CHAPTER 5-1
TARDIGRADE SURVIVAL

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, The Water Bear). Tardigrades are just the right size to move among the bryophyte leaves, they lumber along slowly like bears, and they are downright cute!

Tardigrades can be found in marine, aquatic, and terrestrial habitats. On land they frequently live in association with bryophytes (Figure 3; Figure 4) and lichens (Miheleie 1967; Mehlen 1969; Utsugi 1984; Meiningier et al. 1985; Mancardi 1988; Szymanska 1994; Bertolani & Rebecchi 1996; Tarter et al. 1996; Miller 1997; Jarez Jaimes et al. 2002; Boeckner et al. 2006; Bartels et al. 2009; Meyer & Hinton 2009; Rossi et al. 2009; Simmons et al. 2009).

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, 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.

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.

Most of the terrestrial tardigrades are bryophyte inhabitants (Nelson 1991a). These terrestrial bryophyte taxa have a life span ranging from 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* (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).

**Suitability of Bryophytes as Habitat**


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 6). 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.)

Ramazzotti and Maucchi (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 they 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.

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) on Encalypta streptocarpa [= E. contorta] (Figure 7) 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 8) 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! Perhaps tardigrades were the inspiration for the Rip Van Winkle story.

Moisture seems to be the greatest determinant of the species distribution among bryophytes. Their 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.

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. Like the insects, they have chitin, in this case in the innermost layer of the
The chitinous armor of some terrestrial tardigrades (heterotardigrades) may slow drying and offer protection from damage while dry. Of course their small size is essential for living in the miniature world of bryophytes. And their claws (Figure 9 - Figure 11) may permit them to clamber about more easily among the leaves and branches of the bryophytes.

Their light weight facilitates their dispersal. For many, the stylets permit them to suck the contents out of bryophyte cells, among other things. Their bodies are flexible, permitting them to nestle in leaf axils or move in small spaces. But most of these as adaptations to the bryophyte habitat are speculation. There have been no tests to determine if any of these traits actually increases their survival in 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 12) among bryophytes on a mountain top in Hungary. This cute little bear has two horns on its head (Figure 13) and a nice salmon color. But it is slow and clumsy, out-classed by the faster-moving and more abundant *Ramazzottius oberhaeuseri* (Figure 19). 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.

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 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 fleshy body must be hydrated for oxygen to enter it.

**Physical Adaptations**

The soft-bodied tardigrades appear to have few structural adaptations to survive drought. Some, like *Echiniscus*, have long hairs (Figure 14 - Figure 15), 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. Hmmmm...What might their function be?

Others may have a bit more protection. Some bryophyte-dwelling species such as *Cornechiniscus cornutus* (Figure 16) and some members of the genera *Echiniscus* (Figure 17 - Figure 18) and *Ramaszottius* (Figure 19 - Figure 20) (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!

It is possible that the wonderful colors of some tardigrades (Figure 21 - Figure 22) are adaptations against UV damage to DNA during prolonged periods in a cryptobiotic state. Terrestrial tardigrades come in green, brown, yellow, orange, pink, red, purple, or black, whereas aquatic ones are white (Hebert 2008). Such pigmentation advantages have been demonstrated in bryophytes (Martinez 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.
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.

Literally meaning "hidden life," cryptobiosis is a state of suspended animation in which the organism is able to survive unfavorable conditions without expending much 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: Tardigrades). But in a cryptobiotic state, as discussed below, tardigrades can survive not only desiccation, but temperatures as low as 0.05K (-272.95ºC) for 20 hours or -200ºC for 20 months (Miller 1997). They have even survived 151ºC 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 tardigrades will be the ones to survive when everything else is deceased.
The ability of tardigrades to undergo cryptobiosis is more widely known than their encystment behavior. True cryptobiotic states are survived as a tun.

**Tun Formation**

When they undergo desiccation, the tardigrades form a tun (Figure 23 - Figure 33) (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 23. Tun of *Ramazzottius oberhaeuseri*. Photo by Martin Mach.

Figure 24. Tardigrade tun – water bear in a state of anhydrobiosis. Photo by Janice Glime.

Figure 25. Tardigrade tun – water bear in a state of anhydrobiosis. Note the buccal apparatus (resembles a tuning fork on left end). Photo by Janice Glime.

Figure 26. Tun of *Hypsibius* sp. Photo by Martin Mach.

Figure 27. Tun of *Echiniscus*. Photo by Martin Mach.

Figure 28. Tun of *Echiniscus* on moss leaf. Photo by Martin Mach.

Figure 29. Multiple tuns of *Echiniscus* on a single moss leaf. Photo by Martin Mach.
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Figure 30. Tun of Echiniscus on moss leaf. Photo by Martin Mach.

Figure 31. Tun of Echiniscus on a moss leaf. Photo by Martin Mach.

Figure 32. Tun of Echiniscus. Photo by Martin Mach.

Figure 33. Tun of Echiniscus. Photo by Martin Mach.

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. Despite the waxy protection, the water content is reduced to less than 1% (Lindahl & Balser 1999) and the tun becomes shrivelled and wrinkled (Hingley 1993). Echiniscus testudo, 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 their successful recovery from the tun state.

Tun formation is essential to tardigrade survival under desiccating conditions. For Paramacrobiotus areolatus, 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.

They revive (Figure 34) 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 34. Echiniscus sp. rehydrated after four years of desiccation. Photo by Martin Mach.

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. 2009), ultimately accumulating too much for successful repair. Hence, the tun does not completely protect them, and their chances of survival decrease with time.

**Effects of Size**

Jönsson et al. (2001) found that size influenced survival of cryptobiotic tardigrades, but that direction of influence differed among species. The common *Ramazzottius oberhaeuseri* (300 µm length; Figure 35) had a much higher survival rate (66%) (Figure 36) than did *Richtersius coronifer* (40%) (up to 1 mm length; Figure 37). *Ramaazzottius oberhaeuseri* has a high ability to retain water, perhaps contributing to an advantage for larger individuals with lower surface area to volume ratio.

Within *Richtersius coronifer* (Figure 37), large individuals were less likely to survive cryptobiosis than medium-sized ones (Figure 38); 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 35.** *Ramazzottius oberhaeuseri*. Photo by Martin Mach.

**Figure 36.** 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.

**Figure 37.** *Richtersius coronifer*, clinging to an algal cell. Photo by Martin Mach.

**Figure 38.** Probability of survival from anhydrobiosis for large and medium-sized *Richtersius coronifer* as a function of storage cell size. Probability is based on the predicted values from a logistic regression model, using buccal tube length, category, storage cell size, and interaction between the last two categories. Redrawn from Jönsson & Rebecchi 2002, in Bertolani et al. 2004.

Jönsson and Rebecchi (2002) likewise found that medium-sized tardigrades had a better chance of survival than did large ones in *Richtersius coronifer*. Large storage cell size was an important parameter to predict greater survival in the large tardigrades (Figure 38).

Reuner et al. (2010) described the storage cells as free-floating cells in *Milnesium tardigradum* (Figure 39),
**Paramacrobiotus tonollii** 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**, 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.

**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 and Bertolani 2001)! But, sadly, this record has been called into question, and the tardigrade never fully recovered. 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 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**, **Echiniscus testudo**, and **E. trisetosus** (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** survived up to 1604 days, whereas **Echiniscus** spp. lived only 1085 days.

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** 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 40) on house roofs (Corbet & Lan 1974) or among branches of the epiphyte **Orthotrichum cupulatum** (Figure 41) (Jönsson et al. 2001).

Like the rotifers, tardigrades suspend their aging clock while they are dormant (Hengherr et al 2008). **Milnesium tardigradum** 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 42) and **Macrobiotus** (Figure 43) 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.
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. 2009). In other cases, or in consort, oxidative damage may occur. Using Paramacrobiotus richtersi 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.

\section*{Anhydrobiosis}

The most common of the cryptobiotic states is \textit{anhydrobiosis} (state of dormancy brought on by dehydration). In their state of \textit{anhydrobiosis}, tardigrades can remain inactive during unfavorable conditions such as prolonged dryness (Kinchin 1987b). \textit{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 37) and Echiniscus spiniger failed to respond to the treatment, whereas Milnesium tardigradum declined under increased hydration. But even Richtersius coronifer experienced reduction in the density of eggs (Figure 44 - Figure 45) 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.

\textit{Richtersius coronifer} can increase its survival rate by forming \textit{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.
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Figure 44. Egg of *Richtersius coronifer*. Photo by Martin Mach.

Figure 45. *Macrobiotus magdalenae* egg showing the highly decorated nature that is typical of eggs laid free from the *exuvia* (shed body shell). In this state it can survive as well as in a tun. Photo by Łukasz Kaczmarek and Łukasz Michalczyk.

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).

**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 46. 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 47). Even animals in a molt can enter anoxybiosis (Figure 48).

Figure 46. *Macrobiotus hufelandi* male in anoxybiotic state, showing lack of tun formation. Photo by Martin Mach.

Figure 47. 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.

Figure 48. Tardigrade induced into anoxybiosis during its molt. Photo by Martin Mach.

Revival to normal state (Figure 49) 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).

**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 1992; Ramlovs & 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.

![Tardigrade](image)

Figure 49. 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.

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 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.

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* is reduced by 50% following ca 7x10⁶ 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* and *Bertolanius [=Amphibolus] nebulosus* and compared their cryoprotective strategies. *Richtersius coronifer* lives in drought-resistant mosses and overwinters in a frozen or dry state (cryobiosis). *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.

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 50). *Ramazzottius oberhaeuseri* seems to be almost indestructible down to -80°C, whereas *Hypsibius dujardini* had only 20% survival at that temperature. In fact, it had less than 80% survival at -9°C.

![Survival Graph](image)

Figure 50. Comparison of survival of three bryophytedwelling tardigrades subjected to sub-zero temperatures. Redrawn from Bertolani et al. 2004.

**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 cryobiosis 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 51 - Figure 54) 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 anoxbiosis. 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 (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, the new cuticle is similar to that found on the non-encysted organisms, whereas in Dactylobiotus parthenogeneticus the ultrastructure of the new cuticle differs. The tardigrade retracts within the cuticle (Nelson 1991a).

Figure 51. *Dactylobiotus* sp. Photo by Yuuji Tsukii.

Figure 52. *Dactylobiotus dispar*. Photo by Martin Mach.

Figure 53. Eggs of *Dactylobiotus dispar*. Photo by Martin Mach.

Figure 54. Egg of *Dactylobiotus dispar*. Photo by Martin Mach.

Figure 55. 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.
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Using *Bertolanius volubilis* from the mosses *Racomitrium sudeticum* (Figure 59) and *R. elongatum* (Figure 60) 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 factors serve as stimuli.

Eggs

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* 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 that are laid externally are typically ornamented (Figure 61 - Figure 62) (Nelson 1991a). These may be laid singly or in groups.

Figure 61. Egg of a tardigrade, a stage that helps it survive desiccation. Photo by Martin Mach.

Figure 62. Macrobiotus szeptycki egg showing the highly decorated surface of eggs laid free from the exuvia. Photo by Łukasz Kaczmarek and Łukasz Michalczyk.

Developmental Stages

Schill and Fritz (2008) examined the effects of humidity levels (10, 20, 31, 40, 54, 59, 72, 81%) on five different developmental stages of Milnesium tardigradum (Figure 63). They determined that the younger stages were more sensitive to desiccation. During the first three days of development, low humidity decreased the hatching rate following rehydration. When embryos were subjected to low humidity, development was delayed and they experienced a reduction in hatching rates following rehydration. At least in this case, further development affords greater survival of drought.

Figure 63. Milnesium tardigradum, a bryophyte dweller whose younger stages are the most susceptible to desiccation. Photo by Yuuji Tsukii.

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 the slow-moving tardigrades.

Nelson and Adkins (2001) examined this depth relationship in cushions of the moss Schistidium rivulare [=Schistidium alpicola] (Figure 64). 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. (Hmm... Could the green that gives it its name indicate it has a photosynthetic symbiont that requires light, or just a penchant for green food?)

Figure 64. Schistidium rivulare, a moss where excessive hydration can cause death to its tardigrade inhabitants. Photo by Michael Lüth.

None of the species appeared to use migration as a means to escape reduction in moisture. Nelson and Adkins (2001) 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.
Summary

Tardigrades (water bears) are common in both aquatic and terrestrial bryophytes. The land dwellers require a water film and thus are called limnoterrestrial tardigrades. Despite their worldwide distribution, they are not well known.

The bryophyte habitat offers sufficient oxygen, wetting and drying, sufficient food, a dispersal vehicle, and protection. Moisture is probably the most important factor in their distribution. Species of bryophytes do not seem to affect the types of tardigrades species.

Tardigrades are adapted to the bryophyte habitat by their small size, styles that permit sucking contents from bryophyte cells, flexible bodies, and a very responsive life cycle. Colored pigments in some may offer UV protection, especially during dry periods. Tardigrades can encyst or go into a cryptobiotic state as a tun. Cysts may differ between summer and winter. Tardigrades must dry slowly to survive the cryptobiotic state. While in it, they are resistant to high and low temperature extremes, absence of water, extreme pressure, vacuum, and radiation. Anhydrobiosis is induced by diminishing hydration; cryobiosis is induced by low temperatures near 0ºC; osmobiosis is induced by a change in salinity; anoxybiosis is induced by low oxygen. Tardigrades form trehaloses that protect the cell membranes while dehydrated or at low temperatures. They typically can survive about 10 years in the tun, but one specimen resumed physiological activity after 120 years on a herbarium moss specimen, then died. Nevertheless, DNA damage accumulates during cryptobiosis; survival seems to be based on DNA repair. Furthermore, high temperatures and high humidity destroy trehalose.

Another means of long-term survival is by producing resistant eggs. Variable hatching times may provide a form of bet-hedging in some species.

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Literature Cited


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