Tardigrade Anhydrobiosis: Sleeping Beauty or Rip van Winkle?

Salma Abdel-Raheem, College of William & Mary

Hengherr, S., Brümmer, F., and Schill, R.O. 2008. Anhydrobiosis in tardigrades and its effects on longevity traits. *Journal of Zoology*. 275:216-220.

Several phyla of microscopic invertebrate metazoans are known to live in habitats that are prone to drought. As a consequence, taxa such as nematodes, rotifers, and tardigrades possess an impressively high tolerance to harsh environmental conditions. In particular, these animals are capable of entering into an anhydrobiotic state, where the animal undergoes a period of reversible desiccation by suppressing metabolic activity and resumes normal processes only when conditions allow for rehydration. Interestingly, the aforementioned taxa possess this capability in all stages of life. This unique capability to withstand extreme water loss has led researchers to question the effects such an adaptation has on the life cycle and longevity of these animals. Three models have been proposed to explain the effects of anhydrobiosis on the longevity of these animals:

1. The animal does not register time spent in the anhydrobiotic state due to a complete halt of the internal clock. It enters a state of hibernation in which it does not age. You can think of this hypothesis as being similar to the story of Sleeping Beauty: the beautiful [tardigrade] princess sleeps peacefully until her Prince Charming awakens her with a [rehydrating] kiss.
2. The animal partially registers time spent in the anhydrobiotic state, and the internal clock of the animal slows down. The animal enters a state of hibernation in which it ages, but at a much slower rate than if it were active.
3. The animal completely registers the time spent in the anhydrobiotic state, and the internal clock does not slow down. It enters a state of hibernation in which it ages normally, as if it were still active. This is in contrast to the story of Sleeping Beauty, and can instead be thought of as the story of Rip van Winkle. He went off to the mountains and napped for much longer than he had thought (20 years), and woke up to find himself a very old man[/tardigrade].

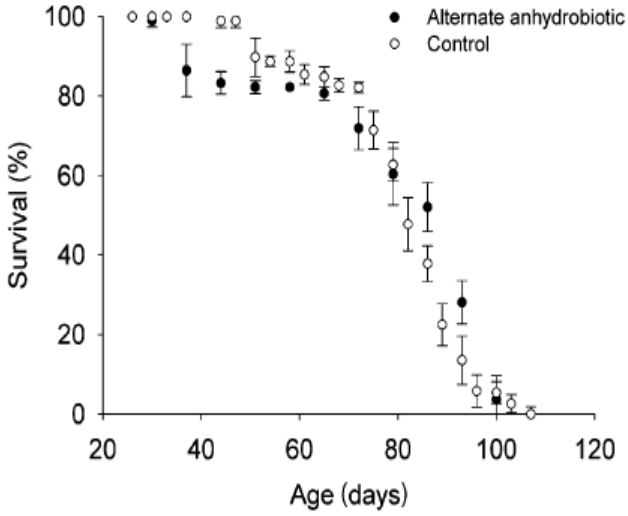
It has been shown that certain rotifer species exhibit the “Sleeping Beauty” model, while one species of nematode is known to exhibit the “Rip van Winkle” model.

The effect of desiccation on longevity in the tardigrade species, *Milnesium tardigradum,* was studied by Hengherr *et al.* (2008). Collected animals were maintained and followed until eggs were laid and collected. Eggs were then maintained until hatching and cultured for experimentation. Newly hatched animals were maintained for 26 days and split into 12 cohorts of 30 individuals each. Six cohorts were isolated and air-dried in test tubes for 7 days; the remaining six cohorts were kept in the hydrated state in agarose petri dishes for control purposes. Dehydrated animals were rehydrated via the addition of 20 microliters of water and were observed for up to two hours. Most animals resumed motility 20 to 30 minutes after the addition of water and were assumed dead if movement was not observed in 2 hours after the addition of water. Dead animals were removed, while rehydrated animals that survived were left to feed for seven days before they were dehydrated once again. This cycle of rehydration/dehydration continued until all animals died. Because the animals used in this study were collected as eggs and maintained, the lifespan for all animals was known with certainty.

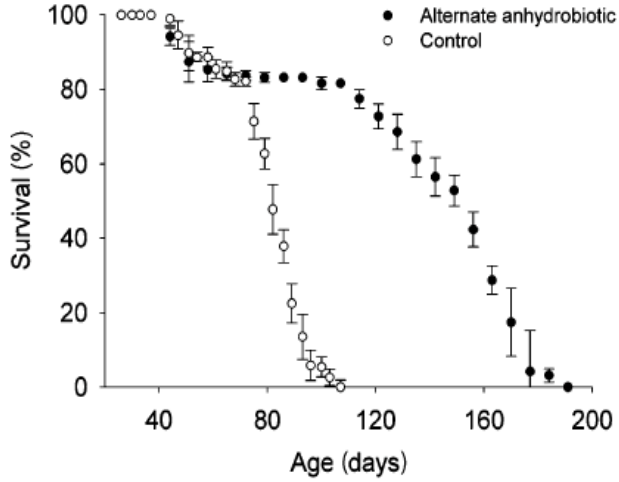
Results were analyzed using a repeated measure ANOVA (a statistical analysis used to measure differences between the means of multiple experimental treatments measured on multiple occasions). Longevity of the control cohorts was not significantly different from the cohorts subjected to rehydration/dehydration cycles (Figure 1a). Both treatments had animals that reached a maximum longevity of 107 days. However, these data deduct the time spent in the desiccated state by the experimental group. When time spent in the anhydrobiotic state was included in the statistical analysis, it was found that there was a significant difference between the cohorts [Figure 1b]. Animals that entered the anhydrobiotic state were documented to have lived for a maximum of 191 days, an increase of 78 percent! Observations on fecundity, reproduction, and normal physiological processes, such as feeding rate, were made and the experimental cohorts did not differ from the control cohorts.

This study beautifully demonstrated the effect, or lack thereof, of anhydrobiosis in a moss-dwelling tardigrade. Like some rotifers, this species of tardigrade exhibits the "Sleeping Beauty" model, where the biological clock of desiccated animals halted during the anhydrobiotic state. Future research on other rotifer or tardigrade species, possibly those that do not experience frequent bouts of drought, would provide an interesting picture of the responses to and the effects of decreased water availability on the life processes of this taxon. Future studies should look into testing the effects of anhydrobiosis on various life history traits, e.g. developmental time, fecundity, rate of reproduction, age to sexual maturity, and egg size, in order to further explore the possible effects of anhydrobiosis on the life cycle of tardigrades.

Other studies on this species have reported drastically different life spans in response to a dehydrated environment. However, animals used in this study and past studies were collected from different field sites and represented different populations. Therefore, it would be interesting to look further into possible variation in this trait within and across tardigrade species. Previous studies have also documented that some tardigrades can survive desiccation for a period of no more than 10 years. While it is unlikely that a habitat would remain dry for that length of time, why would there be an upper limit on tardigrade survival if the animal does not register time spent in the anhydrobiotic state? How can this "upper limit" hypothesis be tested?



a)



b)

Figure 1: a) Survival rate of control and alternating anhydrobiotic tardigrades controlling for time spent in the anhydrobiotic state. b) Survival rate of control and alternating anhydrobiotic tardigrades including the time spent in the anhydrobiotic state Adapted from Hengherr *et al.,* 2008.