Hypoxic coma as a strategy to survive inundation in a salt-marsh inhabiting spider

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Spiders constitute a major arthropod group in regularly inundated habitats. Some species survive a flooding period under water. We compared survival during both submersion and a recovery period after submersion, in three stenotopic lycosids: two salt-marsh species *Arctosa fulvolineata* and *Pardosa purbeckensis*, and a forest spider *Pardosa lugubris*. Both activity and survival rates were determined under controlled laboratory conditions by individually surveying 120 females kept submerged in sea water. We found significant differences between the three species, with the two salt-marsh spiders exhibiting higher survival abilities. To our knowledge, this study reports for the first time the existence of a hypoxic coma caused by submersion, which is most pronounced in *A. fulvolineata*, the salt-marsh spider known to overcome tidal inundation under water. Its ability to fall into that coma can therefore be considered a physiological adaptation to its regularly inundated habitat.

Keywords: survival; salt marsh; stress; interspecific comparison; anoxia

1. INTRODUCTION

The occurrence of terrestrial arthropods in periodically flooded habitats suggests that these species have evolved specific adaptations to cope with adverse habitat conditions. Behavioural, morphological and physiological adaptations have indeed been found, explaining the partial or complete persistence of arthropod species in submerged habitats (Plum 2005). Even if spiders constitute a major group of arthropods in regularly flooded habitats (e.g. floodplains: Rothenbücher & Schaefer 2006, salt marshes: Pétilion *et al.* 2008), to date few studies have examined their survival strategies under water. In spiders, risk-avoiding strategies are commonly found and result either in horizontal migration (long-term immigration and emigration in seasonally flooded habitats) or in vertical migration (active climbing on vegetation: Adis & Junk 2002). In addition to aerial refuges, spider species may use large airspace under a shell or

in an underwater web system as underground refuges and could also derive benefit from the air in the interstices of large soil pores to breathe (Foster & Treherne 1976; see also the possibility of ‘physical gill’ created by nests: Rovner 1987). However, oxygen availability quickly decreases in the last case, even though Hebets & Chapman (2000) found that the non-tracheate *Phrymus marginemaculatus* (Arachnida: Amblypygi) used dissolved oxygen from the surrounding water through cuticular modifications for plastron respiration.

Finally, a reduction in oxygen consumption by lowering metabolic rates may help in limiting the associated anoxia, as was suggested for both larval and adult stages in some insects (Hoback & Stanley 2001). A shift to an anaerobic metabolism as soon as the spiders are submerged may also be hypothesized, but it leads to the accumulation of major end products that can be toxic for organisms (Hochachka & Somero 2002).

In the present study, we determined whether spiders were able to withstand submersion, i.e. whether they developed physiological adaptations to endure flooding periods. To address this question, we compared survival both during and after flooding in three wolf-spider species (Araneae: Lycosidae): two salt-marsh species *Arctosa fulvolineata* and *Pardosa purbeckensis* and one forest species *Pardosa lugubris*. We hypothesized that the forest species would exhibit no particular ability to survive flooding, because individuals of *P. lugubris* never endure such conditions in their native habitat. As we found that spiders could survive submersion by falling into a non-reactive, but non-dormant, state (coma), we then tested the hypothesis that such a trait would be found mainly in species regularly subjected to flooding by comparing its occurrence between species varying in auto-ecology.

2. MATERIAL AND METHODS

Experiments were carried out during May 2007, using hand-collected individuals in the field: adults of *P. lugubris* were sampled in a deciduous forest (Paimpont, France, 48°1'0' N, 2°10'60' W) and those of *A. fulvolineata* and *P. purbeckensis* (Araneae: Lycosidae) in an intertidal salt marsh (Mont St Michel, France, 48°40' N, 1°40' W). The spiders were then kept for 1 day under controlled conditions at a constant $20 \pm 1^\circ\text{C}$ before being used for the experiments.

Tolerance to total submersion was determined by individually placing 120 females of each species, *A. fulvolineata*, *P. lugubris* and *P. purbeckensis*, in sealed plastic vials (6 cm diameter, 7 cm depth), without food. Vials were filled with salted water (33‰) and placed under controlled conditions (15 L : 9 D, temperature: $20 \pm 1^\circ\text{C}$). A circular mesh was placed 2 cm under the water level to avoid spiders breathing at the surface.

Every 2 h until the death of the individuals, the behaviour of all the spiders was monitored, and they were classified either as reactive or non-reactive. They were considered non-reactive if they were unresponsive (no leg movement) to mechanical stimulation with brushes.

The survival of the spiders was checked every 4 h as follows: 10 individuals of each species were randomly removed from the water. Just after removal, the reactivity of the spiders was assessed, and the animals were classified as being either reactive (responsive to mechanical stimulation: alive individuals) or non-reactive (unresponsive to mechanical stimulation: coma or dead individuals). To determine whether the non-reactive spiders were in a coma or dead, females were placed on a dry substrate and reassessed for reactivity after an 8 h recovery period (8 h was defined as the time needed for individuals that went into coma to recover mobility once the stress was stopped; Castañeda *et al.* 2005). After that period, spiders in a coma recovered and were again responsive, whereas dead animals remained unresponsive (non-reactive). Thirty

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Figure 1. Per cent of reactive individuals according to the duration of submersion in the three wolf-spider species. Diamonds, *P. lugubris*; squares, *P. purbeckensis*; triangles, *A. fulvolineata*.

voucher specimens were reassessed 24 h after they were removed from the water (none of them had recovered at that time).

Time–mortality regression equations and hours to Lt_{50} and Lt_{90} , along with both the 95 and 99 per cent fiducial limits, were calculated by probit analysis using MINITAB Statistical Software Release 13, (MINITAB Inc., State College, PA, USA). All data are given as means $\pm$ s.e.

3. RESULTS

Differences in submersion tolerance were observed among the three species. When submerged, most individuals of *P. lugubris* quickly became non-reactive, as shown by the very short duration of the first phase of the sigmoid curve (from 0 to 4 h), and the following abrupt decrease in the number of reactive spiders during the second phase (from 4 to 12 h) (figure 1). After 24 h of submersion, almost all *P. lugubris* were non-reactive. In *A. fulvolineata*, a sigmoid relationship was also found between reactivity and duration of submersion, but the curve was more extended. Most of the spiders were still reactive after a 16 h flooding period, and we had to wait approximately 36 h before finding most of them to be non-reactive. *Pardosa purbeckensis* exhibited a response intermediate between *P. lugubris* and *A. fulvolineata* (figure 1).

For the first survival analysis, we considered only the reactive spiders. Again, the highest differences were found between *P. lugubris* and *A. fulvolineata* (figure 2), with *A. fulvolineata* exhibiting significantly higher Lt_{50} and Lt_{90} than *P. lugubris* (probit analysis, $p < 0.05$). Both the Lt_{50} and the Lt_{90} of *P. purbeckensis* were significantly higher than those of *P. lugubris* ($p < 0.05$). Even if a trend can be observed, no difference occurred in the survival ability between the two salt-marsh species *P. purbeckensis* and *A. fulvolineata* ($p > 0.05$). In *A. fulvolineata*, 100 per cent mortality occurred after 36 h, whereas it occurred after 28 h in *P. purbeckensis* and after 24 h in *P. lugubris*.

In a subsequent step, non-reactive spiders that recovered after removal (defined as the individuals being in a coma) were included in the survival analysis. When spiders in a coma were included, the most important changes occurred for *A. fulvolineata*, for

which both Lt_{50} and Lt_{90} strongly increased: from 16.81 ± 1.03 to 29.56 ± 1.58 h for Lt_{50} and from 26.58 ± 2.73 to 42.27 ± 2.84 h for Lt_{90} . It also resulted in a significantly higher survival compared with *P. purbeckensis* (probit analysis; figure 2). Only slight and non-significant changes were found for *P. purbeckensis* and *P. lugubris*, suggesting that females of both *P. purbeckensis* and *P. lugubris* either (i) did not fall into a coma during submersion but died directly or (ii) fell into a coma, but then died very quickly when the duration of submersion was prolonged. In *A. fulvolineata*, mean recovery time from coma was 2.11 ± 0.31 h ($n = 27$), with a maximum value of 7 h. The ratio coma/(coma + reactive) was maximum after 20 h of submersion (70% of the spiders in coma).

4. DISCUSSION

As expected, the highest survival ability was found in *A. fulvolineata*, defined as a resident/permanent species in salt marshes. Regarding our data, this territorial species must be considered a non-migratory species: most of the tested females were able to endure a submersion period of approximately 16 h, and remained reactive during that time, i.e. a period significantly longer than the duration they have to cope with during tides. Moreover, we demonstrated that this species can endure prolonged submersion exposures by entering a non-reactive, but non-dormant, state: coma. Reactivity levels mainly depended on the availability of sufficient oxygen reserves (the decrease in spider air storage along time has been shown in figure S1 in the electronic supplementary material) and on the opportunity the species had to use dissolved oxygen from the water. As females of *A. fulvolineata* became progressively less reactive, and then non-reactive as the duration of submersion was prolonged, it is highly probable that they were not able to gain oxygen from the water, neither with nor without a well-developed plastron, as demonstrated in some other arachnid species. Even if the reduced reactivity helps in conserving oxygen

Figure 2. Survival (mean $\pm$ s.e.) (a) Lt_{50} and (b) Lt_{90} of the three wolf-spider species. White bars: Lt_{50} and Lt_{90} only include reactive spiders, and letters a and b indicate statistical differences between species ($p < 0.05$). Black bars: Lt_{50} and Lt_{90} include both reactive spiders and spiders in coma, and letters from x to z indicate statistical differences between species ($p < 0.05$). Statistical comparisons between the two survival analyses (reactive spiders versus spiders in coma + reactive ones) are indicated by an asterisk ($p < 0.05$) and a double asterisk ($p < 0.01$).

stores, mean recovery time from coma, approximately 2 h, argues for a progressive accumulation of injuries. Oxygen availability represents a key parameter and PO_2 should thus be investigated further, in both water and spider haemolymph.

Coma has been previously defined in cold-exposed insect species (e.g. Renault *et al.* 1999). In such studies, this narcosis state can more often be found as chill-coma and precedes the insect's death. The animal maintains a low rate of metabolism, and neuromuscular failure was regularly suggested as one of the main reasons for the onset of chill-coma (Pörtner 2001). In our study, the quasi-absence of coma and the rapid death of *P. lugubris* may result from the inability of this species to use anaerobiosis. We suggest that *P. lugubris* does not tolerate the potential osmotic and pH effects resulting from the accumulation of anaerobic end products.

More than 25 females of *A. fulvolineata* fell into—and recovered from—coma, whereas very few *P. purbeckensis* and no *P. lugubris* exhibited that behaviour. Such a statement partly matches with their environments: *P. lugubris* is a forest-restricted species, whereas *A. fulvolineata* and *P. purbeckensis* are salt-marsh-restricted species (Roberts 1995). Thus, differences in submersion tolerance between these two species cannot be attributed to differences in habitat and micro-habitat distribution (both species occur together; Pétilion *et al.* 2008) nor to their phylogeny (both belong to the subfamily of

Lycosinae: R. Bosmans 2008, personal communication). In field conditions, when the water level rises, *P. purbeckensis* tends to avoid flooding by climbing upon the vegetation, whereas *A. fulvolineata* mostly withstands submersion (J. Pétilion & K. Lamberts 2008, unpublished data). The ability of *A. fulvolineata* to fall into a coma can, therefore, be considered as a physiological adaptation to its regularly inundated habitat, especially when bad soil drainage leads to continuous salt-marsh immersion between two spring tides (i.e. >18 h).

To conclude, we therefore recommend checking for arthropod activity during subsequent recovery when assessing submersion tolerance under controlled conditions, especially when species from frequently flooded habitats are studied. Further studies should investigate whether the differences we found between the species result from (i) their distinct abilities to reduce aerobic metabolic activities, (ii) the amount of oxygen the spiders can store, and (iii) the progressive shift from aerobiosis to anaerobiosis. As the ability to endure anaerobiosis is a time-limited situation, species likely to fall into coma, like *A. fulvolineata*, would then be characterized by higher carbohydrate stores and by a higher tolerance to the accumulation of toxic anaerobic end products such as lactate (Prestwich 1983) when compared with the two other spider species.

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