Chapter 8
Anhydrobiotic Abilities of Tardigrades

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Abstract Tardigrades have been discovered in 1773 and are found in a variety of habitats within marine, freshwater, and terrestrial ecosystems. To survive in habitats that are prone to occasional drought, they possess the ability to enter a reversible state known as anhydrobiosis. The desiccation tolerance allows them to cope with temporal variation of available water and extended lifespan in an anhydrobiotic state with up to 20 years by producing a time shift in the age of tardigrades. The period of anhydrobiosis is limited by cumulative DNA damage and the function of repair pathways during and after rehydration. The same pathways are probably responsible for the tolerance of high doses of radiation. Heat shock proteins serve as molecular chaperones to preserve or restore the protein integrity and late embryogenesis abundant (LEA) proteins LEA proteins play an important role as well. In several desiccated species glass transition has been detected, which support the vitrification hypothesis.

8.1 Discovery of the Tardigrades

In 1773, J. A. E. Goeze (1731–1793), a German pastor from Quedlingburg, Saxony-Anhalt, Germany, was the first to describe tardigrades as "small water bears" (Fig. 8.1). He wrote ". . .this animal is strange because of its extraordinary anatomy and at first glance its appearance has a strong resemblance to a little bear. It is because of this I will name them small water bears. . ." (Goeze 1773). However, another German, J.C. Eichhorn, was maybe the first to discover these animals in 1767 but did not publish his findings until 1781 when he published a description of a "water animal" (Eichhorn 1781), 8 years after Goeze's "Herrn Karl Bonnets

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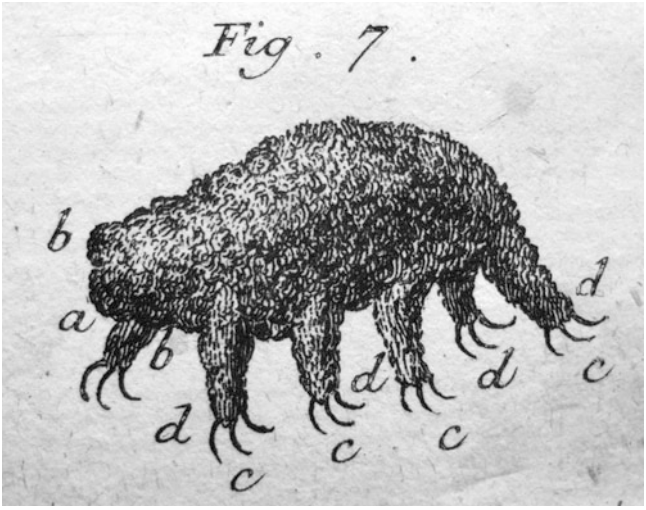

Fig. 8.1 The first drawing of a tardigrade, observed by J. A. E. Goeze and published in 1773

Abhandlungen aus der Insektologie." Shortly afterwards, L. Spallanzani (1729–1799), an Italian biologist and physiologist, also described the tardigrades and discovered their ability to desiccate and revive after "death" (Spallanzani 1776). He observed that these animals did not need a protective cover for this phenomenon and consequently suggested that these animals must slowly lose water to survive desiccation. The name "tardigrade" refers to the animal's way of movement (Lat. *tardus* – slow, *grado* – walker).

In 1834, the first tardigrade, *Macrobiotus hufelandi*, was described by C.A.S. Schultze (Schultze 1834). This species is probably the most well known of all tardigrades and was named after the German medical scientist C. W. Hufeland (1762–1836). He was the author of "Makrobiotik – Die Kunst das menschliche Leben zu verlängern" (The art of extending human life) (Hufeland 1817). In contrast to Spallanzani, Schultze spoke of a "reawakening" or "resurrection" of desiccated tardigrades after rehydration with water. His observations and interpretation were met with general disapproval. Chr. G. Ehrenberg (1795–1876), a German naturalist, zoologist, comparative anatomist, geologist, and microscopist, hypothesized that the animals secreted a medium during the dehydration process, in which they continued living and even reproduced. He believed that revived tardigrades which spent many years in desiccation were merely descendants of those formerly dehydrated (Ehrenberg 1834). Other scientists at the time also believed in abiogenesis (*generatio spontanea*).

The first substantive review of the field, "Mémoire sur les Tardigrades" (in 1840–1842), on the anatomy, systematics, and physiology of tardigrades was published by L. Doyère. His argument that the organism retained the integrity for revival during a slow desiccation process led to a quarrel with F.-A. Pouchet. Pouchet (1800–1872), a French naturalist and a leading proponent of spontaneous

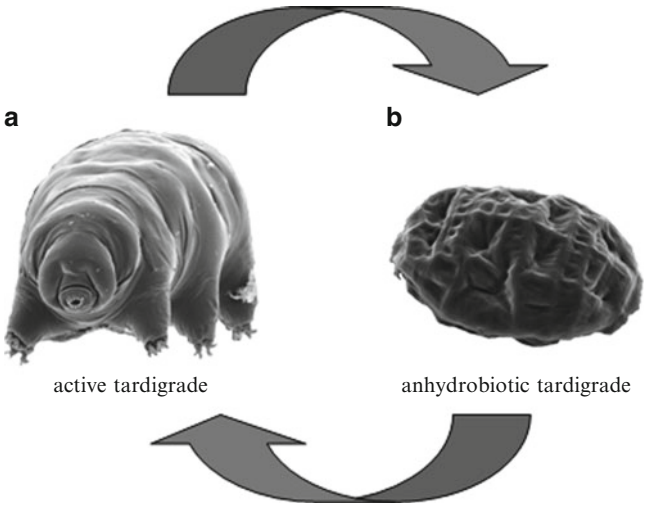

Fig. 8.2 A tardigrade in the active state (**a**) and in the anhydrobiotic, tun state (**b**)

generation of life from nonliving materials, was convinced that nothing could prevent the death of the entire organism after dehydration. This scientific dispute was settled in 1859 by the arbitral court of the Société de Biologie in Paris, France with Doyère as the victor. Since then, debates have focused more on whether tardigrade metabolism slows down (*vita minima*) or shuts down completely. At the start of the last century, the German Jesuit G. Rahm showed that desiccated tardigrades tolerated very low temperatures near absolute zero, and therefore metabolism seemed impossible (Rahm 1921). In contrast, H. Baumann, who studied the morphology and physiology of this desiccated stage worked on the assumption of *vita minima* (Baumann 1922). To this day, the ability of tardigrades to withstand unfavorable environmental conditions (in particular dryness) in a state of latent life or cryptobiosis remains a subject of particular interest (Fig. 8.2).

8.2 Cryptobiosis

A. v. Leeuwenhoek (1632–1723), the pioneering Dutch microscopist, was the first to describe the phenomenon of resurrection in desiccated animals (van Leeuwenhoek 1702). He discovered many small "animalcules" in the dry dust from a roof gutter after subsequent rehydration. These organisms were presumably bdelloid rotifers. Astonished, he confessed "I never thought that there could be any living creature in a substance so dried as this was" (van Leeuwenhoek 1702). Over the last 300 years different terms have been used to describe the resting stage of animals, until D. Keilin coined the term "cryptobiosis." This he defined as "the state of an organism when it shows no visible signs of life and when its metabolic rate becomes

hardly measurable, or comes reversibly to a standstill" (Keilin 1959). According to Keilin's definition, there are four different types of cryptobiosis: states resulting from low temperature (cryobiosis), lack of oxygen (anoxybiosis), high concentrations of salt (osmobiosis), and desiccation (anhydrobiosis), or various combinations. Metabolite research on a variety of organisms suggests that there are similarities between the states of anhydrobiosis and cryobiosis owing to the reduction of free water in the cells; however, it has been suggested that freezing and dehydration are not equivalent phenomena, but involve different mechanisms (Crowe et al. 1992).

8.3 Longevity and Long-Term Anhydrobiotic Ability

8.3.1 Longevity in Tardigrades

Although there have been some studies on the biochemical modifications associated with attainment of an anhydrobiotic state in several organisms (Clegg 1967; Crowe and Clegg 1973, 1978; Tomos 1992), there have been a number of studies concerning the effects of desiccation on the life cycle, longevity, and aging of animals (Ricci 1998; Ricci and Caprioli 1998, 2005; Ricci and Covino 2005; Ricci and Pagani 1997; Ricci et al. 1987; Wharton 2002). Ricci and Pagani (1997) proposed three different hypothetical responses to the effects of anhydrobiosis on aging: The "Sleeping Beauty" model postulates a complete disregarding of the entire time spent in anhydrobiosis. Another model describes a partial discount of the time spent in the dry state, meaning that the physiological age is less than the anagraphical age, due to the slowing down of the metabolism and development. The third model assumes that the anhydrobiotic organism registers the exact time spent in the anhydrobiotic state, resulting in a nonextended longevity. For the tardigrade species *Milnesium tardigradum*, it has been shown that the "Sleeping Beauty" model is the appropriate (Hengherr et al. 2008a). This species shows a complete disregard of the time spent in anhydrobiosis and does not age during anhydrobiosis when compared with control cohorts.

8.3.2 Long-Term Anhydrobiotic Ability of Tardigrades

The ability to survive unfavorable environmental conditions in an anhydrobiotic state at all stages of the tardigrade life cycle is of great importance from an ecological and evolutionary point of view (Schill and Fritz 2008). This ability enables these organisms to inhabit extreme, harsh, and ephemeral environments as well as allowing them to disperse and colonize new habitats. Survival of newly founded populations is also facilitated by the widespread occurrence of parthenogenesis in tardigrades. Being able to survive desiccation in all embryonic states gives tardigrades an obvious advantage over predatory and parasitic organisms, which

may be unable to survive such conditions (Schill and Fritz 2008). Despite the "Sleeping Beauty" model it is unlikely that natural habitats occupied by tardigrades stay dry for several years, decades, or even centuries and it is probable that there is an upper limit to the potential longevity in the anhydrobiotic state (Crowe 1975). Jönsson and Bertolani (Jönsson and Bertolani 2001) took a closer look at the long-term anhydrobiotic abilities and came to the conclusion that there is little support for the claim that tardigrades are capable of century-long survival, as suggested by Franceschi (1948). In fact, the data suggest that a decade is a more realistic estimate of the upper limit of anhydrobiotic survival in tardigrades. The longest known observation of an extended lifespan in an anhydrobiotic state was documented in the species *Echiniscus testudo* with up to 20 years survival (Jørgensen et al. 2007). However, most authors have reported an upper limit of around 10 years (Guidetti and Jönsson 2002; Rebecchi et al. 2006). This is presumably due to the cumulative damaging effects of free radical oxidation processes on cellular and molecular structures and the inactivation of DNA repair enzymes and free radical scavenging enzymes, e.g., superoxide dismutase (SOD) that possesses antioxidant effects (Crowe 1975; Örstan 1998; Wright et al. 1992). A particular problem is the damage to membranes caused by lipid peroxidation (Crowe and Madin 1975; Womersley 1981). The result is a chemical aging (Schöneich 1999) in the anhydrobiotic tun state, which limits the survival capacity of anhydrobiotic tardigrades.

8.4 DNA Damage and Repair Mechanisms

Cumulative damage incurring during cryptobiosis may have lead to selection for particularly efficient repair pathways. These are clearly essential upon rehydration, and DNA repair is presumably the secret of the tremendous ability to tolerate desiccation for a long time. This is evidenced by the fact that tardigrades tolerate high doses of radiation which should, of course, result in DNA damage and subsequently DNA repair. Jönsson et al. (2005) studied the radiation tolerance in the species *Richtersius coronifer*. Desiccated as well hydrated animals which were irradiated with 0.5 and 1 kGy did not deviate in survival from the control. He suggested that radiation tolerance in tardigrades is not due to cellular protection through metabolites or proteins, but to efficient repair mechanisms. For the species *M. tardigradum*, median lethal doses of 5,000 Gy (gamma-rays) and 6,200 Gy [high-linear energy transfer (LET) heavy ions, ^{4}He] in hydrated tardigrades, and 4,400 Gy (gamma-rays) and 5,200 Gy [high-linear energy transfer (LET) heavy ions, ^{4}He] in anhydrobiotic tardigrades at 48 h after irradiation, have been calculated (Horikawa et al. 2006). In *R. coronifer*, irradiation with >1,000 Gy prevents reproduction.

Gladyshev and Meselson Gladyshev and Meselson (2008) demonstrated the extraordinary resistance of bdelloid rotifers to ionizing radiation. The resulting double-strand DNA breakage was accompanied by enhanced either by systems that repair such damage, those that protect the repair systems, or both. They suggested that such breakage and repair systems may have maintained bdelloid

chromosomes as colinear pairs and kept the load of transposable genetic elements low, because accurate repair of double-strand breaks requires the presence of a homologous template. However, the phenomenon seems to be species-specific and a consequence of the evolutionary adaptation of the bdelloid rotifers to survive the desiccation encountered in their characteristic habitats which periodically run dry and become wet after rainfall. In the tardigrade species *M. tardigradum*, the effect of anhydrobiosis on the integrity of DNA has been shown with the technique of microgel electrophoresis of single cells (DNA Comet Assay) (Neumann et al. 2009; Schill et al. 2008). The process of desiccation itself induces only minor DNA damage in cells, while the damage is clearly linked to the time the specimen was maintained in anhydrobiosis. A time-course experiment showed that the maximum level of DNA fragments in the comet tails was reached 90 min after rehydration. The formation and expansion of the comet tails is primarily not due to the damage itself, but instead reflects the excision repair system.

8.5 Protection and Repair with Proteins

8.5.1 Heat Shock Proteins

In the cellular stress response, several families of heat shock/stress proteins (HSPs) serve as molecular chaperones and can be induced by a wide variety of stressors that result in the accumulation of unfolded or misfolded proteins in cells, i.e., proteotoxicity (Hightower 1993). In general, during such proteotoxic conditions, there is a redirection of metabolism, leading to rapid up-regulation of HSP genes. One such family member, Hsp70 is a highly conserved and abundant protein, involved in facilitating protein maturation, stabilization of the conformation of folding intermediates, prevention of the formation of aberrant structures and leading polypeptides to biologically productive pathways (Gething and Sambrook 1992). In cysts of *Artemia* sp., Clegg et al. (1994) showed that protein p26 underwent an extensive stress-induced translocation to nuclei and other sites. These translocations exhibited strong pH dependence (Clegg et al. 1995) in a consistent trend with intracellular pH changes in vivo (Gnaiger et al. 2000; Hand 1998; Hand and Hardewig 1996; van Breukelen and Hand 2000; van Breukelen et al. 2000). Subsequent studies (Liang et al. 1997a, b) showed that p26 is a member of the small heat shock/α-crystallin family of proteins (de Jong et al. 1998; Sun and MacRae 2005) and demonstrated that this protein exhibited molecular chaperone activity in vitro (Liang et al. 1997b), and probably in vivo (Liang and MacRae 1999). In the tardigrade species *R. coronifer* when shifted from an active state to an anhydrobiotic state, Ramløv and Westh (2001) detected an induced protein with a molecular weight of approximately 71 kDa, which potentially belongs to the Hsp70 heat shock protein family. Three heat shock protein (*hsp70* family) genes are known in *M. tardigradum*, which are differently expressed during dehydration, rehydration, the active, and the anhydrobiotic state (Schill et al. 2004). One of these isoforms is significantly induced in the transitional stage between the

active and the cryptobiotic state. The resulting comparatively high number of mRNAs may be stored for repair processes after rehydration. A further hint concerning general mechanisms of stabilization and repair at the protein level has been demonstrated in *R. Coronifer,* with elevated levels of stress proteins (Hsp70) are detected in rehydrated tardigrades after a period of desiccation (Jönsson and Schill 2007). This suggests that Hsp70 may be involved in the biochemical repair system and that it is connected to cellular repair processes after rehydration rather than to biochemical stabilization in the dry state.

8.5.2 LEA Proteins

In plants, and more recently in several animals, induction of high levels of hydrophilic proteins, in particular the late embryogenesis abundant (LEA) proteins, has been associated with water stress. LEA proteins were first identified 20 years ago in plants, where they are produced during seed development (Galau et al. 1986; Grzelezak et al. 1982). However, their precise function is poorly understood. To be classified as molecular chaperones, LEA proteins must not only prevent aggregation, they must additionally form transient, noncovalent complexes (Ellis 2004). Unfavorable protein–protein interactions, however, can lead to irreversible conformational changes and, in enzymes, a loss of catalytic activity (Carpenter et al. 1987). LEA proteins might simply function as "molecular shields", forming a physical barrier between partially unfolded neighboring proteins and preventing contact between them (Tunnacliffe and Wise 2007). LEA protein expression has more recently been demonstrated in other organisms, also linked to desiccation stress and the acquisition of desiccation tolerance. So far these organisms include microorganisms (Battista et al. 2001), nematodes (Browne et al. 2002; Goyal et al. 2003, 2005), rotifers (Tunnacliffe et al. 2005), a chironomid larvae (Kikawada et al. 2006), and Collembola (Bahrndorff et al. 2009). Research on LEA proteins has recently focussed on tradigrades because of the evidence that these proteins play an important role in protecting cellular proteins. In the tardigrade species *M. hufelandi*, putative LEA proteins have been isolated from the tun state (McGee et al. 2004); however, more results will be expected in the future.

8.6 Sugars and Vitrification

8.6.1 Hypothesis of Cell Stabilization

In the early sixties, S.J. Webb postulated the water replacement hypothesis in which accumulated hydrophilic molecules such as nonreducing sugars (e.g., trehalose and sucrose) interact with macromolecules through hydrogen bonds, leading to the replacement of water (Crowe et al. 1987, 1992; Crowe 2002; Webb 1964;

Webb et al. 1965). Over the last three decades considerable research has centered on the role of these sugars, particularly trehalose in animals (Yancey et al. 1982; Vertucci and Farrant 1995; Ingram and Bartels 1996; Chandler and Bartels 1999; Alpert 2000) and sucrose in higher plants (Crowe and Clegg 1973; Potts 2001; Crowe et al. 1997; Alpert 2000). These carbohydrates appeared to be ubiquitous in cryptobiotes and serve multiple additional roles (1) as compatible intracellular osmolytes during desiccation or freeze-dehydration; (2) as stabilizers of protein quaternary structure and lipid bilayer integrity with declining free water activity; and (3) as supercoolants. Trehalose is critical to desiccation tolerance in the cysts of the brine shrimp *Artemia* sp. Many studies have demonstrated its important role in the protection of cellular and macromolecular structures by replacing the water that is normally hydrogen-bonded to polar residues (Clegg 1986). Also of some importance is the vitrification hypothesis proposed by Crowe et al. (Crowe et al. 1998; Crowe 2002). This hypothesis suggests that hydrophilic molecules enter a glassy state during desiccation which prevents denaturation, aggregation, and disintegration because of immobilization. These two proposed hypotheses are not mutually exclusive.

8.6.2 The Role of Sugars in Tardigrades

There are many examples of trehalose accumulation in anhydrobiotic organisms, in adults, juveniles, and different developmental states. The desiccated embryo of brine shrimp *Artemia* sp. in the cyst contains substantial concentrations of trehalose. This can be as high as 13–18% of dry weight (Clegg 1965) and the anhydrobiotic nematode *Aphelenchus avenae* can produce 10–15% of its dry weight as trehalose when dried slowly (Madin and Crowe 1975). Similarly, a large amount of trehalose (18% of dry body mass) is rapidly accumulated in the African chironomid larvae *Polypedilum vanderplanki* (Okuda et al. 2004; Watanabe et al. 2002, 2003) and plays a role in water replacement and intracellular glass formation (Sakurai et al. 2008). Trehalose was also detected in anhydrobiotic states of the freshwater sponge *Trochospongilla* sp. (gemmules), the bryozoan *Cristatella mucedo* (statoblasts) and the mictic eggs of crustaceans *Daphnia magna, D. pulex*, *Triops longicaudatus,* and *T. cancriformis* (Hengherr et al. 2008b). The first confirmation of the presence of trehalose in tardigrades was in the species *Macrobiotus areolatus* (Crowe 1975); later Westh and Ramløv (1988) observed the production of trehalose in cryptobiosis in Arctic tardigrades. However, compared with the level of trehalose in *Artemia* sp., *A. avenae,* and *P. vanderplanki* very little trehalose was detected in the tun state of *R. coronifer* (0.1–2.3% of dry weight body mass) (Westh and Ramlov 1991). Hengherr et al. (2008c) measured the trehalose levels of six eutardigrade species (*M. tardigradum*, *Macrobiotus tonollii*, *Macrobiotus sapiens*, *Paramacrobiotus richtersi*, *Paramacrobiotus* "*richtersi* group" 1, and *Paramacrobiotus* "*richtersi* group" 2) and two heterotardigrade species (*E. testudo* and *E. granulatus*) during the induction of anhydrobiosis, the anhydrobiotic state, the

rehydration phase, and after a 4-h active period. The data reveal great differences between the species. In *Macrobiotus* and *Paramacrobiotus* species, the increase in trehalose takes place during tun formation, and trehalose degradation is induced within 10–15 min following rehydration. *Echiniscus* showed the presence of trehalose but not desiccation-induced changes, while *Milnesium* showed no detectable trehalose. Lapinski and Tunnacliffe (2003) also demonstrated excellent desiccation tolerance of the bdelloid rotifers *Philodina roseola* and *Adineta vaga*, without measurable levels of trehalose and no expression of trehalose synthase genes. It seems that the apparent lack of trehalose in certain organisms does not preclude excellent desiccation tolerance, and that there are multiple strategies for desiccation tolerance in different organisms.

8.6.3 Vitrification in Tardigrades

A glass is a liquid of a quite high viscosity that it is capable of slowing down or inhibiting chemical reactions (see Sect. 6.1). Molecules embedded in a sugar and/or protein glass should gain substantial chemical stability compared with their counterparts diffusing freely in aqueous solutions. Glasses show temperature-dependent transitions during which they change from a glassy mechanical solid state to a state with decreased viscosity and the vitrification (glass transition) temperature and can be measured by differential scanning calorimetry (DSC). Viability studies on seeds resulted in predictions of the maximum temperature at which seeds will survive at a given water content and demonstrated the transition from a glassy mechanical solid to a state with a distinct decreased viscosity (Sun et al. 1984). Several other studies have been carried out on pollen (Hoekstra et al. 1992a, b) and animals (Clegg 1974; Sakurai et al. 2008). If vitrification is responsible for the tolerance in anhydrobiotic organisms, the thermal limits for the formation of amorphous glasses and survival in desiccated organisms should be dictated by the vitrification temperature and dependent on the final concentration of carbohydrates, proteins, and water. One of the first heat tolerance experiments demonstrated that anhydrobiotic tardigrades of the species *M. hufelandi* survived a short exposure to temperatures between 120 and 125°C (Doyère 1842). Later, Baumann (1927) reported that anhydrobiotic tardigrades could tolerate 100°C for 6 h and *R. coronifer* survived temperatures up to approximately 70°C for 60 min without any decrease in survival (Ramløv and Westh 2001). A systematic study with seven eutardigrade species (*M. sapiens*, *P* "*richtersi* group 1," *P*", *richtersi* group 2", *P.* "*richtersi* group 3", *M. tonollii*, *P. richtersi*, *M. tardigradum*) and two heterotardigrades (*E. granulatus* and *E. testudo*) has been completed recently (Hengherr et al. 2009). Exposure to temperatures up to 80°C for 1 h resulted in a moderate decrease in survival of the tardigrades. At 95°C all *Macrobiotus* and *Paramacrobiotus* species died. The heterotardigrade species *E. granulatus* and *E. testudo* survived, though with a low survival rate. However, *M. tardigradum* showed recovery rates of >90% after experiencing 100°C but followed by a steep decrease after exposure to higher

temperatures. The temperature of 102°C for 1 h was the limit of the tolerance. Assuming the formation of an amorphous glass during desiccation by nonreducing carbohydrates and hydrophilic proteins as one of the major mechanisms to protect cellular structures from denaturation, coagulation, and disintegration, glass transition temperature is a limiting factor for high temperature tolerance in tardigrades. By using DSC, glass transition in the desiccated *Macrobiotus* and *Paramacrobiotus* species has been detected and therefore provides the first evidence for vitrification in tardigrades during anhydrobiosis (Hengherr et al. 2009). No glass transition could be detected in the two heterotardigrade species and *M. tardigradum*, which may indicate that these species use other mechanisms to tolerate desiccation and heat stress.

References

Alpert P (2000) The discovery, scope, and puzzle of desiccation tolerance in plants. Plant Ecol 151:5–17

Bahrndorff S, Tunnacliffe A, Wise MJ, McGee B, Holmstrup M, Loeschcke V (2009) Bioinformatics and protein expression analyses implicate LEA proteins in the drought response of Collembola. J Insect Physiol 55:210–217

Battista JR, Park MJ, McLemore AE (2001) Inactivation of two homologues of proteins presumed to be involved in the desiccation tolerance of plants sensitizes *Deinococcus radiodurans* R1 to desiccation. Cryobiology 43:133–139

Baumann H (1922) Die Anabiose der Tardigraden. Zool Jahrb 45:501–556

Baumann H (1927) Anabiosis of tardigrades. Zool Anz 72:175–179

Browne J, Tunnacliffe A, Burnell A (2002) Anhydrobiosis – plant desiccation gene found in a nematode. Nature 416:38

Carpenter JF, Crowe LM, Crowe JH (1987) Stabilization of phosphofructokinase with sugars during freeze-drying characterization of enhanced protection in the presence of divalent cations. Biochim Biophys Acta 923:109–115

Chandler J, Bartels D (1999) Plant desiccation. In: Lerner HR (ed) Plant Responses to Environmental Stresses: From Phytohormones to Genome Reorganization. Marcel Dekker, New York, pp 575–590

Clegg JS (1965) Origin of trehalose and its significance during formation of encysted dormant embryos of *Artemia salina*. Comp Biochem Physiol 14:135–143

Clegg JS (1967) Metabolic studies of crytobiosis in encysted embryos of *Artemia salina*. Comp Biochem Physiol 20:801–809

Clegg JS (1974) Biochemical adaptations associated with the embryonic dormancy of *Artemia salina*. Trans Am Microsc Soc 93:481–490

Clegg JS (1986) The physical properties and metabolic status of *Artemia* cysts at low water contents: the water replacement hypothesis. In: Leopold AC (ed) Membranes, Metabolism and Dry Organisms Cornell University Press, New York, pp 169–187

Clegg JS, Jackson SA, Warner AH (1994) Extensive intracellular translocations of a major protein accompany anoxia in embryos of *Artemia franciscana*. Exp Cell Res 212:77–83

Clegg JS, Jackson SA, Liang P, Macrae TH (1995) Nuclear–cytoplasmic translocations of protein p26 during aerobic–anoxic transitions in embryos of *Artemia franciscana*. Exp Cell Res 219:1–7

Crowe JH (1975) The physiology of cryptobiosis in tardigrades. Memorie dell'Istituto Italiano di Idrobiologia Dott Marco de Marchi 32:37–59

Crowe LM (2002) Lessons from nature: The role of sugars in anhydrobiosis. Comp Biochem Physiol A Mol Integr Physiol 131:505–513
Crowe JH, Clegg JS (1973) Anhydrobiosis dowden. Hutchinson and Ross, Stroudsburg, PA, p 477
Crowe JH, Clegg JS (eds) (1978) Dry biological systems. Academic, New York
Crowe JH, Madin KAC (1975) Anhydrobiosis in nematodes evaporative water loss and survival. J Exp Zool 193:323–334
Crowe JH, Crowe LM, Carpenter JF, Wistrom CA (1987) Stabilization of dry phospholipid-bilayers and proteins by sugars. Biochem J 242:1–10
Crowe JH, Carpenter JF, Crowe LM (1998) The role of vitrification in anhydrobiosis. Annu Rev Physiol 60:73–103
Crowe JH, Crowe LM, Petrelski S, Hoekstra FA, Araujo PD, Panek AD (1997) Anhydrobiosis: cellular adaptation to extreme dehydration. In: Dantzler WH (ed) Handbook of Physiology. Oxford University Press, New York
Crowe JH, Hoekstra FA, Crowe LM (1992) Anhydrobiosis. Annu Rev Physiol 54:579–599
de Jong WW, Caspers GJ, Leunissen JA (1998) Genealogy of the alpha-crystallin–small heat-shock protein superfamily. Int J Biol Macromol 22:151–162
Doyère PLN (1842) Memoires sur les Tardigrades. Sur le facilité que possedent les Tardigrades, les rotiferes, les anguillules des toits et quelques autres animalcules, de revenir à la vie après été completement desséchées. Ann Sci Nat Zool Biol Anim 2e:5–35
Ehrenberg CG (1834) Über das Wiederaufleben der Moosfauna. Okens Isis 6(7):710–713
Eichhorn JC (1781) Beyträge zur Naturgeschichte der kleinsten Wasserthiere, die mit blossem Auge nicht können gesehen werden und die sich in den Gewässern in und um Danzig befinden. F. Nicolai, Berlin and Stettin
Ellis JR (2004) From chloroplasts to chaperones: How one thing led to another. Photosynth Res 80:333–343
Franceschi T (1948) Anabiosi nei tardigradi. Boll Mus Ist Biol Univ Genova 22:47–49
Galau GA, Hughes DW, Dure L III (1986) Abscisic-acid induction of cloned cotton gossypium–hirsutum late embryogenesis-abundant lea messenger rna species. Plant Mol Biol 7:155–170
Gething MJ, Sambrook J (1992) Protein folding in the cell. Nature 355:33–45
Gladyshev E, Meselson M (2008) Extreme resistance of bdelloid rotifers to ionizing radiation. Proc Natl Acad Sci USA 105:5139–5144
Gnaiger E, Méndez G, Hand SC (2000) High phosphorylation efficiency and depression of uncoupled respiration in mitochondria under hypoxia. Proc Natl Acad Sci USA 97:11080–11085
Goeze JAE (1773) Herrn Karl Bonnets Abhandlungen aus der Insektologie aus d. Franz. übers. u. mit einigen Zusätzen hrsg. v. Joh. August Ephraim Goeze Gebauer, Halle. p 414
Goyal K, Tisi L, Basran A, Browne J, Burnell A, Zurdo J, Tunnacliffe A (2003) Transition from natively unfolded to folded state induced by desiccation in an anhydrobiotic nematode protein. J Biol Chem 278:12977–12984
Goyal K, Pinelli C, Maslen SL, Rastogi RK, Stephens E, Tunnacliffe A (2005) Dehydration-regulated processing of late embryogenesis abundant protein in a desiccation-tolerant nematode. FEBS Lett 579:4093–4098
Grzelezak ZF, Sattalo MH, Hanley-Bowdoin LK, Kennedy TD, Lane BG (1982) Synthesis and turnover of proteins and mRNA in germinating wheat embryos. Can J Biochem Physiol 60(3): 389–397
Guidetti R, Jönsson KI (2002) Long-term anhydrobiotic survival in semi-terrestrial micrometazonas. J Zool (1987) 257:181–187
Hand SC (1998) Quiescence in *Artemia franciscana* embryos: reversible arrest of metabolism and gene expression at low oxygen levels. J Exp Biol 201:1233–1242
Hand SC, Hardewig I (1996) Downregulation of cellular metabolism during environmental stress: Mechanisms and implications. Annu Rev Physiol 58:539–563
Hengherr S, Brümmer F, Schill RO (2008a) Anhydrobiosis in tardigrades and its effects on longevity traits. J Zool (1987) 275:216–220

Hengherr S, Heyer AG, Brümmer F, Schill RO (2008b) Trehalose as protecting agent in aquatic invertebrates during dormancy induced by desiccation. Comp Biochem Physiol A Comp Physiol 151:S34

Hengherr S, Heyer AG, Köhler HR, Schill RO (2008c) Trehalose and anhydrobiosis in tardigrades – evidence for divergence in responses to dehydration. FEBS J 275:281–288

Hengherr S, Worland MR, Reuner A, Brümmer F, Schill RO (2009) High-temperature tolerance in anhydrobiotic tardigrades is limited by glass transition. Physiol Biochem Zool 82:749–755

Hightower LEH (1993) A brief perspective on the heat-shock response and stress proteins. Mar Environ Res 35:79–83

Hoekstra FA, Crowe JH, Crowe LM (1992a) Germination and ion leakage are linked with phase transitions of membrane lipids during imbibition of *Typha latifolia* pollen. Physiol Plant 84:29–34

Hoekstra FA, Crowe JH, Crowe LM, van Roekel T, Vermeer E (1992b) Do phospholipids and sucrose determine membrane phase transitions in dehydrating pollen species? Plant Cell Environ 15(5):601–606

Horikawa DD, Sakashita T, Katagiri C, Watanabe M, Kikawada T, Nakahara Y, Hamada N, Wada S, Funayama T, Higashi S, Kobayashi Y, Okuda T, Kuwabara M (2006) Radiation tolerance in the tardigrade *Milnesium tardigradum*. Int J Radiat Biol 82:843–848

Hufeland CW (1817) Makrobiotik – Die Kunst das menschliche Leben zu verlängern. Fleischhauer, Reutlingen

Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annu Rev Plant Physiol Plant Mol Biol 47:377–403

Jönsson KI, Bertolani R (2001) Facts and fiction about long-term survival in tardigrades. J Zool (Lond) 255:121–123

Jönsson KI, Schill RO (2007) Induction of Hsp70 by desiccation, ionising radiation and heat-shock in the eutardigrade *Richtersius coronifer*. J Comp Physiol B 146:456–460

Jönsson KI, Harms-Ringdahl M, Torudd J (2005) Radiation tolerance in the eutardigrade Richtersius coronifer. Int J Radiat Biol 81:649–656

Jørgensen A, Møbjerg N, Kristensen RM (2007) A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. J Limnol 66:77–83

Keilin D (1959) The Leeuwenhoek Lecture. The problem of anabiosis or latent life: History and current concept. Proc R Soc Lond B Biol Sci 150:149–191

Kikawada T, Nakahara Y, Kanamori Y, Iwata K, Watanabe M, McGee B, Tunnacliffe A, Okuda T (2006) Dehydration-induced expression of LEA proteins in an anhydrobiotic chironomid. Biochem Biophys Res Commun 348:56–61

Lapinski J, Tunnacliffe A (2003) Anhydrobiosis without trehalose in bdelloid rotifers. FEBS Lett 553:387–390

Liang P, MacRae TH (1999) The synthesis of a small heat shock/alpha-crystallin protein in *Artemia* and its relationship to stress tolerance during development. Dev Biol 207:445–456

Liang P, Amons R, Clegg JS, MacRae TH (1997a) Molecular characterization of a small heat shock alpha-crystallin protein in encysted *Artemia* embryos. J Biol Chem 272:19051–19058

Liang P, Amons R, Macrae TH, Clegg JS (1997b) Purification, structure and in vitro molecular-chaperone activity of Artemia p26, a small heat-shock/alpha-crystallin protein. Eur J Biochem 243:225–232

Madin KAC, Crowe JH (1975) Anhydrobiosis in nematodes – carbohydrate and lipid-metabolism during dehydration. J Exp Zool 193:335–342

McGee B, Schill RO, Tunnacliffe A (2004) Hydrophilic proteins in invertebrate anhydrobiosis. Integr Comp Biol 44:679–679

Neumann S, Reuner A, Brümmer F, Schill RO (2009) DNA damage in storage cells of anhydrobiotic tardigrades. Comp Biochem Physiol A Mol Integr Physiol 153(4):425–429

Okuda T, Watanabe M, Kikawada T, Fujita A, Forczek E (2004) Cryptobiosis in the african chironomid: Physiological mechanism to survive complete dehydration. Proc Arthropod Embryol Soc Jpn 39:1–7

Potts M (2001) Desiccation tolerance: a simple process? Trends Microbiol 9:553–559
Örstan A (1998) Factors affecting long-term survival of dry bdelloid rotifers: a preliminary study. Hydrobiologia 387/388:327–331
Rahm P (1921) Effect of very low temperatures on the fauna of moss. Proc K Ned Akad Wet Ser C Biol Med Sci 23:235–248
Ramløv H, Westh P (2001) Cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*: Tolerance to alcohols, temperature and de novo protein synthesis. Zool Anz 240:517–523
Rebecchi L, Guidetti R, Borsari S, Altiero T, Bertolani R (2006) Dynamics of long-term anhydrobiotic survival of lichen-dwelling tardigrades. Hydrobiologia 558:23–30
Ricci C (1998) Anhydrobiotic capabilities of bdelloid rotifers. Hydrobiologia 387(388):321–326
Ricci C, Caprioli M (1998) Stress during dormancy: Effect on recovery rates and life-history traits of anhydrobiotic animals. Aquat Ecol 32:353–359
Ricci C, Caprioli M (2005) Anhydrobiosis in bdelloid species, populations and individuals. Integr Comp Biol 45:759–763
Ricci C, Covino C (2005) Anhydrobiosis of *Adineta ricciae*: Costs and Benefits. Hydrobiologia 546:307–314
Ricci C, Pagani M (1997) Desiccation of Panagrolaimus rigidus (nematoda): Survival, reproduction and the influence on the internal clock. Hydrobiologia 347:1–13
Ricci C, Vaghi L, Manzini ML (1987) Desiccation of rotifers (Macrotrachela quadricornifera): Survival and reproduction. Ecology 68:1488–1494
Sakurai M, Furuki T, K-i A, Tanaka D, Nakahara Y, Kikawada T, Watanabe M, Okuda T (2008) Vitrification is essential for anhydrobiosis in an African chironomid, *Polypedilum vanderplanki*. Proc Natl Acad Sci USA 105:5093–5098
Schill RO, Fritz GB (2008) Desiccation tolerance in embryonic stages of the tardigrade *Milnesium tardigradum*. J Zool (Lond) 276:103–107
Schill R, Steinbrück G, Köhler H (2004) Stress gene (*hsp70*) sequences and quantitative expression in *Milnesium tardigradum* (Tardigrada) during active and cryptobiotic stages. J Exp Biol 207:1607–1613
Schill RO, Neumann S, Reuner A, Brümmer F (2008) Detection of DNA damage with single-cell gel electrophoresis in anhydrobiotic tardigrades. Comp Biochem Physiol A Comp Physiol 151:S32
Schöneich C (1999) Reactive oxygen species and biological aging: amechanistical approach. Exp Gerontol 34:19–34
Schultze CAS (1834) *Macrobiotus hufelandii*, animale e crustaceorum classe novum, Berlin, p 8
Spallanzani L (1776) Opuscoli di Fisica Animale e Vegetabile. Modena: Società Tipografica, pp 203–285
Sun Y, MacRae TH (2005) Small heat shock proteins: molecular structure and chaperone function. Cell Mol Life Sci 62:2460–2476
Sun WQ, Irving TC, Leopold AC (1984) The role of sugar, vitrification and membrane phase transition in seed desiccation tolerance. Physiol Plant 90(4):621–628
Tomos D (1992) Life without water. Curr Biol 2:594–596
Tunnacliffe A, Wise MJ (2007) The continuing conundrum of the LEA proteins. Naturwissenschaften 94:791–812
Tunnacliffe A, Lapinski J, McGee B (2005) A putative LEA protein, but no trehalose, is present in anhydrobiotic bdelloid rotifers. Hydrobiologia 546:315–321
van Breukelen F, Hand SC (2000) Characterization of ATP-dependent proteolysis in embryos of the brine shrimp, *Artemia franciscana*. J Comp Physiol B 170:125–133
van Breukelen F, Maier R, Hand SC (2000) Depression of nuclear transcription and extension of mRNA half-life under anoxia in *Artemia franciscana* embryos. J Exp Biol 203:1123–1130
van Leeuwenhoek A (1702) On certain animalcules found in the sediments in gutters of the roofs of houses. Letter 144. The Selected Works of Anton van Leeuwenhoek, London, pp 207–213
Vertucci CW, Farrant JM (1995) Acquisition and loss of desiccation Marcel Dekker: New York

Watanabe M, Kikawada T, Minagawa N, Yukuhiro F, Okuda T (2002) Mechanism allowing an insect to survive complete dehydration and extreme temperatures. J Exp Biol 205:2799–2802

Watanabe M, Kikawada T, Okuda T (2003) Increase of internal ion concentration triggers trehalose synthesis associated with cryptobiosis in larvae of *Polypedilum vanderplanki*. J Exp Biol 206:2281–2286

Webb SJ (1964) Bound water, metabolites and genetic continuity. Nature 203:374–377

Webb SJ, Dumasia MD, Bhorjee JS (1965) Bound Water, Inositol, and the Biosynthesis of Temperate and Virulent Bacteriophages by Air-Dried *Escherichia coli*. Can J Microbiol 11:141–150

Westh P, Ramlov H (1991) Trehalose accumulation in the tardigrade *Adorybiotus coronifer* during anhydrobiosis. J Exp Zool 258:303–311

Westh P, Ramløv H (1988) Cryptobiosis in Arctic tardigrades with special attention to the appearance of trehalose. In: Höpner Petersen G (ed) Grönland Exkursion 2.–25., August 1987. Institut für Polarökologie, Kiel, pp 227–245

Wharton DA (2002) Nematode survival strategies. In: Lee DL (ed) The biology of nematodes. Taylor and Francis, London, pp 389–411

Womersley C (1981) Biochemical and physiological aspects of anhydrobiosis. Comp Biochem Physiol B Biochem Mol Biol 70:669–678

Wright JC, Westh P, Ramlov H (1992) Cryptobiosis in Tardigrada. Biol Rev 67:1–29

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Science 217:1214–1222