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Facts and fiction about long-term survival in tardigrades

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Abstract

Tardigrades have achieved a widespread reputation for an ability to survive more than a century in an inactive, ametabolic state called cryptobiosis. However, a closer look at the empirical evidence provides little support for the claim that tardigrades are capable of century-long survival. Instead, current evidence suggests that a decade may more realistically represent the upper limit of cryptobiotic survival in the most resistant tardigrades.

Key words: Cryptobiosis, anhydrobiosis, tardigrades, long-term survival, ametabolic state

Some organisms more than others challenge our traditional concepts of life. Tardigrades definitely belong to this group. Many semi-aquatic (or semi-terrestrial) species of this taxon and the tidal genus *Echiniscoides* are able to survive total desiccation in a state of completely arrested metabolism called cryptobiosis (Cuènot, 1932; May, 1948; Keilin, 1959; Wright, Westh & Ramløv, 1992). Cryptobiosis induced by desiccation is called anhydrobiosis (Keilin, 1959), but the cryptobiotic state may also be induced by freezing (cryobiosis), osmotic pressure (osmobiosis) and anoxia (anoxybiosis). When exposed to desiccation, tardigrades contract into a so-called tun, and the permeability of the cuticle declines (Crowe, 1972; Wright, 1989). This reduces the rate of transpiration and gives the animal time for synthesis of tissue protectants, e.g. trehalose (Crowe, Crowe & Chapman, 1984; Westh & Ramløv, 1991). According to the main hypothesis, these protectants replace the bound water in membranes and allow structural cell integrity to be maintained in completely dry organisms (Clegg, 1986; Crowe *et al.*, 1997).

Anhydrobiosis allows tardigrades and some other micro-metazoans (e.g. nematodes, rotifers and crustaceans), protozoans and plants, to persist in environments excluded to other organisms. Apart from a protection against desiccation and freezing under natural conditions, anhydrobiosis also allows a resistance against unnatural abiotic extremes. These include temperatures near absolute zero and those well above 100 °C, and very high levels of ionizing radiation and hydrostatic pressure (Rahm, 1923; Becquerel, 1950; May, Maria & Guimard, 1964; Seki & Toyoshima, 1998).

Such findings have astonished biologists ever since the first observations on cryptobiotic rotifers, nematodes and tardigrades, respectively, more than 200 years ago (Leeuwenhoek, 1702; Needham, 1743; Spallanzani, 1776). However, excitement over the remarkable achievements of tardigrades seems to have stimulated exaggerations, with the result that fiction can sometimes replace the facts about the 'toughest animal on the Earth' (Copley, 1999). For instance, some authors have speculated that tardigrades may survive the space environment ('*les Tardigrades sont un matériel de choix pour les études biologiques concernant les espaces interplanetaires.*', May *et al.*, 1964: 365), and be transported around the world by wind in the stratosphere (Copley, 1999). Both claims may be true, but to our knowledge it has never been proven. In fact, we still have little direct evidence that tardigrades or other cryptobiotic metazoans disperse by wind, and the common assumption that cryptobiotic organisms readily disperse by wind has recently received some criticism (Jenkins & Underwood, 1998). In this note, we focus on one of the most frequent tales about tardigrades, that of century-long survival in cryptobiosis, and show a considerable discrepancy between the facts from the original document and subsequent reports.

The story that tardigrades may survive a century in a cryptobiotic state has been most frequent in popular science articles (e.g. Nelson, 1975; Copley, 1998, 1999), but has also been repeated in well-reputed books of invertebrate zoology (Brusca & Brusca, 1990) and senescence (Finch, 1990). This gives the impression of a well-documented phenomenon. However, we have found only one original paper on the subject, written by the

Italian biologist Tina Franchesci in 1948. Franceschi had obtained a sample of moss from an Italian museum. The sample dated back to 1828, and thus was 120 years old at the time of the investigation. Several tardigrades in the 'tun' stage were found in the material. When rehydrated, the tuns extended without any signs of life, but with one exception. Franchesci writes:

'*After twelve days from the beginning of the dampening, in the partly extended specimen (hardly identifiable to species but certainly to attribute to the order Eutardigrada and very likely to the genus* Macrobiotus*) quivers in several zones of its body were noted. In particular, in the front legs an extending movement followed by retraction was observed. In this case, it was not the usual process by which the tun, after hydration, changed into a near normal shape of the animal but without sign of life. Instead, there was a movement, which led us to conclude that an activity of life appeared, even if very slight. Afterwards, no movement was observed in the tardigrade even though for some days the internal organs were still recognisable.*' (p. 48, translation from Italian by the authors).

We would not deny that this observation is of great interest, and that it deserves further investigation. However, the above quotation is difficult to reconcile with statements claiming that after 120 years in the dry moss 'tardigrades were later found crawling all over it' (Copley, 1998). Quite clearly, the single animal that showed any signs of life did not revive successfully. Consequently, it provides no evidence for century-long survival in cryptobiosis. Current evidence rather suggests that the limit of cryptobiotic survival in tardigrades is within the range of a decade. Baumann (1927) revived tardigrades of the genus *Macrobiotus* after nearly 7 years, and Sømme & Meier (1995) reported a high rate of survival of tardigrades from the Antarctic after 8 years of storage at −22 °C. To our knowledge, these studies represent the longest known periods of cryptobiosis with successful revival in tardigrades. It is also worth noting that in some tardigrades (e.g. marine species, apart from the genus *Echiniscoides*) the ability to enter anhydrobiosis is unknown, while in some semi-terrestrial species revival from cryptobiosis is successful only after a relatively short time (1–2 months) in this state (R. Bertolani, pers. obs.). Information on cryptobiosis in limnic tardigrades is sparse. It is known that one eutardigrade species living in permanently wet peat (*Sphagnum*), *Murrayon hastatus* (Macrobiotinae, a limnic subfamily), may survive a beginning dehydration ('*qui peuvent subir un début de dessication à certain moments . . .*', May, 1951: 98). However, many limnic species (and some soil and moss species) of tardigrades have cysts, a not completely dehydrated state with thickened cuticle, quite different from the tun (Bertolani, 1982).

In nematodes, cryptobiotic life-spans of up to 39 years have been reported (see Fielding, 1951), but the problem of contamination from younger material may be unresolved in many of these studies. Many organisms have egg or larval stages that may remain in cryptobiosis for long periods. Perhaps the most well-known is the crustacean *Artemia salina* in which viable egg cysts were documented after 15 years in cryptobiosis (Clegg, 1967). The record for all organisms is held by seeds of the sacred lotus (*Nelumbo nucifera*), reported viable after more than 1000 years (Shen *et al.*, 1995).

Despite the current lack of evidence for successful revival from century-long cryptobiosis, tardigrades may be able to do so under limited and rather specialized conditions. Cryptobiotic survival in cysts of *Artemia salina* and in tardigrades seems to decline with time when stored under normal oxygen conditions, while in a vacuum such decline was not observed (Clegg, 1967; Crowe, 1975). This suggests that viable animals should be more likely to be found when stored under low levels of oxygen. We await with great interest future attempts to find such material. The possible impact on cryptobiotic survival of parasites such as fungi and bacteria remains to be investigated.

The ability of cryptobiotic organisms to survive much longer (and to be exposed to much more severe conditions) than needed under natural conditions appear to be of little ecological or evolutionary importance, and probably arose as a by-product of the adaptation to more relevant conditions. It is nevertheless of great interest from a biotechnological perspective. Animals and plants that are able to revive after several years in cryptobiosis have to possess a very efficient technology to protect their cells against environmental destruction such as oxidation and radiation. Knowledge about the biochemistry, physiology and genetics of this natural technology will be of considerable importance for developing methods of long-term preservation of biological material, e.g. in the food and medical industries. In addition, even if the ability of long-term cryptobiotic survival cannot be considered an adaptation, and has no survival value today, it still represents a pre-adaptation to conditions that may be realized in the future. Thus, the possibility of cryptobiotic survival over decades and centuries should not be dismissed as a biological curiosity. Rather, we need more scientific studies on this phenomenon.

The story about century-long cryptobiotic survival in tardigrades provides a good example of how some biological phenomena, especially those related to extreme life spans, easily give rise to sensational journalism in popular science. More importantly, the story shows that incorrect information, if spectacular enough, may persist for several decades when perpetuated also by the scientific community. In the present case, the fact that the original text was written in Italian may explain, but not excuse, the long life span of the story.

Tardigrades are fascinating animals, and cryptobiosis is indeed one of the most remarkable adaptations among living organisms. It deserves considerable attention, as we still understand relatively little of its general biology and biochemistry, and know practically nothing about its ecological and evolutionary implications. Therefore, although tardigrades may not be able to survive a century in cryptobiosis, they will certainly continue to provide biologists with exciting challenges in the future.

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