Water Bears Associated with Bryophytes in the Pacific Northwest

Rosa Hunter

BIOL 289, Introduction to Bryology

Instructor: Brian Compton, Ph.D.

Spring 2011 Report

22 July 2011

Water Bears Associated with Bryophytes in the Pacific Northwest

Introduction

What are Tardigrades? Since 1773, when J.A.E Goeze reported the first observation of a tardigrade, he called them "water bears" because they looked like little bears (Bordenstein, 2008). Because of their uniqueness, they are classed in their own phylum, Tardigrada. Among the characteristics that make tardigrades unique is their lack of circulatory and respiratory systems. Often described as a minor phylum, tardigrades have been relatively neglected by invertebrate zoologists (Nelson 1991). This is due to the lack of economic importance to humans, and the difficulties of collecting and culturing the organisms. Tardigrades are considered to be related to arthropods, such as insects and millipedes, and are grouped with them in the superphylum Ecdysozoa based on the shared characteristic of the shedding of a cuticle as they grow.

The tardigrade body is composed of five somewhat indistinct segments: a cephalic segment and four trunk segments where each segment supports a pair of legs. Tardigrades have a central nervous system with a dorsal brain that supports many sensory inputs, including light sensitivity to their eyespots. They have a complete digestive system with a complex mouth and pharynx structure. They eat bacteria, detritus, algae, nematodes, rotifers, and each other. They have well-developed muscles and are flexible and articulate in all directions (Smith, 2001).

Tardigrades are common in aquatic and terrestrial habitats, requiring a film of water around their bodies to permit locomotion and gas exchange. Most of the known tardigrades are freshwater aquatic or limno-terrestrial species, but there are marine species as well (*Biosystematics of the Marine*, n.d.; Middleton, n.d.). Limno-terrestrial species are commonly associated with bryophytes that comprise habitat providing sufficient moisture, oxygen, food, and protection. Moisture is probably the most important factor with respect to their habitat preferences (Glime, 2010/n.d.). During moist environmental conditions, bryophytes grow and retain moisture on their bodies sufficient to permit tardigrades to be

active. However, because of their simple form, bryophytes are subject to rapid drying in less favorable conditions, which has implications for their associated tardigrades.

To accommodate fluctuations in water availability, most "terrestrial" forms of tardigrades can enter into a kind of suspended animation in which they dehydrate and remain viable—a situation called anhydrobiosis (see Figure 1 in Schill, et al., 2004). Though they are not able to survive cryptobiosis for centuries, Bertolani, et al. (2004) maintained adult tardigrades in a cryptobiotic state for four years with standard atmosphere (oxygen exposure), and they had a high recovery rate. Tardigrades are even able to survive exposure to the vacuum of space in low earth orbit while in a cryptobiotic state (Jönsson, et al., 2008). Their abilities to survive cryptobiotically have allowed them to exploit all manner of extreme environments from abyssal ocean regions to mountaintops, the poles and hot springs (Kinchin, 1994; Nelson, 2003; and Romano, 2002).

Tardigrades have been known for nearly 200 years and have been considered in more than 300 papers. However, tardigrades of North America have received little attention. Hidalgo and Coombs (1985) reported that 16 states in the USA had no records of tardigrades, and western North America has only a few records of tardigrades, notably those presented by Schuster and Grigarick (1965). More recent research has been conducted in California (*Tardigrades at Hastings*, n.d.). Most tardigrade species found in North America were usually found to be species well known in Europe. Although, nearly one-third of the known species occur in North America, few are recorded and no records are known from Whatcom County, Washington. My research, which I conducted as an individualized studies project for the spring 2011 quarter, involved the documentation of tardigrades in Whatcom County and the bryophytes they inhabit. I conducted the research in collaboration with Jack Dunn, who conducted bryological studies during the spring 2011 quarter, and Dr. Brian Compton, who served as our instructor.

Research documenting tardigrades from western North America that was reviewed during this study does not indicate which bryophytes were associated with those tardigrades (Schuster and Grigarick, 1965; *Tardigrades at Hastings*, n.d.). My research, however, involved a collaborative goal of collecting

and identifying local bryophytes and the tardigrades found to inhabit them to explore and document basic aspects of bryophyte-tardigrade ecology.

Methods and Materials

Bryophyte specimens were collected, documented and identified using standard methods (Lawton, 1971; Malcom & Malcom, 2006; Malcom, et al., 2009; Pojar & MacKinnon, 2004; Schofield, 2001; and Vitt, et al., 1988). Collection sites included the campus of Northwest Indian College on the Lummi Reservation near Bellingham, Washington, Whatcom Lake Park in Bellingham, and near the Nooksack River in Deming, Washington. The bryophyte specimens collected in this study were deposited in the Northwest Indian College Herbarium.

The collection of bryophytes is a standard method to obtain tardigrades (Miller, n,d.). Once the bryophytes were collected, tardigrades were isolated from them using two basic methods. The first method involved soaking the specimens in water to release the tardigrades from the bryophytes. The second method involved the use of a small Berlese (or Berlese-Tullgren) funnel and a lamp to dry the bryophyte specimens and isolate tardigrades from the samples by collecting the materials that passed through the funnel and subsequently adding those materials to distilled water. In both cases, the material obtained was then transferred using micropipettes to glass slides with distilled water to be examined microscopically using bright field and phase contrast methods for the presence of tardigrades (cf. Bordenstein, 2008). Isolated tardigrades were identified using standard identification keys (Lindahl & Balser, 1999; Schuster & Grigarick 1965). Bryophyte specimens were kept separate from one another and the micropipettes used to transfer the water to slides were cleaned regularly to help avoid the possibility of transferring tardigrades from one sample to another.

Once a tardigrade was found through microscopic examination, a solution of magnesium chloride and distilled water (7.5 g of $MgC1_2$—$6H_20$ in 100 ml of distilled water) was used as a narcotizing agent to slow down the tardigrade for better viewing and subsequent identification (cf. Glime, 2010/n.d., p. 79). Where possible, digital images were obtained of the tardigrades. Specimens of tardigrades were retained

as vouchers by mounting them on glass microscope slides with the cover slips sealed with fingernail polish and retained at Northwest Indian College.

Scientific nomenclature and taxonomy applied in this study to bryophytes is based upon the Plant List <http://www.theplantlist.org/> with common names as applied by the Plants Database <http://plants.usda.gov/>. The Integrated Taxonomic Information System <http://www.itis.gov/> was used similarly for tardigrades and other microinvertebrates.

Results

A total of 15 species of bryophytes were collected and identified, including 14 mosses and one leafy liverwort (see Table 1).

Table 1: Bryophytes Identified[1]

Collection Number	Division	Family	Species	Common Name
RHu 1	Bryophyta (Mosses)	Bryaceae	*Bryum argenteum* Hedw.	silvergreen bryum moss
JD 12	Bryophyta (Mosses)	Brachytheciaceae	*Eurhynchium pulchellum* (Hedw.) Jenn. (syn.: *Eurhynchiastrum pulchellum* (Hedw.) Ignatov & Huttunen)	eurhynchium moss
JD 16	Bryophyta (Mosses)	Brachytheciaceae	*Homalothecium fulgescens* (Mitt. ex Müll. Hal.) A. Jaeger	tree mat homalothecium moss
JD 11	Bryophyta (Mosses)	Brachytheciaceae	*Isothecium myosuroides* Brid.	cat's tail moss
RHu 4	Bryophyta (Mosses)	Dicranaceae	*Dicranoweisia cirrata* (Hedw.) Lindb.	dicranoweisia moss
RHu 5	Bryophyta (Mosses)	Ditrichaceae	*Ceratodon purpureus* (Hedw.) Brid.	ceratodon moss
RHu 12	Bryophyta (Mosses)	Hylocomiaceae	*Rhytidiadelphus squarrosus* (Hedw.) Warnst.	square goose neck moss
RHu 7	Bryophyta (Mosses)	Hylocomiaceae	*Rhytidiadelphus triquetrus* (Hedw.) Warnst.	rough goose neck moss
JD 15	Bryophyta (Mosses)	Leskeaceae	*Bryohaplocladium microphyllum* (Hedw.) R. Watan. & Z. Iwats. (syn.: *Haplocladium microphyllum* (Hedw.) Broth.)	bryohaplocladium moss
RHu 2	Bryophyta (Mosses)	Leskeaceae	*Claopodium crispifolium* (Hook.) Renauld & Cardot	claopodium moss
RHu 3	Bryophyta (Mosses)	Mniaceae	*Plagiomnium insigne* (Mitt.) T.J. Kop.	plagiomnium moss
RHu 6	Bryophyta (Mosses)	Orthotrichaceae	*Orthotrichum speciosum* Nees	lanceolateleaf rock moss
RHu 9	Bryophyta (Mosses)	Plagiotheciaceae	*Plagiothecium undulatum* (Hedw.) Schimp.	undulate plagiothecium moss
JD 1	Bryophyta (Mosses)	Pottiaceae	*Syntrichia princeps* (De Not.) Mitt.	twisted moss
RHu 8	Marchantiophyta (Liverworts)	Lepidoziaceae	*Lepidozia reptans* (L.) Dumort.	little hands liverwort

[1] The following abbreviations denote collectors: JD (Jack Dunn) and RHu (Rosa Hunter).

Each bryophyte was examined to identify it and for the presence of tardigrades and other microinvertebrates. Bryophytes that were found to harbor tardigrades were examined in detail to document as many tardigrades as possible. Four mosses were found to have tardigrades living on them. In total, twenty tardigrades were isolated from those bryophytes. Of these 16 were identified positively or tentatively to the species level (see Table 2 and Table 3).

Table 2: Tardigrades Identified

Sample Identification	Class	Order	Family	Species
RHu 4-1	Eutardigrada (Eutardigrades)	Parachela	Calohypsibiidae	*Haplomacrobiotus* ?*hermosillensis* May, 1948
RHu 12-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12	Eutardigrada (Eutardigrades)	Parachela	Macrobiotidae	*Macrobiotus* ?*richtersi* Murray, 1911
RHu 1-1 RHu 4-2	Heterotardigrada (Heterotardigrades)	Echiniscoidea	Echiniscidae	*Echiniscus blumi* Richters, 1903

During the research, additional microinvertebrates were observed in association with the bryophytes that were examined for tardigrades. In those cases, the presence of those organisms was noted and where possible they were photographed and identified to phylum (in the case of rotifers, probably representing Order Bdelloidea) (see Table 3).

Table 3: Bryophytes and Associated Microinvertebrates[2]

Bryophyta (Bryophytes)	Tardigrada (Tardigrades)	Rotifera (Rotifers)	Nematoda (Nematodes)	Other Unidentified Species (Protista, Protists?)
Bryum argenteum	X *Echiniscus blumi*	X	X	X
Clapodium	X	X	X	X

[2] Each "X" in the table indicates the observation of a microinvertebrate found in association with a bryophyte species. The numeral "12" indicates the number of individuals that were counted in a single specimen. Where identification of tardigrades was possible at or near the species level, the species name is provided, in some cases with question marks to denote a tentative identification. Where other organisms were observed that were not the focus of the project, their presence was noted with an "X" for each individual tallied and they were identified with as much specificity possible (i.e., to kingdom, phylum or order).

crispifolium				
Plagiomnium insigne		X	X	
Dicranoweisia cirrata	X (*E. blumi*) X (*Haplomacrobiotus ?hermosillensis*)	X	X	X
Ceratodon purpureus				
Orthotrichum speciosum				
Rhytidiadelphus triquetris		X	X	X X X
Lepiodozia reptans				X
Plagiothecium undulatum				
Rhytidiadelphus squarrosus	12 (*Macrobiotus ?richtersi*)			
Eurhynchiastrum pulchellum		X		

Discussion and Conclusions

This project is the first at Northwest Indian College to address bryophytes and their associated microinvertebrates. It was exploratory and involved the rapid learning by the project team of concepts, terminology, techniques and other matters associated with those organisms. This included discovering the challenges associated with the isolation, observation and identification of bryophytes, tardigrades and other organisms as well as the need for creativity and refinement of methods where useful or necessary.

Methodological considerations—In contrast to the reference regarding the use of distilled water in isolating tardigrades by Bordenstein (2008), Glime (2010/n.d., p. 79) indicated that this would pose problems due to the osmotic uptake of water by tardigrades. The current research has demonstrated the severity of problems associated with exposing tardigrades to distilled water which regularly resulted in osmotic over-pressure and subsequent lysis of the tardigrades. The substitution of dechlorinated tap water in place of distilled water resulted in fewer instances of tardigrade lysis.

Despite being regarded as "slow walkers," the tardigrades observed were often very mobile making their observation and identification difficult. Similarly to the effects of distilled water, the use of the magnesium chloride solution also apparently produced lysis in many tardigrades.

Additional problems were encountered when the pressure of microscope cover slips crushed tardigrades prior to their identification. Small amounts of Vaseline were applied to the corners of some cover slips to help prevent this problem (Lindahl & Balser, 1999)

Other studies have indicated the value of using a staining agent to enhance taxonomic features helpful in identifying small tardigrades (e.g., Evans, 1992), although staining agents were not used in the current study. Future efforts may require refinement of the isolation and narcotizing methods as well as the use of staining and refined mounting methods to assist in the identification and preservation of tardigrade specimens. And the use of larger Berlese funnels and more substantial bryophyte collections may yield more tardigrades.

Ecological considerations—Glime (2010/n.d., p. 45) reported that some studies have included identifications of bryophytes where tardigrades were found as well as differences in the types of tardigrades found amongst different bryophytes. The current research demonstrated some patterns of bryophyte-tardigrade associations, notably the presence of tardigrades with *B. argenteum*, *C. crispifolium*, D. *cirrata*, and *R. squarrosus* with especially abundant tardigrades (*M.* ?*richtersi*) in the last species. This last moss is a tall lawn-inhabiting moss that was collected at a relatively high elevation location near the Nooksack River in Deming, WA in a relatively moist situation. Because of its location and association with lawns, it may retain more moisture than other of the sampled mosses, which may help to account for the higher abundance of tardigrades found with it (cf. Glime 2010/n.d., p. 45).

Bryum argenteum and *D. cirrata* were collected from a dryer situation in the woods behind Northwest Indian College, and yielded fewer tardigrades. The specimen of *D. cirrata* had two tardigrades (*E. blumi* and *H.* ?*hermosillensis*). However, a collection of *Rhytidiadelphus squarrosus* was collected at a higher elevation location near the Nooksack River in Deming, WA.

Concluding comments—This initial exploratory project has yielded results that may be explored further as the focus of a baccalaureate program of study at Northwest Indian College to contribute to the knowledge of tardigrades in western Washington.

Works Cited

Berlese-Tullgren extractor.http://140.211.62.101/lessons/makentake/berlese1.html

Bertolani, R., R. Guidetti, I. Jönsson, T. Altiero, D. Boschini, and L. Rebecchi. 2004. Experiences with dormancy in tardigrades. *J., Limnol*. 63 (Suppl. 1): 16-25.

Biosystematics of the marine heterotardigrades. (n.d.). Retrieved June 23, 2011, from Garey Lab, University of South Florida, website: http://chuma.cas.usf.edu/~garey/tardigrade.html

Bordenstein, S. (2008, December 17). Tardigrades (water bears). In *Microbial life education resources*. Retrieved June 23, 2011, from Carlton College website: http://serc.carleton.edu/microbelife/topics/tardigrade/

Degma, P., Bertolani, R., and Guidetti, R. 2010. *Zootaxa* 845,- 1-46 (2005) Actual updated checklist of *tardigrades*.

Evans, G. O. 1992. *Principles of Acarology*. CAB International, Cambridge.

Glime, J. M. (n.d.). *Bryophyte ecology: Vol. 2. Bryological interactions*. (Original work published 2010) Retrieved from http://www.bryoecol.mtu.edu/

Jönsson, K. I., E. Rabbow. R. O. Schill, M. Harms-Ringdahl, and P. Rettberg. 2008. Tardigrades survive exposure to space in low Earth orbit. *Current Biology*, 18(17): R729-R731.

Kinchin, I. M. (1994). *The biology of tardigrades*. Chapel Hill, NC: Portland Press.

Lawton, E. (1971). *Moss flora of the Pacific Northwest. Suppl. No. 1, Journal of the Hattori Botanical Laboratory*. Nichinan, Miyazaki, Japan: Hattori Botanical Laboratory.

Lindahl, K., & Balser, S. (1999, October 1). *Species distribution project*. Retrieved June 22, 2011, from Illinois Wesleyan University, Bloomington, IL website: http://www.iwu.edu/~tardisdp/

Malcom, B., & Malcom, N. (2006). *Mosses and other bryophytes: An illustrated glossary* (2nd ed.). Nelson, New Zealand: Micro-Optics Press. (Original work published 2000)

Malcom, B., Malcom, N., Shevock, J., & Norris, D. (2009). *California mosses*. Nelson, New Zealand: Micro-Optics Press.

Middleton, R. (n.d.). Tardigrada (water bears, tardigrades). In *Biodiversity explorer*. Retrieved June 23, 2011, from iziko Museums of Cape Town, South Africa website: http://www.biodiversityexplorer.org/metazoa/tardigrades/

Miller, W. R. (n.d.). Tardigrades: Bears of the moss. In *Tardigrades*. Retrieved 1999, from KanCRN Collaborative Research Network website: http://www.pathfinderscience.net/tardigrades/cbackground.cfm

Nelson, D. R. 1991. Tardigrada. In J. H. Thorp, & A. P. Covich*, Ecology and classification of North American freshwater invertebrates.* New York, NY: Academic Press.

Nelson, D. R. 2002. Current status of the Tardigrada: evolution and ecology. *Integ. and Comp. Biol*. 42-(3), 652-659.

Pojar, J., & MacKinnon, A. (Eds.). (2004). *Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia & Alaska* (Rev. ed.). Renton, WA: Lone Pine. (Original work published 1994)

Romano, F. A. 2003. On water bears. *Florida Entomologist*., 86(2): 134-137.

Sayre, R.M. and Brunson, L.K. (1971, February). Microfauna of moss habitats. *Amer. Biol. Teacher*, 100-102, 105.

Schill, R. O., G. H. B. Steinbruck, and H. R. Kohler. 2004. Stress gene (hsp70) sequences and quantitative expression in *Milnesium tardigradum* (Tardigrada) during active and cryptobiotic stages. *Journal of Experimental Biology*, 207: 1607-1613.

Schofield, W. B. (2001). *Introduction to bryology* (Reprint of First Edition, Copyright 1985 ed.). Caldwell, NJ: Blackburn.

Schuster, R. O. and Grigarick, A. A. (1965). Tardigrada from western North America, with emphasis on the fauna of California. *University of California Publications in Zoology,* 76, 1-67.

Tardigrades at Hastings. (n.d.). Retrieved June 22, 2011, from Hastings Natural History Reservation website: http://www.hastingsreserve.org/invertebrates/Tardigrades/HastingsTardigrades.html

Upton, M.S. 1991. *Methods for Collecting, Preserving and Studying Insects and Related Forms.* Australian Entomological Society: Brisbane.

Upton, M.S. 1993. *Aqueous gum-chloral slide mounting media: an historical review*. Bulletin of Entomological Research 83: 267-274. Great mounting information

Vitt, D. E., Marsh, J. E., & Bovey, R. B. (1988). *Mosses lichens and ferns of northwest North America*. Auburn, WA: Lone Pine.