibit mechanisms to survive long periods in these conditions, whereas the swimmers show hydrophobic surfaces which allow them to escape flooding by swimming or gliding over the water’s surface until they attain firm substrate again. *Platynothrus peltifer* was classified as diver and *G. lanceata* as swimmer (Smrž 1996) and these classifications clearly coincide with our observations. In the present study *Z. exilis* was also found several times drifting on the water’s surface and hence may also belong to the swimmers. As a possible swimmer *Z. exilis* is able to avoid inundation, but even in the case individuals are accidently captured under water they are able to survive 34 days on average under these unfavourable conditions. The activity of these submerged specimens is reduced, therefore the submersion tolerance of *Z. exilis* can be classified as predisposition well supported by the passive behaviour during submergence.

The present study also showed that overflow tolerances of terrestrial mites are basically remarkably high compared to survival times of littoral mites (Schuster 1979, Pfingstl 2013) and mites of typical floodplains (Franklin et al. 2001). Littoral mites are submerged only for a few hours but on a daily base and mites from Amazonian floodplains are inundated seasonally for several months, therefore resistance to flooding is clearly an adaptive trait for these taxa. Typical terrestrial habitats, on the other hand, are flooded sporadically and only for short periods and these are clearly exceeded by the presently inferred survival times. Therefore, terrestrial mites may show a basic predisposition for overflow tolerances, meaning they already possess this ability without having adapted to flooding events.

Compared with other soil invertebrates, oribatid mites show relatively high overflow tolerances; earthworms can survive from 40 to 270 days submerged, isopods approx. 72 days, diplopods 22 days and dipteran larvae show the lowest survival in water with only a few days (Plum 2005). However, this comparison is only based on very few experimental studies and further investigations are necessary in the future to get comprehensive insights into overflow tolerances of soil arthropods in general.

The opposite experiment with the aquatic *Hydrozetes lemnae* in atmospheric air also showed interesting results. *Hydrozetes* is classified as truly aquatic (Behan-Pelletier & Eamer 2007) but is known to use elaborate plastron respiration (Krantz & Baker 1982, Pugh et al. 1987, Messner et al. 1992) (see also Fig. 2). Being out of the water should not be a problem for *Hydrozetes* in terms of respiration, as it always breathes air and this is corroborated by our results showing that *H. lemnae* can easily survive periods of 130 days on average. *Hydrozetes* has been reported from several meters below the waterline to several centimetres above (Buford 1976) suggesting that the water as well as the area above

Figure 2. Photograph (stacked stereomicroscopic images) of a submerged *Hydrozetes lemnae* adult showing the obvious layer of air kept on the body surface (plastron, seen as silvery shimmer).

are used as habitat. Nevertheless, all tested specimens of *H. lemnae* ceased activity nearly completely when exposed to atmospheric air indicating that this is an unfavourable environment. Our results thus indicate that adjacent terrestrial environments are only used to a limited extent and that the animals most likely spend their time in atmospheric air by reducing their activity to withstand periods of drought. Additionally, this ability to survive long periods under terrestrial conditions, though with reduced activity, may represent an ancestral trait.

4.1 Conclusions

Typical terrestrial mites are able to survive periods of inundation for several days up to several months. Resistances to flooding differ between the species and hence are species dependent. There may also be a correlation with the ecological needs of each species, but further experiments are necessary to prove this in detail. Overflow tolerances may be a basic predisposition of typical terrestrial mites that has allowed the distribution into semiaquatic habitats as for example floodplains or the marine littoral.

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