

2012

The ecology of tardigrades

Northcote-Smith, E.

Northcote-Smith, E. (2012) 'The ecology of tardigrades', *The Plymouth Student Scientist*, 5(2), p. 569-580.

<http://hdl.handle.net/10026.1/14001>

The Plymouth Student Scientist
University of Plymouth

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

The ecology of tardigrades

Emma Northcote-Smith

Project Advisor: [Paul Ramsay](#), School of Biomedical & Biological Sciences,
Plymouth University, Drake Circus, Plymouth, PL4 8AA

Introduction

Tardigrades are hydrophilic microscopic invertebrates belonging to the phylum Tardigrada and are more commonly known as 'water bears'. They were first discovered in 1773 by Goeze and since this time many species have been described (Romano 2003). They require a film of water to remain active and are found in marine, freshwater and terrestrial ecosystems ranging from deep sea abysses to the tops of mountains (Ramazzotti and Maucci 1983, Kinchin 1994). Tardigrades have a bilaterally symmetrical body plan with four pairs of lobopodous legs terminating in claws or sucking disks. The adults average 200- 500µm in size, although some adults reach 1200 µm and juveniles can be much smaller. Reproduction is either sexual or by parthenogenesis and males are generally smaller in size than females. The fluid-filled body cavity functions in respiration and circulation and they have a full digestive system (Nelson 2002).

Tardigrade life span is estimated at 3-30 months. This does not include periods of latency that may increase the life span greatly. Species with the ability to enter cryptobiosis are thought to have longer life spans than those that do not. Terrestrial species from the genera *Macrobiotus* and *Hypsibius* live for 4-12 years whereas the freshwater species from these genera live for 1-2 years, this is because of the inability of the freshwater species to enter cryptobiosis (Ramazzotti and Maucci 1983). Throughout life, tardigrades undergo periods of molting which last from 5-10 days (Walz 1982). Body size increases with each molt until the maximum size is reached and they will continue to molt and grow even after sexual maturity (Nelson 2002). During molting the old cuticle, claws and foregut and hindgut lining are shed and the tardigrade is unable to feed (Walz 1982).

There are two major classes in the Phylum; Eutardigrada and Heterotardigrada (table 1). The Eutardigrada primarily include the 'unarmoured' freshwater and terrestrial species, they are identifiable by their claw and cuticle structures and by the buccal apparatus (Nelson and Marley 2000). The Heterotardigrada mainly include the 'armoured' terrestrial and the

marine species, the key morphological features include the cephalic appendages, cuticular extensions and the patterns of dorsal cuticular plates (Nelson and Marley 2000). There is a third class; Mesotardigrada (table 1) which consists of one species, *Thermozodium esakii* Rahm. This species was found in hot springs near Nagasaki in Japan, however the type locality and specimens were destroyed during an earthquake and no other mesotardigrades have been found since (Nelson 2002).

Table 1: Subdivision of the Phylum Tardigrada with habitat classifications (adapted from Nelson 2001)

| Class | Order | Family | Habitat | Main genera | | |
|--------------------------|------------------|-----------------------|------------|--|---|---|
| Heterotardigrada | Arthrotardigrada | | M | Many, except <i>Styraconyx hallasi</i> (F) | | |
| | | Echiniscoidea | M, F, T | | | |
| | | Echiniscoididae | M | <i>Echiniscoides</i> ; <i>Anisonyches</i> | | |
| | | Oreellidae | T | <i>Oreella</i> | | |
| | | Carphaniidae | F | <i>Carphania</i> | | |
| | | Echiniscidae | T, some F | <i>Echniscus</i> ; <i>Hypechiniscus</i> ; <i>Pseudechiniscus</i> | | |
| Mesotardigrada (dubious) | Thermozodia | Thermozodiidae | Hot spring | <i>Thermozodium</i> | | |
| Eutardigrada | Parachela | | M, F, T | | | |
| | | Macrobotidae | T, F | <i>Adorybiotus</i> ; <i>Calcarobotus</i> ; <i>Dactylobiotus</i> ; <i>Macrobotus</i> ; <i>Macroversum</i> ; <i>Minibiotus</i> ; <i>Murrayon</i> ; <i>Pseudodiphascon</i> ; <i>Pseudohexapodibius</i> ; <i>Richtersius</i> ; <i>Xerobiotus</i> | | |
| | | Calohypsibiidae | T | <i>Calohypsibius</i> ; <i>Haplohexapodibius</i> ; <i>Haplomacrobotus</i> ; <i>Hexapodibius</i> ; <i>Parhexapodibius</i> | | |
| | | Eohypsibiidae | T, F | <i>Eohypsibius</i> ; <i>Amphibolus</i> | | |
| | | Micrphypsibiidae | T | <i>Microhypsibius</i> ; <i>Fractonotus</i> | | |
| | | Hypsibiidae | M, T, F | <i>M</i> = <i>Halobiotus</i> ; <i>Ramajendas</i> ; <i>Isohypsibius</i> ; <i>T & F</i> = <i>Acutuncas</i> ; <i>Astatuman</i> ; <i>Diphascon</i> ; <i>Doryphoribius</i> ; <i>Eromobiotus</i> ; <i>Hebesuncus</i> ; <i>Hypsibius</i> ; <i>Isohypsibius</i> ; <i>Itaquascon</i> ; <i>Fujiscon</i> ; <i>Mesocrista</i> ; <i>Mixibius</i> ; <i>Paradiphascon</i> ; <i>Parascon</i> ; <i>Platicrista</i> ; <i>Pseudobiotus</i> ; <i>Ramajendas</i> ; <i>Ramazottius</i> ; <i>Thulinia</i> | | |
| | | Necopinatidae | T | <i>Necopinatum</i> | | |
| | | <i>Incertae sedis</i> | T | <i>Apodibius</i> | | |
| | | | Apochela | Milnesiidae | T | <i>Milnesium</i> ; <i>Limmenius</i> ; <i>Milnesioides</i> |

M – marine; F – freshwater; T - terrestrial

Latency in tardigrades

All species of tardigrades are aquatic as they require a film of water to be active but many species are able to enter a latent state when environmental conditions become unfavourable. By entering this latent state they are able to withstand extremes in temperature, desiccation, low oxygen and salinity variations. During these periods of latency, reproduction, metabolism and growth are suspended or slowed (Bertolani et al. 2004). Whilst in a latent state tardigrades have been subjected to a vacuum (Jönsson et al. 2008) and ionizing radiation and survived. In 2007 *Milnesium tardigradum* and *Richtersius coronifer* were sent into space and survived the exposure to low temperatures, cosmic and solar radiation and microgravity; however egg production was reduced (Jönsson et al. 2008). In laboratory experiments, *Richtersius coronifer* had a survival rate of 96-100 percent when exposed to a vacuum and survived instant freezing to -195.8°C (Persson et al. 2009). It is suggested that the ability of tardigrades to survive these extreme conditions is a result of efficient DNA repair (Jönsson et al. 2005).

Five types of latency have been identified; anhydrobiosis, cryobiosis, anoxybiosis, osmobiosis and encystment (Crowe 1975). Latency strategies

can be divided into two categories; diapause and quiescence. Diapause are those with endogenous control which are induced by environmental factors and are maintained and ended by an internal physiological response (Hand 1991). Quiescence are those with exogenous control which are induced and maintained by environmental conditions and end when the conditions are again favourable (Keilin 1959, Hand 1991). Within tardigrades, cryptobiosis represents quiescence and encystment represents diapause (Guidetti and Rebecchi 2011).

Cryptobiosis

Cryptobiosis is mostly seen in terrestrial tardigrades although it is also seen in a few marine species including species from the heterotardigrada genus *Echiniscoides* and the eutardigrade, *Halobiotus criskea* (Grøngaard et al. 1990, Wright et al. 1992). The ability to enter cryptobiosis enables tardigrades to survive in harsh areas where conditions are not always favourable. Tardigrades can enter cryptobiosis during any phase of their life cycle and the duration of the state can vary depending of the length of the adverse environmental conditions and the species ability to survive long periods of cryptobiosis (Bertolani et al. 2004). A study by Bertolani et al. (2004) showed that *Rammazottius oberhaeuseri* had better long-term survival than *Echiniscus* sp. in anhydrobiosis. This portrays the variation in cryptobiotic capabilities between different species. Cryptobiosis can be divided into four distinct types that relate to the environmental conditions that trigger the latent state.

Anhydrobiosis is seen in terrestrial tardigrades and echiniscids and is induced by evaporative water loss. It is the most studied form of cryptobiosis (Bertolani et al. 2004). To enter anhydrobiosis, tardigrades form a tun by contracting their body and in doing so lose most of their bound and free water (>95%) whilst synthesizing cell protectants, for example heat shock proteins and trehalose (Westh and Ramløv 1991, Ramløv and Westh 2001), and they either suspend or reduce their metabolism (Clegg 2001, Wright 2001). This enables limno-terrestrial tardigrades to enter an environmentally resistant state during which they can survive periods of desiccation (Bertolani et al. 2004) which may occur often to tardigrades living in bryophyte cushions, lichens, leaf litter and tree bark. Whilst in anhydrobiosis, individuals are able to withstand extremes of high and low temperatures.

Species abilities to withstand below freezing in a desiccated state have been seen in many experiments. High survival rates were seen in three desiccated Antarctic species when the species were exposed to -22°C for eight years (Somme and Meier 1995) and also in species of Antarctic soil tardigrades exposed to -70°C for over six years (Newsham et al. 2006). Ramløv and Westh (2001) observed no decreases in survival in desiccated *Richtersius coronifer* when exposed to 70°C for one hour in a moss cushion but at temperatures above 70°C survival was reduced. These experiments demonstrate increased tolerance to stresses whilst in an anhydrobiotic state which allow these organisms to survive in highly variable and extreme conditions.

There is uncertainty surrounding the factors that maintain survival whilst the organism is in an anhydrobiotic state. Crowe (1975) showed increased levels in the bioprotectant trehalose in individuals of *Macrobotus areolatus* whilst in an anhydrobiotic state, although Jönsson and Persson (2010) found no significant changes in levels of trehalose in active and anhydrobiotic individuals of *Milnesium tardigradum*. Other studies have also shown little change in trehalose levels during anhydrobiosis in other species (Hengherr et al. 2008). Heat shock proteins are also thought to protect the cells during anhydrobiosis but studies also show contradicting results between different species (Guidetti and Rebecchi 2011).

Cryobiosis is induced by low temperatures and is the ability of active animals to survive freezing and thawing. Cryobiotic tardigrades have been seen to survive temperatures as low as -196°C (Ramløv and Westh 2001). It is seen in freshwater and terrestrial species found in polar regions (Somme and Meier 1995) and in alpine areas in temperate and tropical regions (Halberg et al. 2009, Hengherr et al. 2009, Rebecchi et al. 2009). This ability to survive periods of freezing and thawing allows tardigrades to inhabit polar and mountain regions where they would otherwise not survive.

Anoxybiosis is induced by low oxygen tensions in the environmental water (Nelson 2002) and length of survival in this state depends on the species. Truly aquatic species can only survive short periods, from a few hours to three days, whereas *Echiniscoides* species have been seen to survive for months in seawater containing decaying barnacles (Wright et al. 1992).

Osmobiosis is caused by high osmotic pressures, some limno-terrestrial and freshwater species form contracted tuns in varying salt solutions, whereas some terrestrial euryhaline and intertidal marine species can survive varying levels of salinities (Wright et al. 1992). All the cryptobiotic states allow essentially aquatic organisms to survive in terrestrial habitats with highly variable environmental conditions.

Encystment

Terrestrial tardigrades living in soil and moss and more commonly freshwater species are able to enter a state of encystment (Westh and Kristensen 1992). Cysts are composed of a number of cuticles surrounding the animal and metabolism is reduced, although not as much water is lost as in cryptobiosis. Encystment is maintained by internal physiological responses and is not an obligatory part of a tardigrades life cycle (Bertolani et al. 2004). Cysts are drought resistant but not able to withstand high temperatures due to the high water content and they can last a year before exhausting their food reserves (Westh and Kristensen 1992).

Habitats

Tardigrades are found in a number of different niches in a range of marine, freshwater and terrestrial ecosystems (Nelson 2002).

Marine

Within marine systems, tardigrades are found within intertidal areas to deep sea abysses (Renaud-Mornant 1982). Within the subtidal zones, tardigrade species can be semibenthic, epibenthic or mud and deep-sea ooze inhabitants. Epibenthic species are found on coral and algae species and subtidal tardigrades can be found in many types of substrate (Nelson 2002). *Halobiotus crispae* is a marine eutardigrade found in Greenland which undergoes cyclomorphosis, an annual cycle of morphological change that allows survival during harsh environmental conditions and short breeding seasons. During this cycle there are two distinct forms, pseudosimplex 1 and pseudosimplex 2. Pseudosimplex 1 is the sexually immature, hibernating winter morph. Individuals of this species gather to survive the harsh winter in sheltered areas and undergo synchronized development of the gonads. This results in the summer pseudosimplex 2 morphs reaching sexual maturity at the same time and then breeding. The process enables the species to survive the freezing winter and breed successfully (Kristensen 1982). Cyclomorphosis is also seen in some cryoconite and moss-dwelling species (Dastych 1993, Rebecchi and Bertolani 1994). On sandy shores in intertidal zones, *Echiniscooides* species and some other tardigrade species are found in algae and acorn barnacles (Kristensen and Hallas 1980, Grimaldi de Zio et al. 1983). In the intertidal zone, individuals have to cope with low tide and periods of desiccation; some species are able to enter cryptobiosis whilst others have to move to areas that retain water (Nelson 2002).

Freshwater

Hydrophilous tardigrades are found only in permanent freshwater ecosystems, they are benthic and primarily found on aquatic plants or in sandy sediments in streams, rivers, lakes and ponds (Nelson 2002). Some are found in deep lakes (up to 150m) but the majority dwell in the littoral zone (Ramazzotti and Maucci 1983). Tardigrades are also found in cryoconite holes in glaciers where they have to deal with freezing on a regular basis, a species of *Hysibius* is found here which undergoes cyclomorphosis to cope with the harsh conditions (Dastych 1993). Hygrophilous tardigrade species are usually found living in moist moss cushions but can also be found in freshwater habitats. Eurytopic species, such as *Milnesium tardigradum* and *Macrobotus hufelandi* can survive in varying moisture conditions and although they are a terrestrial species they are also found in freshwater systems. Few studies focus on the edges of freshwater systems, where community composition of hydrophilous, hygrophilous and eurytopic species may show the effects of seasonality on population composition and densities.

Terrestrial

The majority of tardigrade species inhabit terrestrial ecosystems and can be found in bryophyte cushions, lichens, bark, leaf-litter and soil (Ramazzotti and Maucci 1983, Kinchin 1994). In terrestrial systems tardigrades have to cope with alternating periods of wet and dry and many species can enter cryptobiosis as a result. This has enabled species to populate harsh, extreme environments such as polar and high altitude regions (Bertolani et al. 2004). Studies have tried to identify the factors that determine tardigrade distribution and density but in many cases the results between studies have been

contradictory. Factors such as altitude, bedrock type, rainfall, temperature and plant species have been suggested as affecting tardigrade distributions. Dastych (1980) found that the number of individuals increased with altitude whilst the number of species decreased slightly in his study in Tatra National Park in Poland. However, Dastych (1987) and Rodríguez (1951) found that the number of tardigrade species increased with altitude. In a study by Guil et al. (2009) tardigrade richness was highest at 1000-2000 m. asl and lowest at 0-500 m. asl, however in other studies, richness was at its highest between 0-500 m. asl (Utsugi et al. 1997, Collins and Bateman 2001). This suggests that other variables contribute to the distribution and diversity of tardigrades in these areas. Some authors have categorised tardigrade species into groups based on altitudinal ranges and localities (Ramazzotti and Maucci 1983, Dastych 1987) whilst others have found no relationships in these species between altitude and distribution (Kathman and Cross 1991, Kaczmarek et al. 2011). In a study by Herrera-Vásquez and Vargas (2003), clear altitudinal preferences for areas above 1000 m. asl were seen in *Echiniscus angolensis* and *Echiniscus bigranulatus* however some species including *Milnesium tardigradum* showed no altitudinal preferences. The varied results from these studies may be due to other variables within the study areas or due to a lack of replicas within the study (Kaczmarek et al. 2011).

Some studies have suggested a relationship between bryophyte or lichen species and tardigrade abundance and diversity. Hofmann (1987) found associations between five moss species and four tardigrade species and Dastych (1987, 1988) found weak associations between tardigrade distribution and bryophyte species and type, substrate type and habitat type. Other studies have not shown there to be associations between moss species and tardigrade distribution (Nelson 1975, Ramazzotti and Maucci 1983, Kathman and Cross 1991). Kaczmarek et al. (2011) studied the ecological factors affecting the distribution of tardigrada in Costa Rica and found that altitude had the most influence on distribution. They also saw some correlation between plant formation, with 40.6% (26 species) showing a clear correlation with plant formation type and 7.8% showing no correlation. A higher diversity was found in mosses than in lichen and liverworts and also in samples collected from rocks or trees as opposed to samples from soil and leaf litter, however these results may be due to uneven samples taken from the bryophyte species and the substrates.

It is suggested that the tardigrade distribution is influenced by the climatic conditions that occur at various altitudes as they show a preference for areas of high humidity. Plant communities are determined by humidity and temperature and are therefore also determined by altitude, which is why we may see a correlation between tardigrade distribution and plant species (Kaczmarek et al. 2011). A number of factors have been suggested for influencing distribution in moss inhabiting tardigrada including season and temperature (Schuster and Greven 2007), food availability (Greven and Schüttler 2001) and moisture within the moss cushion (Wright 1991, Jönsson 2007). The inconsistencies between the studies show a need for further, more in depth studies where a large number of samples with a large number of replicas are taken. These studies need to consider the other variables that

may affect tardigrade distribution, such as altitude, habitat and substrate as it has been suggested that tardigrade distribution and density is determined by microhabitat factors (Kathman and Cross 1991, Guil et al. 2009).

Biogeography and Dispersal

As a phylum, Tardigrada have a worldwide distribution however the biogeography remains unknown (McInnes 1994). This is a result of the uncertain species identification and a bias of taxonomic studies in selected habitats and geographic areas (Guil 2002, Guil and Cabrero-Sañudo 2007). There are a number of species with high tolerances and wide ecological requirements that are considered to be cosmopolitan (Nelson 2002). *Milnesium tardigradum*, *Minibiotus interedius* and *Macrobotus hufelandi* and considered to be cosmopolitan species however it may be that they are species complexes (Bertolani and Rebecchi 1993, Claxton 1998). These species have wide distributions, which may be due to their ability to enter cryptobiosis and thus survive in a range of conditions where species without this ability would not be able to survive. There are also a number of rare and endemic species; these tend to be species that have narrow tolerances for environmental conditions (Nelson 2002). A number of species from the homothermic springs in Greenland are considered endemic (Kristensen 1982). Further studies are needed to determine the biogeography of Tardigrada and whether there truly are cosmopolitan species.

Little is known on the dispersal of aquatic tardigrades although it may be possible that in periods of high rainfall, active tardigrades are distributed within river systems. As they rarely have the ability to enter cryptobiosis they are unable to disperse in this form unlike terrestrial species (Nelson and Marley 2000). Cysts, tuns and eggs of terrestrial tardigrades are usually passively dispersed by wind however active and latent states can also be dispersed by floodwater, melting snow and rain (Ramazzotti and Maucci 1983). The ability to enter cryptobiosis and thus be passively dispersed by the wind enables terrestrial tardigrades to disperse over wider distances than they would be able to in their active forms.

Population and Community Interactions

Tardigrades have a buccal apparatus that they use for feeding on plant cell fluids, algae, protozoa, bacteria and small tardigrades and invertebrates (Kinchin 1994). It is suggested that the type of buccal apparatus corresponds to the type of food consumed by the tardigrade (Nelson 2002). There are carnivorous species, such as *Milnesium tardigradum* that feeds on rotifers and nematodes (Suzuki 2003), and omnivorous species, such as *Batillipes* species which feed on diatoms and algae (Marcus 1928). Larger species of *Milnesium* and *Macrobotus* have been seen to feed on smaller eutardigrades, nematodes and rotifers (Nelson 2002). Fungi and parasites have been known to infect tardigrade populations and predators include invertebrates such as mites, larvae, spiders, nematodes and other tardigrades (Ramazzotti and Maucci 1983). *Milnesium tardigradum* and *Ramazzotius oberhaeuseri* have been seen to carry the protozoan *Pykidium tardigradum* on their surface and although this relationship is seen as symphoriont (Morgan 1976). In some cases there can be as many as 35 protozoans on the surface of an individual

tardigrade which obstructs movement and could therefore be considered a parasite (Vicente et al. 2008). The fungus *Lecophagus antarcticus* attacks tardigrades on cyanobacterial mats in lake sediments (McInnes 2003) and the fungus *Ballocephala pedicellata* is known to attack moss-dwelling *Hypsibius dujardini* and *Diphyscon pingue* (Pohlad and Bernard 1978).

Population densities of tardigrades tend to be patchily distributed and highly variable and changes have been linked to environmental conditions. Studies have associated air pollution (Steiner 1994), food availability (Hallas and Yeates 1972) temperature and moisture (Briones et al. 1997, Morgan 1997) with changes in population densities of tardigrades and it may be that predation, parasitism and competition also have an affect (Nelson 2002). A reduction in tardigrade density within epiphytic moss species was seen with poor air quality in Ohio, USA (Meininger et al. 1985). However further research is needed to assess which of these factors have the most effect on population densities and on the specific environmental and feeding requirements of the various species of tardigrades.

Conclusions

Further research is required to determine the biogeography of tardigrada and to assess whether cosmopolitan species are in fact species complexes (Bertolani and Rebecchi 1993, Claxton 1998). Tardigrada have a global distribution including areas that have variable and extreme conditions as the ability to enter latent states allows colonization in otherwise inhospitable areas (Nelson 2002). However, the factors affecting distribution and population density still remain unanswered and future research is needed in this area. To understand the effects of these factors, sampling needs to occur in a variety of different locations with varying climatic conditions, altitudes and microhabitats and high numbers of samples and replicas need to be collected. Future research is needed in these areas to understand the ecology of this group, which is currently poorly understood (Nelson 2002).

References

- Bertolani, R., R. Guidetti, K. I. Jonsson, T. Altiero, D. Boschini, and L. Rebecchi. 2004. Experiences with dormancy in tardigrades. *J. Limnol.* **63**:16-25.
- Bertolani, R. and L. Rebecchi. 1993. A revision of the *Macrobiotus hufelandi* group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades. *Zool. Scripta* **22**:127–152.
- Briones, M., P. Ineson, and T. Pearce. 1997. Effects of climate change on soil fauna; responses of enchytraeids, Diptera larvae and tardigrades in a transplant experiment. *Appl. Soil Ecol.* **6**:117–134.
- Claxton, S. 1998. A revision of the genus *Minibiotus* (Tardigrada: Macrobiotidae) with descriptions of eleven new species from Australia. *Records Aust. Mus.* **50**:125–160.
- Clegg, J. S. 2001. Cryptobiosis — a peculiar state of biological organization. *Comp. Biochem. Physiol.* **128**:613-624.
- Collins, M. and L. Bateman. 2001. The ecological distribution of tardigrades in Newfoundland. *Zool Anz* **240**:291–297.

- Crowe, J. H. 1975. The physiology of cryptobiosis in tardigrades. *Mem. Ist. ital. Idrobiol.* **32 Suppl.**:37-59.
- Dastych, H. 1980. *Macrobotus kurasi* sp. nov., a new specie of Tardigrada from Mountains of Uganda. *B Acad Pol Sci Biol* **XXVII**:653–657.
- Dastych, H. 1987. Altitudinal distribution of Tardigrada in Poland. Pages 169–214. *in* R. Bertolani, editor. *Biology of Tardigrades: Selected Symposia and Monographs*, Vol. 1:Unione Zoologia Italia. Mucchi, Modena.
- Dastych, H. 1988. The Tardigrada in Poland. *Monogr Faun Pol* **16**:1–255.
- Dastych, H. 1993. Redescription of the cryoconital tardigrade *Hypsibius klebelsbergi* Mihelcic, 1959, with notes on the microslide collection of the late Dr. F. Mihelcic. *Verf. Mus. Ferdinandeum* **73**:5–12.
- Greven, G. and L. Schu'ttler. 2001. How to crawl and dehydrate on moss. *Zool Anz* **240**:341–344.
- Grimaldi de Zio, S., M. D'Addabbo Gallo, and M. Morone De Lucia. 1983. Marine tardigrades ecology. *Oebalia* **9**:15–31.
- Grøngaard, A., N. M. Kristensen, and M. K. Petersen. 1990. Tardigradfaunaen pa Disko. Pages 155–179. *in* P. F. Andersen, L. Düwel, and O. S. Hansen, editors. *Feltkurs i Arktisk biologi*, Godhavn, Zoological Museum, University of Copenhagen, Copenhagen.
- Guidetti, R. and L. Rebecchi. 2011. On dormancy strategies in tardigrades. *Journal of Insect Physiology* **57**:567-576.
- Guil, N. 2002. Diversity and distribution of tardigrades (Bilateria, Tardigrada) from the Iberian Peninsula, Balearic Islands and Chafarinas Islands. *Graellsia* **58**:75–94.
- Guil, N. and F. Cabrero-Sañudo. 2007. Analysis of the species description process for a little known invertebrate group: the limnoterrestrial tardigrades (Bilateria, Tardigrada). *Biodiversity and Conservation* **16**:1063–1086.
- Guil, N., J. Hortal, S. Sanchez-Moreno, and A. Machordom. 2009. Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment. *Landscape Ecology* **24**:375-390.
- Halberg, K. A., D. Persson, H. Ramløv, P. Westh, R. M. Kristensen, and N. Møbjerg. 2009. Cyclomorphosis in Tardigrada: adaptation to environmental constraints. *Journal of Experimental Biology* **212**:2803–2811.
- Hallas, T. E. and G. W. Yeates. 1972. Tardigrada of the soil and litter of a Danish beech forest. *Pedobiologia* **12**:287–304.
- Hand, S. C. 1991. Metabolic dormancy in invertebrates. *Adv. Comp. Environm. Physiol.* **8**:1-50.
- Hengherr, S., A. G. Heyer, H. R. Köhler, and R. O. Schill. 2008. Trehalose and anhydrobiosis in tardigrades—evidence for divergence in response to dehydration. *FEBS* **275**:281–288.
- Hengherr, S., M. R. Worland, A. Reuner, F. Brümmer, and R. O. Schill. 2009. Freeze tolerance, supercooling points and ice formation: comparative studies on the subzero temperature survival of limno-terrestrial tardigrades. *Journal of Experimental Biology* **212**:802–807.
- Herrera-Vásquez, J. and M. Vargas. 2003. Tardigrades (Tardigrada) from the western part of the Central Valley, Costa Rica with some ecological annotations. *Brenesia* **59-60**:69–76.

- Hofmann, I. 1987. Habitat preference of the most frequent moss-living Tardigrada in the area of Giessen (Hessen). Vol 1. *Unione Zoologia Italia*. Pages 211–216. *in* R. Bertolani, editor. *Biology of Tardigrades: Selected Symposia and Monographs*. Mucchi, Modena.
- Jönsson, K. I. 2007. Long-term experimental manipulation of moisture conditions and its impact on moss-living tardigrades. *J Limnol* **66**:119–125.
- Jönsson, K. I., M. Harms-Ringdahl, and J. Torudd. 2005. Radiation tolerance in the eutardigrade *Richtersius coronifer*. *Internat. J. Radiation Biol.* **81**:649-656.
- Jönsson, K. I. and O. Persson. 2010. Trehalose in three species of desiccation tolerant tardigrades. *Open Zool J* **3**:1–5.
- Jönsson, K. I., E. Rabbow, R. O. Schill, M. Harms-Ringdahl, and P. Petra Rettberg. 2008. Tardigrades survive exposure to space in low Earth orbit. *Current Biology* **18**: R729-R731
- Kaczmarek, L., B. Goldyn, W. Welnicz, and L. Michalczyk. 2011. Ecological factors determining Tardigrada distribution in Costa Rica. *J Zool Syst Evol Res* **49**:78-83.
- Kathman, R. D. and S. F. Cross. 1991. Ecological distribution of moss-dwelling tardigrades on Vancouver island, British Columbia, Canada. *Can J Zool* **69**:122–129.
- Keilin, D. 1959. The problem of anabiosis or latent life: history and current concept. *Proc. R. Soc. Lond. B.* **150**:149- 191.
- Kinchin, I. M. 1994. *The biology of tardigrades*. Portland Press, London.
- Kristensen, R. 1982. New aberrant eutardigrades from homothermic springs on Disko Island, West Greenland. Pages 203–220 *in* D. R. Nelson, editor. *Proceedings of the third international symposium on the Tardigrada*, August 3–6, 1980, Johnson City, Tennessee. East Tennessee State University Press, Johnson City.
- Kristensen, R. and T. E. Hallas. 1980. The tidal genus *Echiniscoides* and its variability, with erection of *Echiniscoididae* fam. n. (Tardigrada). *Zool. Scripta* **9**:113–127.
- Marcus, E. 1928. *Spinnentiere oder Arachnoidea IV: Bärtierchen (Tardigrada)*. Springer, Jena.
- McInnes, S. J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. *Journal of Natural History* **28**:257–352.
- McInnes, S. J. 2003. A predatory fungus (Hyphomycetes: Lecophagus) attacking Rotifera and Tardigrada in maritime Antarctic lakes. *Polar Biol.* **26**:79-82.
- Meininger, C. A., G. W. Uetz, and J. A. Snider. 1985. Variation in epiphytic microcommunities (tardigrade-lichen-bryophyte assemblages) of the Cincinnati, Ohio area. *Urban Ecol.* **9**:45-62.
- Morgan, C. 1997. Population dynamics of two species of Tardigrada, *Macrobiotus hufelandi* (Schultze) and *Echiniscus (Echiniscus) testudo* (Doyere), in roof moss from Swansea. *J. Anim. Ecol.* **46**:263–279.
- Morgan, C. I. 1976. Studies on the British tardigrade fauna. Some zoogeographical and ecological notes. *J. Nat. Hist.* **10**:607-632.

- Nelson, D. R. 1975. Ecological distribution of tardigrades on Roan Mountain, Tennessee, North Carolina. *Mem Ist Ital Idrobiol. Dott Marco Marchi* **32**: 225–276.
- Nelson, D. R. 2001. Tardigrada. Pages 527–550 in J. Thorp and A. Covich, editors. *Ecology and classification of North American freshwater invertebrates*. Academic Press, San Diego.
- Nelson, D. R. 2002. Current Status of the Tardigrada: Evolution and Ecology. *Integ. And Comp. Biol.* **42**:652-659.
- Nelson, D. R. and N. J. Marley. 2000. The biology and ecology of lotic Tardigrada. *Freshwater Biology* **44**:93-108.
- Newsham, K. K., N. R. Maslen, and S. J. McInnes. 2006. Survival of Antarctic soil metazoans at -80°C for six years. . *CrioLetters* **2**:269-280.
- Persson, D., C. Ricci, K. A. Halberg, and R. M. Kristensen. 2009. Extreme stress tolerance in tardigrades — Surviving space conditions in low earth orbit. *in* H. Greven, K. Hohberg, and R. O. Schill, editors. 11th International Symposium on Tardigrada. Conference Guide., Tübingen, Germany,.
- Pohlad, B. R. and E. C. Bernard. 1978. A new species of Entomophthorales parasitizing tardigrades. *Mycologia* **70**:130-139.
- Ramazzotti, G. and W. Maucci. 1983. Il phylum Tardigrada. III edizione riveduta e aggiornata. *Mem Ist Ital Idrobiol. Dott Marco Marchi* **41**:1–1012.
- Ramløv, H. and P. Westh. 2001. Cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*: tolerance to alcohols, temperature and de novo protein synthesis. . *Zool. Anz.* **240**:517-523.
- Rebecchi, L. and R. Bertolani. 1994. Maturative pattern of ovary and testis in eutardigrades of freshwater and terrestrial habitats. *Invert. Reprod. Develop.* **26**:107–117.
- Rebecchi, L., D. Boschini, M. Cesari, V. Lencioni, R. Bertolani, and R. Guidetti. 2009. Stress response of a boreo-alpine species of tardigrade, *Borealibius zetlandicus*(Eutardigrada, Hypsibiidae). *Journal of Limnology* **68**:64–70.
- Renaud-Mornant, J. 1982. *Species diversity in marine Tardigrada.*, East Tennessee State University Press, Johnson City.
- Rodríguez, R. J. 1951. Algunos datos sobre la distribución de los tardígrados españoles. . *Bol Real Soc Esp Hist Nat Biol* **49**:75–83.
- Romano, F. A. 2003. On Water Bears. *Florida Entomologist* **86**:134-137.
- Schuster, R. and H. Greven. 2007. A long-term study of population dynamics of tardigrades in the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst. . *J Limnol* **66**:141–151.
- Somme, L. and T. Meier. 1995. Cold hardiness of Tardigrada from Dronning Maud Land, Antarctica. *Polar Biol.* **15**:221-224.
- Steiner, W. 1994. The influence of air pollution on moss-dwelling animals: 4. Seasonal and long-term fluctuations of rotifer, nematode and tardigrade populations. *Rev. Suisse Zool.* **101**:1017– 1031.
- Suzuki, A. C. 2003. Life history of *Milnesium tardigradum* Doyere (Tardigrada) under a rearing environment. *Zool Sci (Tokyo)* **20**:49– 57.
- Utsugi, K., T. Hiraoka, and N. Nunomura. 1997. On the relations between tardigrade fauna and bryophyte flora in Toyama Prefecture. *Bull Toyama Sci Mus* **20**:57–71.

- Vicente, F., L. Michalczyk, L. Kaczmarek, and M.J. Boavida. 2008. Observations on *Pyxidium tardigradum* (Ciliophora) a protozoan living on Eutardigrada: Infestation, morphology and feeding behaviour. *Parasitol. Res.* **103**:1323-1331.
- Walz, B. 1982. Molting in Tardigrada. A review including new results on cuticle formation in *Macrobiotus hufelandi*. Pages 129–147. *in* D. R. Nelson, editor. Proceedings of the third international symposium on the Tardigrada, August 3–6, 1980. East Tennessee State University Press, Johnson City., Johnson City, Tennessee.
- Westh, P. and R. M. Kristensen. 1992. Ice formation in the freeze-tolerant eutardigrades *Adorybiotus coronifer* and *Amphibous nebulosus* studied by differential scanning calorimetry. *Polar Biology* **12**:693–699.
- Westh, P. and H. Ramløv. 1991. Trehalose accumulation in the tardigrade *Adorybiotus coronifer* during anhydrobiosis. *J. Exp. Zool.* **258**:303-311.
- Wright, J. C. 1991. The significance of four xeric parameters in the ecology of terrestrial Tardigrade. *Journal of Zoology* **224**:59–77.
- Wright, J. C. 2001. Cryptobiosis 300 years on from van Leuwenhoek: what have we learned about tardigrades? . *Zool. Anz.* **240**:563-582.
- Wright, J. C., P. Westh, and H. Ramløv. 1992. Cryptobiosis in Tardigrada. *Biological Review* **67**:1–29.