# CHAPTER 5-1
## TARDIGRADE SURVIVAL

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CHAPTER 5-1
TARDIGRADE SURVIVAL

Figure 1. *Dactylobiotus* sp. on the green alga *Spirogyra*. Photo by Yuuji Tsukii, with permission.

**Tardigrades – Water Bears**

Tardigrades (*tardus* = slow, *gradus* = step, or slow walkers), also known as water bears or moss piglets, are close relatives of the arthropods (Garey *et al.* 1996, 1999; Giribet *et al.* 1996).

Water bears resemble small bears (0.1-1 mm), complete with claws, but a few too many legs (4 pairs) (Figure 1). They are either armored (*Heterotardigrada*) or unarmored (*Eutardigrada*). The aquatic ones are usually a translucent white, whereas the terrestrial ones are often colored. Each of the eight legs has claws, which, when combined with their slow gait, makes them look very much like miniature polar bears with some extra legs. The very common *Macrobiotus hufelandi* (Figure 2) lumbers along at a maximum of 17.7 cm h⁻¹ (Ramazzotti & Maucci in Mach 2010). Tardigrades are just the right size to move among the bryophyte leaves, they lumber along slowly like bears, and they are downright cute!

Tardigrades, comprising about 900 species (Garey *et al.* 2008), can be found in marine, aquatic, and terrestrial habitats (Goeze 1773; May 1948; Greven 1980; Maucci 1986; Kinchin 1994). On land they frequently live in association with bryophytes (Figure 3; Figure 4) and lichens (Mihelčič 1967; Mehlen 1969; Utsugi 1984; Meininger *et al.* 1985; Mancardi 1988; Szymanska 1994; Bertolani & Rebecchi 1996; Tarter *et al.* 1996; Miller 1997; Jerez Jaimes *et al.* 2002; Boeckner *et al.* 2006; Bartels *et al.* 2009; Meyer & Hinton 2009; Rossi *et al.* 2009; Simmons *et al.* 2009). In water, algae, as well as bryophytes, provide homes.

Figure 2. *Macrobiotus hufelandi*, a common tardigrade that is among those inhabiting mosses. Photo by Paul Bartels, with permission.

These terrestrial tardigrades depend on the water drops that adhere to mosses and liverworts (Hingley 1993) and are therefore often termed *limnoterrestrial* (living in terrestrial habitats, but requiring a water film). Aquatic bryophytes can also house tardigrades (Hallas 1975; Kinchin 1987b, 1988; Steiner 1994a, b), as do the algae. However, of the ~1000 tardigrades reviewed by Guidetti
and Bertolani (2005) and Garey et al. (2008), only 62 were truly aquatic. The others depend on water associated with the interstitial spaces of terrestrial algae, lichens, bryophytes, soil, and leaf litter. Water bears are found in habitats from hot springs to layers under the ice (in cryoconite holes in glaciers) and occupy every continent of the world.

Figure 3. This tardigrade resided among the leaves of the moss *Hypopterygium arbuscula* (Figure 4). Photo courtesy of Filipe Osorio.

Figure 4. *Hypopterygium arbuscula*, a known bryophyte habitat for tardigrades in Chile. Photo by Juan Larrain through Creative Commons.

Most of the terrestrial tardigrades are bryophyte inhabitants (Nelson 1991a). These terrestrial bryophyte taxa have a life span ranging 3-4 months (Franceschi et al. 1962-1963), 3-7 months for *Macrobiotus hufelandi* (Figure 2; Morgan 1977), up to about 3 months for roof-moss-dwelling *Echiniscus testudo* (Figure 5; Morgan 1977), to about 2 years (Altiero & Rebecchi 2001) of active life (not counting dormant periods). The bryophyte-inhabiting taxa are more common in temperate and polar zones than in the tropics (Nelson 1991a). Some, as for example *Echiniscus testudo* (Figure 5), live almost exclusively on bryophytes (Corbet & Lan 1974).

Despite their cosmopolitan distribution (Romano 2003), broad habitat requirements, and relative visibility (compared to protozoa, for example), the tardigrades remain poorly known. As late as 1985, Hidalgo and Coombs reported that 16 states in the USA had no records of tardigrades. Species not previously described are easily discovered by those who know where to look for them.

The environmental conditions that affect tardigrades are likewise poorly studied (Guil et al. 2009), despite the extensive studies on a few species that have become travellers of the universe in space. Among those conditions that have been studied, altitudinal gradients and micro-environmental variables (including substrate choice among vegetation, bryophytes, and leaf litter) seem to have gotten the bulk of the attention (Guil et al. 2009). Guil and coworkers found a unimodal distribution relative to altitude for species richness, but it was closely tied to habitat variation. The best predictor of the distribution of tardigrades in the Sierra de Guadarrama Mountain Range, Spain, was bioclimatic classification. Soil, climate, vegetation structure, and leaf litter type worked both separately and in combination to determine species richness, explaining nearly 60% of the species richness in micro-scale plots. Abundance, on the other hand, was significantly influenced only by soil composition and leaf litter type. The macro-environmental gradients seemed to be unimportant in determining abundance (e.g. Figure 6).

Figure 5. *Echiniscus testudo* dormant stage (tun), demonstrating the rigid nature of its armor that prevents it from extensive changes in size. Photo by Power & Syred through Creative Commons.

Figure 6. *Echiniscus* species (*E. testudo* occurs almost exclusively on bryophytes) seem to be unresponsive to moisture changes. Photo by Martin Mach, with permission.

**Suitability of Bryophytes as Habitat**

The importance of bryophytes as a tardigrade habitat is evident by the number of publications on "moss" tardigrade fauna: Mihelčič 1967; Hallas 1975; Pilato & Sperlinga 1975; Morgan 1976; Bruegmann 1977; Morgan 1977; Maucci 1978, 1980; Bertolani 1983, 2001; Binda 1984;

Unfortunately, the authors rarely name the bryophytes from which their prizes were extracted. However, some evidence suggests that little specificity exists for bryophyte species, and lichens are as suitable as bryophytes, with no apparent differences in tardigrade species (Meyer & Hinton 2007). I have to wonder, however, why reports on tardigrades from liverworts are so scant (Figure 7).

Perhaps it is just as suggested to me by Łukasz Kaczmarek, that most zoologists do not understand the differences between mosses and liverworts. (Neither do my students when they begin looking at them.)

Figure 7. SEM view of tardigrades on the lower sides of leaves of a leafy liverwort. Photo by Łukasz Kaczmarek and Łukasz Michalczyk, with permission.

Ramazzotti and Maucci (1983) considered mosses suitable habitat based on three needs of the limnoterrestrial tardigrades:

1. a structure that allows sufficient oxygen diffusion  
2. the ability to undergo alternate periods of wetting and drying resulting from solar radiation and wind  
3. a medium that contains sufficient food.

Based on these criteria, bryophytes are particularly good habitats for tardigrades in several ways (Ramazzotti & Maucci 1983; Claps & Rossi 1984; Adkins & Nelson 1996). Their structure permits sufficient oxygen diffusion, both in aquatic and terrestrial habitats. Bryophytes experience drying, which most do slowly, permitting the tardigrades likewise to dry slowly, and both have a tolerance to dehydration that permits them to survive adverse conditions (Kinchin 1994). Furthermore, the tardigrades have a prolonged life span when it is interrupted by such a dormancy period. And bryophytes contain food items, such as algae, protozoa, and nematodes, as well as the bryophytes themselves, sufficient for the tardigrades. Most likely, the small chambers among the bryophyte branches also afford protection from larger would-be predators. And when fragments of bryophytes disperse, they may carry tardigrades with them.

It is the interstitial water of bryophytes that provides the suitable habitat for tardigrades (Hallas 1975). This water is typically found in leaf sheaths of bryophytes. Hallas investigated the drying of "cushions" of Hypnum cupressiforme (Figure 8). It required 19 hours for the moss to dry to a stable weight. The water retention relative to the weight of the cushion decreased linearly with the density of the cushion. However, the rate of drying can change with the temperature, saturation of the air, and air movement. He concluded that all compartments dry at the same rate, independent of the initial water content of each pocket, and the small variation in drying time is of no consequence for the tardigrades and other inhabitants. The tardigrades were concentrated in the living, chlorophyllous layer (compared to deeper, senescent layers) where there was more moisture. The water pockets connect vertically from one leaf to the next, but only connect horizontally when it rains. Therefore, horizontal migration of the tardigrades is only possible when the moss becomes saturated during rainfall. In H. cupressiforme (Figure 8), such bridges are formed only when rainfall exceeds 3 mm. Hallas considered that nighttime and morning dew were not sufficient for the most common tardigrade [Macrobiotus hufelandii (Figure 2) – a species that comprised 91% of the 386 tardigrades] to become active, suggesting that it would take ten times that amount of water to bring the moss to saturation levels.

Figure 8. Mat of Hypnum cupressiforme. Photo by Dick Haaksma, with permission.

But bryophytes do pose their problems for the tiny tardigrades. These animals are quite light weight, so imagine their struggle to control their movements when they encounter fully hydrated bryophytes with a continuous bath of water surrounding them. Greven and Schüttler (2001) observed these slow-moving creatures [Macrobiotus sp., Echiniscus testudo (Figure 5) on Encalypta streptocarpa [=E. contorta] (Figure 9) when the bryophyte was fully hydrated. The poor bears could barely
move and had difficulty maintaining the direction of their movements in the water. They could easily become dislodged by rainwater unless they are able to nestle in a leaf axil or other protected niche. And that is often a good place to look for them.

On the other hand, Polytrichastrum (=Polytrichum) formosum (Figure 10) did not sustain a continuous water film and the tardigrades seemed also unable to move in this "dry" habitat (Greven & Schüttler 2001). Rather, they seemed confined to the leaf axils, where water collected. As water receded, the animals ceased movement and formed a tun (protective dormant stage of tardigrade that is altered both chemically and physically) right there, permitting it to survive without water for up to 10 years (Jönsson & Bertolani 2001)! Perhaps tardigrades were the inspiration for the Rip Van Winkle story.

Moisture seems to be the greatest determinant of species distribution among bryophytes. Richness among epiphytic bryophytes in the Cincinnati, Ohio, USA area was greatest in areas of high humidity (Meininger et al. 1985). Hofmann and Eichelberg (1987) found that the tardigrades lacked correlation with bryophyte species but that their distribution could be predicted by the degree of moisture they prefer. It is therefore not surprising that some bryophytes housed no tardigrades.

Tardigrades in association with roads along the Alaska pipeline demonstrate a moisture relationship (Meininger & Spatt 1988). Dust resulting from gravel roads associated with the pipeline alters the habitat for both mosses and tardigrades. Those tardigrades living among mosses near roads were species adapted to xeric habitats. These species typically fed on fungi and algae, whereas those farther from the road were more likely to be omnivores or carnivores, presumably because they had more freedom to move about in a somewhat more moist environment.

Adaptations of Tardigrades

One might ask if these bryophyte-dwelling creatures have any special adaptations that permit them to live where they do. Their greatest adaptation is that they live in a habitat that permits them to dry slowly and go into a dormant state, as we will discuss shortly – a kind of behavioral/physiological adaptation. In fact, it appears that limnoterrestrial species actually require a habitat where they have dormant periods. And for many, the stylets permit them to suck the contents out of bryophyte cells, among other things, making them one of the few organisms specially adapted for obtaining nutrients from bryophytes.

Like insects, tardigrades have chitin, in this case in the innermost layer of the cuticle. The chitinous armor of some terrestrial tardigrades (heterotardigrades) may slow drying and offer protection from damage while dry. Of course small size is essential for living in the miniature world of bryophytes. And their claws (Figure 11-Figure 13) may permit them to clamber about more easily among the leaves and branches of the bryophytes. But Bertolani and Bisero (1996) consider that the reduction of claws on the fourth pair of legs is an adaptation to moving among the interstitial spaces in the soil. Does this same adaptation pertain to those among bryophytes?
Figure 13. Claws of *Echiniscus* sp., a genus with many bryophyte-dwelling species. Photo by Martin Mach, with permission.

Their light weight facilitates tardigrade dispersal. Their bodies are flexible, permitting them to nestle in leaf axils or move in small spaces. But most of these adaptations to the bryophyte habitat are speculation. There have been no tests to determine if any of these traits actually increases their survival among bryophytes compared to other habitats. Some very interesting experiments could be designed.

Let's examine one of the bryophyte-dwelling tardigrades as an example of potential adaptations. Martin Mach (The Water Bear) found *Cornechiniscus cornutus* (Figure 14) among bryophytes on a mountain top in Hungary. This cute little bear has two horns on its head (Figure 15) and a nice salmon color. But it is slow and clumsy, out-classed by the faster-moving and more abundant *Ramazzottius* (formerly *Hypsibius*) oberhaeuseri (Figure 25). Do such ornamentations as horns and hairs help to reduce predation in this habitat? Is that an advantage to offset the slower movement? Does the bright color protect the water bear from UV damage, especially while it is dry?

Survival of Hazardous Conditions

The biggest hazard a bryophyte imposes on a tardigrade is intermittent desiccation. But in addition to that desiccation, the organism may be subjected to high or low temperatures, low oxygen conditions, and UV light for prolonged periods. With little ability to move elsewhere, it needs some other type of protection.

Figure 14. *Cornechiniscus cornutus*. Photo by Martin Mach, with permission.

Aquatic organisms rarely need to be concerned with desiccation. However, if an animal is to survive among terrestrial bryophytes, it must be prepared for drying when the bryophyte dries out, and many of the tardigrade habitats are in dry places, including *cryptogamic crusts* (assemblages of Cyanobacteria, algae, lichens, & mosses) in the prairie and desert, and among epiphytes on trees. These bring with them the very hazards mentioned above – UV light in the absence of water for protection, and extremes in temperature. And the watery body must be hydrated for oxygen to enter it.

To unravel the relative importance of these stressors related to desiccation, Wright (1991) studied fifteen species of tardigrades and their responses to insolation, elevation, standardized desiccation rate, and hydration capacity of the plant substrate. There was considerable variation in ecotype among seven species with xeric associations. *Macrobiotus hufelandi* (Figure 2) and *Hypsibius dujardini* (Figure 16), both hygrophilic species, are absent from habitats that desiccate rapidly. On the other hand, the xerophiles *Milnesium tardigradum* (Figure 51) and *Ramazzottius oberhaeuseri* (Figure 25) avoid locations with high insolation and rapid desiccation rate, but also avoid poorly drained sites and sites with prolonged hydration. Despite these differences, *Milnesium tardigradum* often associates with the two *Hypsibius* species and may use them for food. The lack of association among *Macrobiotus hufelandi*, *Paramacrobiotus* (formerly *Macrobiotus*) richtersi (Figure 17), and *Hypsibius prosostomus* may be due to competitive exclusion.

Figure 15. *Cornechiniscus cornutus* showing one of its two head horns. Photo by Martin Mach, with permission.

Figure 16. *Hypsibius dujardini* with the alga *Chlorococcum* in its gut. Photo by Willow Gabriel through EOL Creative Commons.
Physical Adaptations

The soft-bodied tardigrades appear to have few structural adaptations to survive drought. Some, like *Echiniscus*, have long hairs (Figure 18-Figure 19), but the hairs are so few that one can hardly imagine they are of any help to reduce water loss or protect the dry animal. Hmm... What might their function be? Or are they a non-detrimental left-over? One interesting idea is that they might serve as straws to draw water into the body – a hypothesis requiring both experimentation and TEM examination. But suppose that is true. It could be a way to cause water to enter without drowning the animal – i.e., permitting maintenance of an air layer on the surface. On the other hand, they could serve as fine hairs to collect dew and fog on their surface and direct it to the skin, thus increasing surface area and collection surface for the water. Or the hairs cold act like whiskers on a cat, facilitating navigation among the mosses. More interesting experiments are needed!

*Echiniscus viridianus* (Figure 20) seems to do well among bryophytes. It was originally described from mosses in Alabama USA (20 individuals) and later collected in New Mexico and the Azores Islands, again among mosses (Pilato et al. 2007). As in other members of the genus, this species bears armor and long hairs.

Oxygen availability can be a problem, and for this reason the tardigrades avoid dense bryophytes and usually remain in the top few centimeters of soil where more oxygen is available (Ramazzotti & Maucci 1983). Hence, another possibility for the long hairs is that they could be tactile extensions to warn of an environment that is too tight, or chemical sensors to aid in finding food or sensing predators – or sensing a low-oxygen environment. Hypothesis testing is needed!

Spines/hairs and body armor may offer a bit more protection. Some bryophyte-dwelling species such as *Cornechiniscus cornutus* (Figure 21) and some members of the genera *Echiniscus* (Figure 22-Figure 23) and *Ramazzottius* (Figure 24-Figure 25) (and others) have "armor" on their bodies that is somewhat leathery. I am aware of no studies that demonstrate the ability of the armor to reduce water loss, but it would appear to be a good possibility. Other possible advantages of this armor-like cuticle may include protection from fungi and other pathogens and some kinds of predators, particularly while in cryptobiosis, and it most likely would afford limited UV protection. How little we know!
Figure 21. *Cornechiniscus cornutus* showing armor. Photo by Martin Mach, with permission.

Figure 22. *Echiniscus* sp. posterior dorsal side showing a type of armor. Photo by Martin Mach, with permission.

Figure 23. Tardigrade sp. showing a type of armor. Photo by Martin Mach, with permission.

Figure 24. Armor on *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.

Figure 25. *Ramazzottius oberhaeuseri*, a tardigrade with armor. Photo by Martin Mach, with permission.

**Pigments**

Terrestrial tardigrades come in green, brown, yellow, orange, pink, red, purple, or black, whereas aquatic ones are white (Hebert 2008). Bonifacio et al. (2012) pointed out that despite the many studies on tardigrades relative to space travel, little is known about the nature or function of their pigments. They described the carotenoid nature of the pigments and the ability of these pigments to decrease under high oxidative stress. They hypothesized that these pigments had an anti-oxidant function and could possibly protect the animals during extreme environmental conditions.

It is possible that the wonderful colors of some tardigrades (Figure 26-Figure 27) are adaptations against UV damage to DNA, especially during prolonged periods in a cryptobiotic state. Such pigmentation advantages have been demonstrated in bryophytes (Martínez Abaigar & Olivera 2007) and copepods (Byron 1982), so it is reasonable to expect them to serve similar functions in tardigrades, particularly in those more open habitats such as cryptogamic crusts. It would be an interesting study to examine the relationship of color with habitat in tardigrades. I am aware of no such study, but Martin Mach (pers. comm. 18 October 2012) pointed me to the publication of Ernst Marcus (1929). Marcus suggested that pigments were a response to UV light. He cited as support the findings that pigmentation varies with winter vs summer UV radiation. Experiments are needed to support this hypothesis, and one must wonder how this relates to those living among bryophytes. Marcus pointed out that insolation does not penetrate well into the moss colony, making bright pigments unnecessary for these tardigrades.

**Physiological Adaptations**

**Light Response**

Tardigrades have a pair of eyes, although at times they may be "ghost eyes" (Figure 28) that cannot be seen.
through ordinary observation (Mach 2012). These eyes respond to light, and at least in *Macrobiotus hufelandi* (Figure 2) the response changes with size and age (Beasley 2001). The smaller, younger size group had a significantly negative response to light. Beasley hypothesized that this behavior serves to conserve body moisture in small individuals that have a larger surface area to volume ratio than do larger ones. The response is not a phototaxis (directional response to light), but rather was photokinesis (non-directed, random movement), resulting in either an increase in speed or a change in direction when exposed to light. Such behavior would seem to support finding a "safe" place away from light.

**Cryptobiosis**

Albert Szent-Gyorgyi, a 20th Century Hungarian biochemist, once stated "Water is life's mater and matrix, mother and medium. There is no life without water." In their cryptobiotic state, tardigrades come close to disproving that statement. Anthony van Leuwenhoek first described cryptobiosis in 1702, but it was not until 1959 that Keilin coined the term *cryptobiosis* (Wright 2001). Cryptobiosis is a reversible ametabolic state that can be induced by dehydration and cooling, and possibly osmotic stress and anoxia.

Metazoans such as tardigrades use glycerol and the disaccharide sugars sucrose and trehalose (Wright 2001) as protectants. Before entering the cryptobiotic state, these substances must be synthesized from glycogen reserves, hence requiring a preparatory period. Certain behavioral adaptations may help to delay the desiccation, thus permitting these compounds to be synthesized.

One of the factors that contributes to the tolerance of desiccation is the ability to reduce surface area during tun formation (Wright 1991), hence slowing the process. Those that are most desiccation tolerant have the greatest infolding. This means those species with thick dorsal plates (Figure 29) are at a disadvantage. As Wright showed for *Echiniscus testudo* (Figure 5), there is very little surface area reduction possible.

Moss cushions help to make survival of this cryptobiotic state possible. The small spaces among mosses hold static air that can slow the dispersion of water vapor (Wright 1989). This permits the slow drying that is necessary for survival of the tardigrade in the desiccated state. Mosses in exposed positions may desiccate rapidly. Some mosses [e.g. *Polytrichum* (Figure 30), *Dawsonia* spp. (Figure 31)] are able to slow this process by folding their leaves (van Zanten 1974).

Wright experimented with tardigrades on mosses in their natural habitat. *Eutardigrada* species were hydrated at least 24 hours before the experiments. As expected, there is considerable variation among species in their ability to tolerate desiccation. But they also differ in the lethal humidities (53-78%) for initial desiccation. Those species that are best able to tolerate rapid initial drying are also those most able to acquire tolerance to low humidities of 25-31% following drying in high humidity.
Typically, tardigrades are desiccated in 80% relative humidity (Wright 1991). In this condition, they dehydrate rapidly, then abruptly reduce water loss (the permeability slump). This slump occurs in both live animals prior to tun formation and in extended dead animals, so it is not a physiological phenomenon. This slump permits the animals to retain considerable water in their desiccated state. Crowe (1972) examined the humidity effects on Paramacrobiotus areolatus (Figure 32). He found that at humidities lower than 70% this species became flattened and crumpled. Above this level, dehydrating animals form tuns. This appears to be an active process that is not as effective in anesthetized animals. Tuns of active animals lose water at only 0.3 times the rate of anaesthetized animals. The anaesthetized animals reach moisture equilibration with the environment within one hour, whereas tuns do not reach that equilibrium within 100 hours. In dry air, tardigrades can reach as little as 2-3% water content without dying if they are able to dry properly.

Literally meaning "hidden life," cryptobiosis is a state of suspended animation in which the organism is able to survive unfavorable conditions while expending little energy. During that state, the organism does not feed, reproduction stops, and metabolism is extremely reduced and may possibly even cease. For the limnoterrestrial (living in water films on land) tardigrade, it appears to be an essential part of survival and life, and it stops the aging clock.

Despite the apparent absence of structural adaptations, desiccated tardigrades, like their mossy habitats, have great survival capabilities. They have two forms of dormancy: cryptobiosis and encystment (Guidetti et al. 2006). The cryptobiosis of tardigrades is exhibited in several forms:

- **anhydrobiosis** (induced by loss of water)
- **cryobiosis** (induced by declining temperatures)
- **anoxybiosis** (induced by insufficient oxygen)
- **osmobiosis** (induced by loss of water due to higher external salt concentrations) (Bertolani et al. 2004).

To be active, tardigrades must stay in a water film in order to breathe (Bordenstein 2008). But in a cryptobiotic state, as discussed below, tardigrades can survive not only desiccation, but temperatures as low as 0.05K (-272.95°C) for 20 hours or -200°C for 20 months (Miller 1997). They have even survived 151°C for a few minutes (Lindahl & Balser 1999). They become active again after living with 0% hydration (Lindahl & Balser 1999). This desiccated dormant state also permits them to survive pressures of 6000 atmospheres (Seki & Toyoshima 1998), i.e. six times the pressure of the deepest part of the oceans! Yet they can also survive the vacuum and UV radiation of space (Jönsson et al. 2008), a feat not known for any other animal. The ability of tardigrades to undergo cryptobiosis is more widely known than their encystment behavior. True cryptobiotic states are survived as a tun (Figure 33-Figure 43). The tardigrades will be the ones to survive when everything else is deceased.

**Tun Formation**

When they undergo desiccation, the tardigrades form a tun (Figure 33-Figure 43) (Lindahl & Balser 1999). The tun is a barrel-shaped, dry, dormant tardigrade. Tuns are
formed in the process of entering true cryptobiosis, *i.e.*, in anhydrobiosis, osmobiosis, and cryobiosis, but not in anoxybiosis. Although the stimulus differs among these, each ultimately involves the loss of free water.

![Figure 33. Tun of *Ramazzottius oberhaeuseri*. Photo by Martin Mach, with permission.](image1)

![Figure 34. *Tardigrade* tun – water bear in a state of anhydrobiosis. Photo by Janice Glime.](image2)

![Figure 35. *Tardigrade* tun – water bear in a state of anhydrobiosis. Note the buccal apparatus (resembles a tuning fork on left end). Photo by Janice Glime.](image3)

![Figure 36. Tun of *Hypsibius* sp. Photo by Martin Mach, with permission.](image4)

![Figure 37. Tun of *Echiniscus* sp. Photo by Martin Mach, with permission.](image5)

![Figure 38. Tun of *Echiniscus* sp. on moss leaf. Photo by Martin Mach, with permission.](image6)

![Figure 39. Multiple tuns of *Echiniscus* sp. on a single moss leaf. Photo by Martin Mach, with permission.](image7)
This tun is a little ball in which the tardigrade can survive 0% relative humidity! However, it only requires a reduction to 70-95% humidity to trigger the tun formation, a resting form in a cryptobiotic state in which the tardigrade appears to be dead (Crowe 1972). During tun formation, loss of free and bound water is greater than 95% (Bertolani et al. 2004). The body folds and the appendages are withdrawn (Lindahl & Balser 1999). Wax is extruded onto the surface and most likely reduces water loss (Wright 1988a, b). Those tardigrades with the most variability in the thickness of this cuticle, making them more pliable, are those able to have the greatest surface area reduction when they form tuns (Wright 1988a, 1989). The thin areas would permit greater infolding. Lipids of the inner cuticle are thickest in the species that are best able to tolerate rapid drying. Crowe (1972) demonstrated that the cuticle of Paramacrobiotus areolatus (Figure 32) is removed from air contact during tun formation and becomes less permeable to water. Crowe likewise suggested that this loss of permeability might be due to a lipid phase change.

Despite the waxy cuticular protection, the water content is reduced to less than 1% (Lindahl & Balser 1999) and the tun becomes shrivelled and wrinkled (Hingley 1993). Echiniscus testudo (Figure 5), an armored tardigrade, has much thicker dorsal (back) plates, apparently compensating for its limited ability to reduce surface area as it is drying (Wright 1988a, 1989).

The tardigrade bodies synthesize cell protectants such as trehalose, glycerol, and heat shock proteins that contribute to successful recovery from the tun state (Wright 1989). Trehalose is typically found in high concentrations in animals in a state of cryptobiosis (Crowe & Crowe 1984). Trehalose is able to bond with DPPC and alter the spacing of polar head groups, apparently stabilizing the dry cell membranes. Hengherr et al. (2008b) determined that levels of trehalose varied considerably among species. In fact, they detected no changes in trehalose levels in any Heterotardigrada, and Milnesium tardigradum (Figure 51) apparently had no trehalose at all. They did demonstrate that tardigrade embryos can accumulate high levels of trehalose, seemingly explaining the high level of desiccation tolerance in that life cycle stage.

Tun formation is essential to tardigrade survival under desiccating conditions. For Paramacrobiotus areolatus (Figure 44), and probably most tardigrades, if the humidity is low (<70%) or anoxic (lacking oxygen) during its desiccation, it is unable to form a tun and cannot be revived (Crowe 1972). It must have sufficient energy (requiring oxygen), hydration, and time to enter the tun stage.

This tun is a little ball in which the tardigrade can survive 0% relative humidity! However, it only requires a reduction to 70-95% humidity to trigger the tun formation,
Chapter 5-1: Tardigrade Survival

Tardigrades revive (Figure 45) almost as quickly as a moss when water returns (Crowe & Higgins 1967), in as little as 4 minutes (Hingley 1993), or several hours, depending on how long they have been dehydrated (Lindahl & Balser 1999). One marine tardigrade has been induced to alternate between a cryptobiotic state and activity on a 6-hour cycle.

![Figure 45. Echiniscus sp. rehydrated after four years of desiccation. Photo by Martin Mach, with permission.](image)

### Dangers in a Tun

One concern that comes to mind is the possible damage that could happen to these organisms while in the tun stage. I am reminded of the frozen frogs and toads during the winter. They are very susceptible to physical damage if they are disturbed. I would think an animal such as the amphibians hiding under a rock or clump of moss would experience no more physical abuse than the tiny tardigrade among the moss leaves. Ice crystals could poke holes in cells, larger animals could eat them, or they could get knocked off into a hole where conditions were not favorable to their maintenance and survival. I have to wonder just what dangers these dormant organisms do face, and how many actually survive these in the wild to become once again active. It seems we currently have no idea.

Certain dangers include cell degradation and DNA damage. As the tardigrades exist longer and longer, they accumulate cell degradation and DNA damage (Rebecchi et al. 2009b), ultimately accumulating too much for successful repair. Hence, the tun does not completely protect them, and chances of survival decrease with time.

### Effects of Size

Jönsson et al. (2001) found that size influenced survival of cryptobiotic tardigrade species, but that direction of influence differed among species. The common Ramazzottius oberhaeuseri (300 µm length; Figure 46) had a much higher survival rate (66%) (Figure 47) than did Richtersius coronifer (40%) (up to 1 mm length; Figure 48). Ramazzottius oberhaeuseri (Figure 46) has a high ability to retain water, perhaps with a physiological adaptation to reduce water loss in this smaller organism with a high surface area to volume ratio.

Within Richtersius coronifer (Figure 48), large individuals were less likely to survive cryptobiosis than medium-sized ones (Figure 49); reproductive state had no effect (Jönsson & Rebecchi 2002). Better energetic conditions increased survival. Jönsson and Rebecchi suggested that larger organisms had greater energy constraints when entering and leaving anhydrobiosis, decreasing survival rate.

![Figure 47. Comparison of survival during encystment for Richtersius coronifer and Ramazzottius oberhaeuseri from Italy and Sweden. Vertical line represents standard error. Redrawn from Bertolani et al. 2004, based on Jönsson et al. 2001.](image)

![Figure 48. Richtersius coronifer, clinging to an algal cell. Photo by Martin Mach, with permission.](image)
Jönsson and Rebecchi (2002) likewise found that medium-sized tardigrades had a better chance of survival than did large ones in *Richtersius coronifer* (Figure 48). Large storage cell size was an important parameter to predict greater survival in the large tardigrades (Figure 49).

Reuner *et al.* (2010) described the storage cells as free-floating cells in *Milnesium tardigradum* (Figure 51), *Paramacrobiotus tonollii* (Figure 50), and *Macrobiotus sapiens* that apparently store and release energy as glycogen, protein, and fat. These stores provide energy during cryptobiosis. Storage cell size did not relate to body size, except that the largest tardigrade, *Milnesium tardigradum* (Figure 51), also had the largest storage cells. After seven days of anhydrobiosis (tun stage resulting from desiccation), this species had decreased cell size, but the other two species did not. Food sources used in the study did not seem to affect cell size.

Figure 50. *Paramacrobiotus tonollii*. Photo by P. J. Bartels, with permission.

Longevity

Tardigrades are often credited with century-long survival in a cryptobiotic state. This is due to the report that one herbarium specimen of a moss housed a tardigrade that began cellular activity after 120 years of being dry in the herbarium (Franceschi 1948; Brusca & Brusca 1990; Jönsson & Bertolani 2001)! But, sadly, this record has been called into question, and the tardigrade never fully recovered despite its cellular activity. At the very best, even this faint degree of survival is probably a rare occurrence (see Jönsson & Bertolani 2001). Jönsson and Bertolani (2001) reviewed the evidence and considered that ten years is a more realistic estimate of survival time for tardigrades in a cryptobiotic state.

Rebecchi *et al.* (2008) decided to test this claim of longevity further, using five species of tardigrades from lichens. They collected wet lichens with active tardigrades and permitted them to dry in the ambient conditions of the lab. Among these, *Ramazzottius oberhaeuseri* (Figure 25), *Echiniscus testudo* (Figure 5), and *E. trisetosus* (Figure 18), species that also occur on bryophytes, were sufficiently abundant to permit statistical conclusions. At the beginning of the experiment 91% of *R. oberhaeuseri* and 72% of *Echiniscus* spp. were active. *Ramazzottius oberhaeuseri* (Figure 25) survived up to 1604 days, whereas *Echiniscus* spp. lived only 1085 days. Nevertheless, this may not reflect going into the tun stage under natural conditions, which is likely to be slower among mosses. This could be particularly important for tardigrades that increase trehalose levels.

To test the longevity of tuns vs eggs under anhydrobiosis, Guidetti and Jönsson (2002) examined 63 different moss samples from stored collections, ranging in anhydrobiotic state 9-138 years. Eggs survived longer than dry adults (tuns), with those of *Ramazzottius oberhaeuseri* (Figure 25) surviving nine years. Much more work is needed to determine what factors account for such differences in survivorship and how it relates to individual species and habitats. The ability to survive unfavorable conditions permits the tardigrades to live in such places as *Grimmia pulvinata* tufts (Figure 52) on house roofs (Corbet & Lan 1974) or among branches of the epiphyte *Orthotrichum cupulatum* (Figure 53) (Jönsson *et al.* 2001).
Like the rotifers, tardigrades suspend their aging clock while they are dormant (Hengherr et al. 2008a). Milnesium tardigradum (Figure 51) that was subjected to alternating periods of drying and activity exhibited similar longevity of active periods to that of animals of the species that had not experienced dry periods.

Ramazzotti and Maucci (1983) estimated that freshwater species such as those of Hypsibius (Figure 54) and Macrobiotus (Figure 55) live about 1-2 years. Terrestrial bryophyte-inhabiting species of the same genera live much longer, averaging 4-12 years. This extended life is due largely to their periods of cryptobiosis, during which the biological clock stops.

One contributing factor in their survival of drying is the ability of tardigrades to alter their cell membranes (Brave New Biosphere 1999). They replace the water in the cell membranes with sugar, thus preventing radiation from causing ionization. Like the nematodes and rotifers, some tardigrades prepare for desiccation by producing disaccharide sugars, including trehaloses (Bordenstein: Tardigrades; Westh & Ramløv 1991). Disaccharides like trehalose and sucrose, as well as glycerol, are used as membrane protectants by metazoans such as tardigrades, whereas plants typically use oligosaccharides such as stachyose and raffinose (Wright 2001).

This water replacement by sugars also protects invertebrates during freezing because crystallization cannot occur (Brave New Biosphere 1999). The accumulation of trehalose of 0.1-2.3% of dry weight occurred within 5-7 hours during desiccation in Richtersius coronifer (Figure 48; Westh & Ramløv 1991). This accumulation was reversed within 6 hours upon rehydration. Both water loss and sugar replacement prevent the rupture of the cell membrane that would result in death. But trehalose has multiple properties that help to stabilize desiccated cells (Table 1).

Table 1. Properties of trehalose that benefit dehydrating cells. From Watanabe 2006.

<table>
<thead>
<tr>
<th>Property</th>
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<tbody>
<tr>
<td>Non-reducing activity</td>
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<tr>
<td>Low tendency to crystallize</td>
</tr>
<tr>
<td>Stable glass formation</td>
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<tr>
<td>High vitrification temperature</td>
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<tr>
<td>High ability of water replacement</td>
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<tr>
<td>Structuring activity of intracellular water with HSP</td>
</tr>
<tr>
<td>Stabilization of dry membranes</td>
</tr>
<tr>
<td>Antioxidant activity of protein and fatty acids</td>
</tr>
<tr>
<td>Free-radical scavenger</td>
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Nevertheless, tardigrades accumulate trehalose at the low end of the scale for anhydrobiotic organisms – about 2% (Watanabe 2006). This lower level in tardigrades and absence of trehalose in rotifers is coupled with their ability to enter anhydrobiosis within one hour, whereas organisms with larger accumulations (up to 40%) can take at least two days.
Trehalose is not a cure-all for desiccation effects in tardigrades. High temperatures and high humidity may lead to destruction of trehalose (Rebecchi et al. 2009a). In other cases, or in consort, oxidative damage may occur. Using *Paramacrobiotus richtersi* (Figure 17) as an experimental organism, Rebecchi et al. demonstrated that DNA changes can occur during desiccation. Neumann et al. (2009) likewise demonstrated a slight increase in DNA damage during drying, but they also found that DNA damage increased with duration of anhydrobiosis. Furthermore, high temperatures and relative humidity have negative effects on both survival and time to recover after rehydration, with effects increasing with duration of exposure. One reason for this is that damages are not repaired during anhydrobiosis and therefore accumulate with time.

**Anhydrobiosis**

The most common of the cryptobiotic states is **anhydrobiosis** (state of dormancy brought on by dehydration). In their state of anhydrobiosis, tardigrades can remain inactive during unfavorable conditions such as prolonged dryness (Kinchin 1987b). **Anhydrobiosis** is usually restricted to animals less than 1 mm in length (Watanabe 2006). Hence, some invertebrates are only able to enter this state during early developmental stages. Tardigrades and rotifers, being less than 1 mm when fully developed, are able to do so at any developmental stage.

In order to survive anhydrobiosis, tardigrades must dry very slowly (Hingley 1993; Collins & Bateman 2001). To form the tun, they must retract their head, legs, and hind end, forming a rounded tun, thus reducing surface area. In this state of anabiosis, they are able to withstand extremes of temperature and desiccation. Nevertheless, water arouses them in as little as four minutes.

It appears that continuously hydrated conditions may be detrimental to the survival of tardigrades (Jönsson 2007). Using bryophyte populations from Island Öland, Sweden, Jönsson subjected the tardigrades to two treatments of 6-month duration over an 18-month period. These experimental treatments increased hydration, decreased hydration, or remained as controls. The total population was significantly smaller (barely so) under increased hydration. But effects were not the same for all tardigrades. *Richtersius coronifer* (Figure 48) and *Echiniscus spiniger* failed to respond to the treatment, whereas *Milnesium tardigradum* (Figure 51) declined under increased hydration. But even *Richtersius coronifer* experienced reduction in the density of eggs (Figure 56–Figure 57) under the watering treatment. Hydration did not significantly increase density in any of the tardigrades. This adds further support to the idea that periods of dormancy (cryptobiosis) are necessary to increase longevity of the tardigrade. This would, in turn, increase variability of conditions, offering an array of conditions for reproduction.

*Richtersius coronifer* (Figure 48) can increase its survival rate by forming **aggregates**, a mechanism barely known for tardigrades but common in nematodes (Ivarsson & Jönsson 2004). The clustering reduces exposed surface area and thus slows drying. It is possible that this is used more in tardigrades than is realized; its use among bryophyte fauna is as yet unknown.

Schill and Fritz (2008) examined the desiccation tolerance of the embryonic stages of *Milnesium tardigradum* (Figure 58), a potential means of surviving desiccation that has been neglected. They rehydrated this species after treatment at eight different humidity levels (10, 20, 31, 40, 54, 59, 72, 81%). They found that the less developed stages were more susceptible to desiccation damage. In the first three days of development, low humidity caused a decrease in hatching rates following rehydration. Later stages fared better, but when older embryos were dried fast at low humidity levels, development was delayed and hatch rates were lower after rehydration. Nevertheless, older embryos fared better than younger ones.

Even in 2011, Schokriae et al. still considered the survival mechanisms of tardigrades to be poorly understood. They considered the possibility that heat shock proteins (HSPs) might protect them from irreversible aggregation and degradation during anhydrobiosis. They found that *Milnesium tardigradum* (Figure 51) had HSPs in several major chaperone families, suggesting the ability...
of the tardigrade to use these for protection when dehydrated.

Figure 58. *Milnesium tardigradum*, a bryophyte dweller whose younger stages are the most susceptible to desiccation. Photo by Yuuji Tsukii, with permission.

The cryptobiotic state of anhydrobiosis has a significant impact on the ecological role of the tardigrades. It affects their role in the food chain, their ability to disperse, and their survival through a longer period of time (see reviews by Pilato 1979; Wright *et al.* 1992; Kinchin 1994). Bryophytes often play a significant role in achieving that state.

**Osmobiosis**

Osmobiosis is a special case of cryptobiosis that permits some species to tolerate high salinity and to form a tun (Lindahl & Balser 1999). It is initiated when the animal experiences an external salt concentration that is higher than that inside the organism. However, for tardigrades, while possible, osmobiosis is typically not necessary as most tardigrades already have a high salt tolerance.

**Anoxybiosis**

Anoxybiosis is another special case where the tardigrade has the ability to survive low oxygen (Lindahl & Balser 1999). Tardigrades are very sensitive to changes in oxygen tension, and prolonged reduction of oxygen leads to osmoregulatory failure.

Anoxybiosis is not a true state of cryptobiosis and does not involve tun formation (Figure 59). Unlike true cryptobiosis, anoxybiosis involves the uptake of water. The lack of oxygen results in the inability to control osmosis, causing water to enter the cells in excess. The animals become turgid, immobile, and retain fully extended bodies that are perfectly bilaterally symmetrical (Figure 60). Even animals in a molt can enter anoxybiosis (Figure 61).

Figure 59. *Macrobiotus hufelandi* male in anoxybiotic state, showing lack of tun formation. Photo by Martin Mach, with permission.

Figure 60. Tardigrade showing anoxybiosis, where water has entered through the cuticle by osmosis and caused swelling and turgidity. Note the extended legs and perfectly symmetrical body. The animal cannot move in this state. Photo by Martin Mach, with permission.

Revival to normal state (Figure 62) relates to the duration of the dormant state. However, the success of that recovery is controversial (Wright *et al.* 1992), with some researchers finding that they can survive for only 3-4 days (Crowe 1975) and others finding survival of *Echiniscoides* (a tidal zone genus) up to six months in closed vials (Kristensen & Hallas 1980).

Figure 61. Tardigrade induced into anoxybiosis during its molt. Photo by Martin Mach, with permission.

Figure 62. This tardigrade was caught by low oxygen during molt and entered anoxybiosis. Here it has recovered and is moving within the swollen cuticle to complete its molt. Photo by Martin Mach, with permission.
Cryobiosis

Cryobiosis is another special case of cryptobiosis that results when the temperature decreases and the water in the cells has frozen (Wikipedia: Cryptobiosis 2009). Molecular mobility stops (Wikipedia: Cryptobiosis 2009), permitting the tardigrades to survive very low temperatures (Westh et al. 1991; Westh & Kristensen 1991; Ramløv & Westh 1992; Somme 1996; McInnes & Pugh 1998). They do this by actually freezing, but the freezing is ordered (Lindahl & Balser 1999) and the result once again is a tun.

Cryobiosis in tardigrades differs from anhydrobiosis (Wright 2001). First of all, tun formation in not necessary for survival. The important process is that intracellular freezing must be avoided. But tardigrades, unlike many arthropods, are freeze-tolerant. This can be accomplished by ice-nucleating proteins that are located outside the cells. These proteins act like water magnets, drawing water out of the cell. Also unlike many other arthropods, the extracellular freezing, promoted by the ice-nucleating proteins, occurs at temperatures near 0°C. In Richtersius coronifer (Figure 48), trehalose synthesis is not part of this process, although there may be other cryoprotectants in the cell. The details of freeze protection are not fully understood, but the loss of water from the cells may cause the cells to become “unfreezable.” Glycerol contributes as an antioxidant. Being imbedded in ice probably also prevents oxidation damage. In this cryobiotic state, the tardigrades can survive for decades.

Tardigrades often experience wide temperature fluctuations while in an active state. In particular, they can be subjected to subzero temperatures. Their ability to tolerate these sub-zero conditions requires either tolerance of freezing body water or having a mechanism to lower the freezing point. Hengherr et al. (2009) subjected nine species from polar, temperate, and tropical regions to cooling by 9, 7, 5, 3, and 1°C h⁻¹ down to -30°C, then returning them to ambient temperature at a rise of 10°C h⁻¹. Survival was better at fast and slow cooling rates, with low survival rates at intermediate cooling rates. Hengherr et al. suggested that this relationship may indicate a physical effect during fast cooling and possible synthesis of cryoprotectants during slow cooling. The increased survival with slower cooling indicates that tardigrades protect their cellular structure from freezing injury without altering their freezing temperature.

As indicated above, at least some protection seems to be accomplished by using ice-nucleating proteins in the body fluids (Westh et al. 1991). Such proteins serve as centers for crystal formation, a technique used to make snow for ski hills. This cryoprotective mechanism permits tardigrades to survive rapid freezing and thawing cycles such as those experienced in the Arctic and Antarctic. Usually this type of protection means that the nucleating centers are small, permitting only small crystals to form, consequently reducing damage to the cell membranes.

The ice-nucleating activity in the body fluid from Richtersius coronifer (Figure 48) is reduced by 50% following ca 7x10⁵ times dilution (Westh et al. 1991). Heating to temperatures above 68°C induces an abrupt decrease in the activity, suggesting that the nucleators are proteinaceous.

Westh and Kristensen (1992) examined Richtersius coronifer (Figure 48) and Bertolanius nebulosus (= Amphibolus nebulosus) (see Figure 63) and compared their cryoprotective strategies. Richtersius coronifer (Figure 48) lives in drought-resistant mosses and overwinters in a frozen or dry state (cryptobiosis). Bertolanius nebulosus, on the other hand, lives among moist mosses and algae and spends its winter frozen in a cyst or as eggs. Both species can supercool to as low as -7°C. But these two species have distinctly different heat stability, resulting from differences in ice-nucleating proteins. In both cases, ice formation is rapid, but crystallization most likely stops within a minute of nucleation. This protects the cells from damage caused by large, sharp crystals. Nevertheless, ice constitutes 80-90% of the body water. Winter acclimatization of R. coronifer results in a 10% lower ice formation than summer acclimatization. The thaw point was unaffected by winter vs summer, suggesting that there is no accumulation of low molecular weight cryoprotective substances.

Figure 63. Bertolanius volubilis type A cyst. Photo by Roberto Bertolani from Bertolani et al. 2004, with permission.

Despite their seeming indestructibility, not all tardigrade individuals fare well at low temperatures, and some species fare better than others. Bertolani et al. (2004) demonstrated this for three species of tardigrades (Figure 64). Ramazzottius oberhaeuseri (Figure 25) seems to be almost indestructible down to -80°C, whereas Hypsibius dujardini (Figure 16) had only 20% survival at that temperature. In fact, it had less than 80% survival at -9°C.
magnitude less than that of terrestrial insects. This suggests that the other invertebrates may use an entirely different mechanism for surviving desiccation and cooling. Dehydration results because the water vapor pressure of supercooled water is higher than that of ice under the same temperature. A drop in temperature of even a few degrees of supercooling will cause considerable water loss. This loss continues until the vapor pressure of body fluids and that of surrounding ice are equal. When this is achieved, there is no longer any danger of ice formation in the tissues, permitting the invertebrate to survive at subzero temperatures. Holmstrup et al. (2002) showed that soil invertebrates do not use supercooling to survive. Instead, they dehydrate, changing their body-fluid melting point to that of the ambient temperature. This mechanism works even in the extreme cooling conditions and rapid rates seen in the polar soils.

Tardigrades live in many habitats that experience such cold temperatures. The widely fluctuating temperatures of the polar regions subject them to extremes while they are still in the active state. Thus they must either have the ability to tolerate the freezing of their body tissues or a means to lower their tissue freezing point (Hengherr et al. 2009).

**Temperature**

Rebecchi et al. (2009) pointed out that most stress studies on tardigrades had involved terrestrial tardigrades. Hence, they examined the limnic boreo-alpine species Borealibius zetlandicus for its stress responses. This species is able to survive freezing in lab experiments by entering a cryptobiotic state. Faster cooling rates lead to greater death from freezing. It furthermore is able to survive relatively "hot" water, having an experimental heat-shock LT50 of 33.0 ± 0.5°C. On the other hand, no members of this species were able to survive desiccation in the experiments. It is likely that in its natural habitat it is able to survive drying conditions by behavior – finding locations among moss leaves or in other plants or in muds where it does not dry as completely.

Ramløv and Westh (2001) studied Richtersius coronifer (Figure 48) and found that when this species enters anhydrobiosis it accumulates the disaccharide trehalose, with the maximum content of 2.3% of the dry weight. This species was able to survive temperatures up to 70°C in a moss cushion for an hour with no effect on survival rate. However, above that temperature, survival decreased rapidly, with no survival at 100°C. Ramaløv and Westh suggested that heat shock proteins may be involved in the high temperature survival.

**Diapause (Encystment)**

Tardigrades are especially endowed with the physiological ability to survive. They are among the few organisms that can use both anhydrobiosis and diapause (encystment) as a means of dormancy to survive unfavorable conditions (Guidetti et al. 2008). Diapause is common among aquatic tardigrades, but there are some terrestrial species that experience diapause (Westh & Kristensen 1992; Nelson 2002). Whereas cryptobiosis is well studied, the role of diapause (encystment) is not well known in tardigrades. It appears that it is not an essential part of the life cycle – only a means to survive some unfavorable conditions.

Węglarska (1957) found that Dactylobiotus dispar (Figure 65-Figure 68) was induced to encyst by environmental conditions that gradually became worse. Interestingly, when there was a rapid change to poor conditions, this tardigrade went into anoxybiosis. When a tardigrade is about to encyst, it ingests large amounts of food that is stored in the body cavity cells (Nelson 1991a). The remaining material in the gut is defecated.

Encystment is more complex than tun formation (Bertolani et al. 2004). The cysts are ovoid and are composed of a series of cuticles that surround the sleeping animal (Figure 69; Guidetti et al. 2006). They are described as resembling an onion or a Matrioshka Russian doll.
During encystment, new cuticular structures are synthesized (Guidetti et al. 2006). Encystment starts with the discharge of the sclerified portions of the buccal-pharyngeal apparatus without the loss of cuticle. Rather, they produce two or three new cuticles. In *Bertolanius* [=Amphibolus] *volubilis* (Figure 69), the new cuticle is similar to that found on the non-encysted organisms, whereas in *Dactylobiotus parthenogeneticus* (Figure 70-Figure 72) the ultrastructure of the new cuticle differs. The tardigrade retracts within the cuticle (Nelson 1991a).

Figure 68. Egg of *Dactylobiotus dispar*. Photo by Martin Mach, with permission.

Tardigrade encystment is known for only a few species, although it may be more widespread than is currently known. There are at least three types of cysts (Guidetti et al. 2006). *Bertolanius volubilis* has two types (Figure 69); *Dactylobiotus parthenogeneticus* (Figure 70-Figure 72) exhibits only one. Having two types of cysts in the same species seems to be a terrestrial character (Bertolani et al. 2004). Type 2 cysts have an additional layer of cuticle compared to type 1 cysts. Although only a few species have been described, it appears that a type 1 cyst never shows a modified buccal-pharyngeal apparatus, whereas a type 2 cyst does.

Figure 69. **Upper:** Type 1 cyst. **Lower:** Type 2 cyst (surrounded by several layers of cuticle), both of *Bertolanius volubilis*. Photos by Roberto Bertolani in Bertolani et al. 2004, reproduced with permission.

Figure 70. *Dactylobiotus* sp., a member of a genus with only one type of diapause. Photo by Yuji Tsukii, with permission.

Figure 71. *Dactylobiotus* sp., a tardigrade with only one type of diapause cyst. Photo by Martin Mach, with permission.

Figure 72. *Dactylobiotus* sp. cyst. Photo by Roberto Bertolani in Bertolani et al. 2004, reproduced with permission.
Conditions that cause emergence from the cysts are not understood. Unlike those in an anhydrobiotic state, the encysted tardigrades are not drought-resistant. Nor can they withstand high temperatures, because they have continuous water content. Nevertheless, the cysts can survive in nature for more than a year on their food reserves (Westh & Kristensen 1992).

Using the tardigrade *Bertolanius volubilis* (Figure 69) from the mosses *Racomitrium sudeticum* (Figure 73) and *R. elongatum* (Figure 74) on sandstone in the Northern Apennines of Italy, Guidetti *et al.* (2008) examined the factors involved in the inducement of diapause. They learned that in *B. volubilis* the type of diapause cysts produced in April differed from those produced in November. The April cysts are produced during a warm season, whereas the other type is present during the cold season. Temperature is responsible for induction, maintenance, and termination of the cyst. Both exogenous (temperature) and endogenous (physiological) factors serve as stimuli.

As already noted, eggs can provide a long-lasting escape from unfavorable conditions. At least some tardigrades can produce both *subitaneous* (non-resting) and *resting eggs* (Bertolani *et al.* 2004). Altiero *et al.* (2009) examined the eggs of *Paramacrobiotus richtersi* (Figure 17) and found that the percentage of hatching was high (75-93%), but that four different patterns were discernible. Subitaneous eggs hatched in 30-40 days. Delayed hatching eggs hatched in 41-62 days. Some eggs required 90 days or more if the culture was wet and 13% of these (*diapause resting eggs*) required a dry period followed by rehydration. The remainder (87% of this last >90-day category) never hatched. They considered this variable hatching time to be a form of *bet-hedging*.

**Eggs**

Eggs that are laid externally are typically ornamented (Figure 75-Figure 76) (Nelson 1991a). These may be laid singly or in groups.

**Migration?**

Anhydrobiosis is not the only strategy available to organisms to escape drying conditions. Some organisms migrate to deeper levels of the moss or soil to escape...
drought. However, it appears that this option might not be available to many of the slow-moving tardigrades.

Wright (1991) found that those tardigrades living in the interstices of the moss habitat migrate vertically to the soil C-zone (layer just above bedrock) as a means of escaping or slowing desiccation. The exception to this behavior is *Echiniscus testudo* (Figure 5).

Nelson and Adkins (2001) examined this depth relationship in cushions of the moss *Schistidium rivulare* (=Grimmia alpicola; Figure 77). They found that among five species, only one (*Echiniscus viridissimus*) was more frequent in the top layer, regardless of the wet or dry condition of the moss. (Hmmm... Could the green that gives it its name indicate it has a photosynthetic symbiont that requires light, or just a penchant for green food?)

Nelson and Adkins (2001) concluded that none of the *Schistidium* (Figure 77) inhabitants used migration as a means to escape reduction in moisture. They speculated that for tardigrade inhabitants of xeric mosses, there was no advantage to migration. Rather, they stayed put and went into a state of anhydrobiosis in both upper and lower layers.

**Figure 77.** *Schistidium rivulare*, a moss where excessive hydration can cause death to its tardigrade inhabitants. Photo by Michael Lüth, with permission.

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### Literature Cited


