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Authors: Villella, John, Miller, Jesse E. D., Young, Alexander R., Carey, Greg, Emanuels, Andrew, et. al.

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John Villella[1], Siskiyou BioSurvey LLC, 265 Ball Rd., Eagle Point, Oregon 97520

Jesse E. D. Miller, Department of Biology, Stanford University, 371 Jane Stanford Way, Stanford, California, 94305

Alexander R. Young, Forest and Natural Resource Management, State University of New York, 1 Forestry Drive, Syracuse, New York 13210

Greg Carey, Siskiyou BioSurvey LLC, 265 Ball Rd., Eagle Point, Oregon 97520

Andrew Emanuels and **William R. Miller,** Department of Biology and Chemistry, Baker University, P.O. Box 65, Baldwin City, Kansas, 66006

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Abstract

Tardigrades live in many ecosystems, but local dispersal mechanisms and the influence of ecological gradients on tardigrade communities are not fully understood. Here we examine tardigrade communities in nests of the red tree vole (*Arborimus longicaudus* True), an arboreal mammal occupying the canopy of coniferous forests in western Oregon and northwestern California. We found 12 species of tardigrades from resin ducts sampled from 43 nests along a transect that spanned the east-west range of the red tree vole in southern Oregon. Tardigrade occurrence was more likely in larger trees and species numbers were significantly higher in areas that received more precipitation. At sites where they occurred, tardigrades were more abundant in red tree vole nests at greater heights within the forest canopy. Of the 12 species of tardigrades that were found, seven have not been previously reported in Oregon. Our results suggest that tardigrades in forest canopies in the Pacific Northwest are affected by regional precipitation gradients as well as local environmental variables, and that nest building by small mammals may facilitate dispersal of tardigrades within the forest canopy.

Keywords: tree crowns, red tree vole nests, metazoans, invertebrates, cryptic diversity

Introduction

Tardigrades are aquatic micro-metazoans that occur globally across a range of marine, freshwater and terrestrial habitats (Miller 1997). Tardigrade occupancy of tree crowns in the forest canopy is well documented (Miller et al. 2013), and they can be reliably encountered in epiphytic vegetation such as lichens and bryophytes (Miller et al. 2004). Tardigrades have been observed in the nests and on the feathers of tree dwelling birds (Mogle et al. 2018) and new species have been recently described from the upper canopy (Haefke et al. 2014, Young et al. 2016, Schlabach et al. 2018). Species otherwise known only from other continents have been located in the forest canopies of North America (Spiers et al. 2013, Chappell et al. 2105, Tibbs et al. 2016). Although tardigrades are difficult to study in natural environments, regional and hemispherical diversity patterns are beginning to emerge due to regional compilation studies (Meyer 2013; Kaczmarek et al. 2016a, 2016b).

The anhydrobiotic nature of tardigrades allows them to persist in the seasonally dry temperate rainforest canopies of Douglas-fir forests (Voegtlin 1982), where distinct community assemblages can develop within different canopy strata and epiphyte types (Young et al. 2018). Lack of detailed knowledge of within tree dispersal mechanisms is a limiting factor for understanding small scale species distribution patterns considering these animals' small size and the absence of long-distance self-propelled travel through water (McInnes 1994, McInnes and Pugh 2007). Tardigrades likely have

[1] Author to whom correspondence should be addressed: john.c.villella@gmail.com

specific local environmental needs, and in forested systems they may be sensitive to microclimate gradients such as humidity (Wright 1991), canopy height (Chang et al. 2015), or epiphyte functional group (Young et al. 2018). Because saturation is required for tardigrade activity, regional precipitation patterns may shape the abundance and species richness of tardigrade communities.

The red tree vole (*Arborimus longicaudus* True) is a small arboreal rodent inhabiting temperate coniferous forests of western Oregon and northwestern California (Forsman et al. 2016). Red tree voles feed primarily on conifer needles and are considered the most canopy-dependent mammal in the Pacific Northwest (Carey 1996). They forage primarily at night when they harvest fresh branch tips and bring them to the nest, where they are stored for later consumption (Forsman et al. 2009).

Resin ducts are linear tubules that occur inside conifer needles and are rich in anti-herbivory compounds such as terpenoid resins (Kelsey et al. 2009). When feeding on conifer needles tree voles remove the resin ducts and eat the rest of the needle. The discarded resin ducts are used to line the living chambers and tunnels inside the nest (Clifton 1960, Maser 1966). Nests are constructed in areas where there is an abundance of easily accessible fresh conifer needles and also a suitable foundation, most frequently in Douglas-fir, but occasionally in other conifers (Benson and Borell 1931, Forsman et al. 2016). Because of the hydrophobic nature of resin ducts (Dell and McComb 1979), red tree vole nests may be less hospitable to limno-terrestrial tardigrade establishment than nearby lichens or bryophytes.

In Douglas-fir forests of the Pacific Northwest tardigrades have been reported in canopy epiphytes and on foliage (Voegtlin 1982, Young et al. 2018), and in ground dwelling mosses (Merrifield and Ingham 1998). Because red tree voles move canopy foliage and epiphytes from place to place they may act as dispersal agents for tardigrades. Nesting behavior of other forest animals such as squirrels (Carey 1996), and birds may also contribute to cryptic dispersal opportunities both at the local and continental scale (Mogle et al. 2018). The nest building behavior of the red tree vole congregates canopy foliage, creating opportunities for increased connectivity of disjunct local tardigrade populations.

We document and compare tardigrade communities from red tree vole nests along the following gradients: 1) a precipitation gradient from the wetter coast into the drier Cascade Mountains, 2) a gradient of height above the ground within the canopy, and 3) a gradient in tree diameter at breast height (DBH) as a proxy for tree age. Due to their aquatic requirements we expected that tardigrade abundance and diversity within nests would be greater in wetter locations.

Methods

Field Surveys

During 2010–2015 we collected samples of resin ducts from 43 tree vole nests in 43 separate Douglas-fir trees. These nests were located in the forest canopy (5 to 35 m above ground) along an approximately 200 km longitudinal transect that crossed the range of the red tree vole in southwestern Oregon ranging from longitude –122.35 to –124.21 Mature forests with Douglas-fir as the dominant or codominant tree were selected for study because these were the primary habitat of the red tree vole in most of western Oregon (Forsman 2016). Individual trees were inspected from the ground using binoculars to locate likely nest structures. Trees were then climbed and inspected from the lower tree crown to heights of 35 meters. When found, a sample of resin duct material was collected (approximately 10 grams) from each tree vole nest. These samples were transported to the ground in zipped plastic bags, then stored in paper bags at room temperature until the nest material was dry, and finally stored long-term in zipped plastic bags. Tree location, nest height, and tree diameter were recorded for each nest encountered. Annual precipitation values for each tree location were obtained from WorldClim (Hijmans et al. 2005).

Tardigrade Community Characterization

One gram of material from each nest was placed into a small plastic cup and hydrated with 20 mL of commercially bottled spring water for 24 hours. Three 1-mL aliquots were examined with replacement under a 20X-dissecting microscope with reflected light (Miller 1997). All observed tardigrades were extracted with an Irwin loop (Schram and Davidson 2012) and deposited into a drop of polyvinyl alcohol medium (Salmon 1951) on a glass slide and covered with a coverslip. After five days slides were sealed with fingernail polish to preserve specimen morphology (Kinchin 1994, Miller 1997). Tardigrade abundance was reported as the number of specimens in a 3-mL aliquot of hydrated nest material. Identification of tardigrades was based on Ramazzotti and Mauchi (1983), Pilato and Binda (2010), and Schuster and Grigarick (1965). Nomenclature was based on Guidetti and Bertolani (2005), Degma and Guidetti (2007), Degma, Bertolani and Guidetti (2019), Guil et al. (2018) and Kaczmarek et al. (2018).

Statistical Methods

We used three separate models to test how number of tardigrades per sample, tardigrade occurrence, and tardigrade abundance at sites where they occurred varied in response to tree diameter at breast height, red tree vole nest height, and site precipitation. We used a generalized linear mixed model with a Poisson distribution to analyze number of tardigrades per sample; this model included an observation-level random effect (Harrison 2014). We used a generalized linear model with a binomial distribution (e.g., a logistic model) for tardigrade occurrence, and a linear model for tardigrade abundance at sites where tardigrades occurred. All statistical analyses were performed in R (version 3.4.3), and the lme4 package (Bates et al. 2015) was used for the mixed model.

Results

Of the 43 nests sampled, 28 (65%) contained at least one tardigrade. A total of 167 individual tardigrades were found, representing nine genera and 12 species: *Claxtonia wendti*, *Echiniscus merokensis*, *Milnesium granulatum*, *Hypsibius convergens*, *Hysibius microps*, *Hysibius pallidus*, *Pilatobius nodulosus*, *Ramazzottius oberhaeuseri*, *Macrobiotus hufelandi*, *Macrobiotus spectabilis*, *Mesobiotus* cf. *harmsworthi*, and *Murrayon* cf. *hibernicus*. *Hysibius pallidus* was the most frequently found species at 52% of specimens. Seven of the 12 species (58%) (*E. merokensis, M. granulatum, H. microps*, *H. pallidus*, *P. nodulosus*, *M. spectabilis, M*. cf. *hibernicus*) are reported for the first time in Oregon, raising the known number of tardigrade species in the state by 26% from 27 to 34 (Kaczmarek et al. 2016b).

The number of tardigrade species found per sample was positively associated with local annual precipitation (P = 0.002), but was not strongly influenced by tree diameter (P = 0.446) or nest height (P = 0.095; Table 1, Figure 1). Tardigrade occurrence increased in larger diameter trees (P = 0.046), trended positively with increasing annual precipitation (P = 0.077), and was not substantially affected by nest height (P = 0.103). Tardigrade abundance (the total number of tardigrades per nest sample) at sites where tardigrades occurred was higher in nests that were higher above the ground (P = 0.004), but was unaffected by precipitation (P = 0.102) or tree size (P = 0.295; Table 1, Figure 1).

Discussion

We documented populations of tardigrades on most of the red tree vole nests we sampled, and tree size, nest height, and local precipitation all affected various attributes of tardigrade communities. Tree size (represented by DBH) had a significant effect on tardigrade occurrence, but not on the number of species per sample or the abundance of tardigrades where they occurred. Nest height also positively influenced tardigrade abundance at sites where they occurred. Larger, older trees have had more time to experience tardigrade dispersal, and the greater habitat heterogeneity of older forests could also drive the response of tardigrades to tree size.

Precipitation was the only significant predictor of the number of tardigrade species that were identified, with number of tardigrade species increasing in wetter areas. This finding is con-

TABLE 1. Model results for a generalized linear mixed model for tardigrade richness, a logistic model for tardigrade occurrence, and a linear model for tardigrade abundance at sites where tardigrades were present. Tree diameter at breast height (DBH), site annual precipitation, and red tree vole nest height were the predictor variables for each model. *P*-values < 0.05 are shaded grey, 40 sites analyzed for tardigrade richness and occurrence, and 26 sites analyzed for tardigrade abundance.

Predictor variables and intercept	Tardigrade richness		Tardigrade occurrence		Tardigrade abundance when present	
	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
(Intercept)	–16.414	**0.003**	–38.065	**0.030**	13.503	**0.039**
Tree DBH	0.677	0.446	6.736	**0.046**	–1.007	0.295
Annual precipitation	7.630	**0.002**	13.658	0.077	–4.520	0.102
Nest height	–0.171	0.095	–0.658	0.103	0.365	**0.004**

sistent with Guil et al. (2009) who showed that macroscale environment factors shape tardigrade communities across the landscape. Precipitation is broadly related to ecosystem productivity, which is positively related to the biodiversity of many organisms. However, tardigrades may be especially responsive to precipitation because they become inactive in dry conditions. Our findings suggest that climatic factors such as annual precipitation may drive broad species distribution patterns, while small-scale environmental and biotic factors could have greater effects at the population level (Kinchin 1994, Zawierucha et al. 2019).

The discovery of seven species of tardigrade new to Oregon increases the state's tardigrade species diversity by 26%. It is not clear if the high number of new state records is a reflection of the few tardigrade studies that have been conducted in Oregon or if the sampled habitat within the canopy has a unique community. This study indicates that there are likely more species to be reported for the state of Oregon. A new state record, *Hypsibius pallidus*, was the most frequently encountered species (52%).

Here, we document for the first time the occurrence of tardigrades in the nests of an arboreal mammal. Biotic interactions such as the red tree vole nesting behavior may be important for canopy tardigrade communities, and there are other possible interactions between these mammals and canopy dwelling micro-metazoans communities that remain unexplored. Other canopy dwelling mammals could disperse tardigrades by the

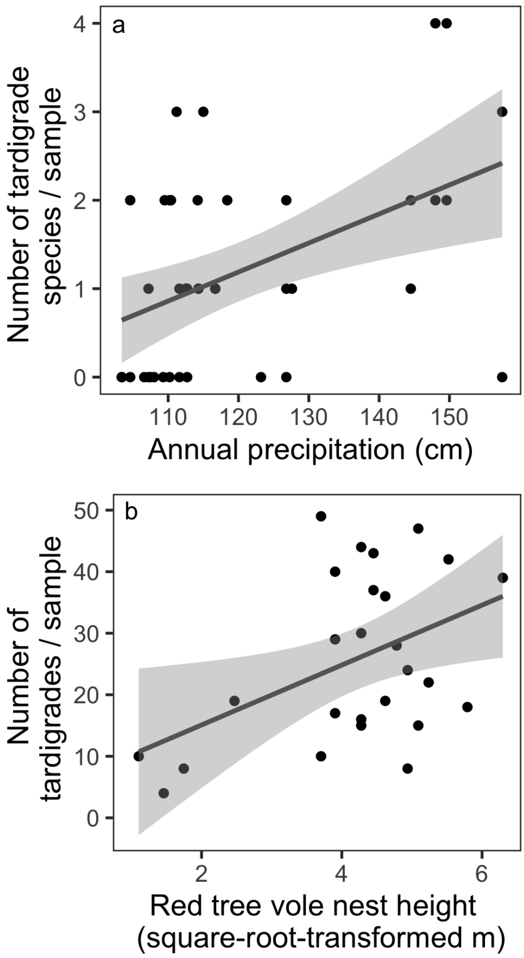

Figure 1. (a) Annual precipitation had a positive effect on the number of tardigrade species per sample. (b) The height of red tree vole nests above the ground had a positive effect on the number of tardigrades per sample at sites.

movement of nesting materials, or by tardigrades adhering to the mammal's fur while scurrying about on trunks and limbs or using their nests, as with birds (Mogel et al. 2018). Dispersal could also come from ingesting the animals and later depositing them with fecal material, as with snails (Fox and Garcia-Moll 1962) or birds (Zawierucha et al. 2016). Fecal material has been shown to affect tardigrade populations by altering habitat chemistry (Porazińska et al 2002, Zawierucha et al. 2016) and this would be an interesting topic for future studies of mammal nest-dwelling tardigrades. It is our hope that future research will garner a greater understanding of the complex trophic interactions between vertebrates and invertebrates within forest canopies.

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Literature Cited

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Benson, S., and A. Borell. 1931. Notes on the life history of the red tree mouse, *Phenacomys longicaudus*. Journal of Mammalogy 12:226-233.

Chang, L., D. Powell, W. R. Miller, and M. D. Lowman. 2015. Tardigrades of the canopy: evidence of stratification. Transactions of the Kansas Academy of Science 118:230-236.

Chappell, B. M., D. Parry, W. R. Miller, and M. D. Lowman. 2015. Tardigrades of the canopy: *Doryphoribius gibber* Beasley and Pliato, 1987 (Eutardigrada: Parachela: Hypsibiidae) new records from eastern Kansas, USA. Transactions of the Kansas Academy of Science118:48-52.

Carey, A. B. 1996. Interactions of Northwest forest canopies and arboreal mammals. Northwest Science 70:70-78.

Clifton, P. L. 1960. Biology and life history of the dusky tree mouse *Phenacomys silvicola* (Howell). Ph.D. Dissertation, Walla Walla College, Walla Walla, WA.

Degma, P., R. Bertolani, and R. Guidetti. 2019. Actual checklist of Tardigrada species 2009–2019, 35th Edition: 31-07-2019. Available online at http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf (accessed 08 October 2019).

Degma, P., and R. Guidetti. 2007. Notes on the current checklist of Tardigrada. Zootaxa 1579:41-53.

Dell, B., and A. J. McComb. 1979. Plant resins—their formation, secretion and possible functions. *In* Advances in Botanical Research, Vol. 6, H. W. Wolhouse (editor), Academic Press, London, UK. Pp. 277-316.

Forsman, E. D., J. K. Swingle, and N. R. Hatch. 2009. Behavior of red tree voles (*Arborimus longicaudus*) based on continuous video monitoring of nests. Northwest Science 83:262-272.

Forsman, E. D., J. K. Swingle, R. J. Davis, B. L. Biswell, and L. S. Andrews. 2016. Tree voles: an evaluation of their distribution and habitat relationships based on recent and historical studies, habitat models, and vegetation change. USDA Forest Service Gen Tech Rep PNW-GTR-948. Pacific Northwest Research Station, Portland, OR.

Fox, I., and I. Garcia-Moll. 1962. *Echiniscus molluscorum*, new tardigrade from the feces of the land snail, *Bulimulus exilis* (Gmelin) in Puerto Rico (Tardigrada: Scutechiniscidae) Journal of Parasitology 48:177-181.

Guidetti, R., and R. Bertolani. 2005. Tardigrade taxonomy: an updated checklist of the taxa and a list of characters for their identification. Zootaxa 485:1-46.

Guil, N., J. Hortal, S. Sánchez-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.

Guil, N., A. Jorgensen, and R. Kristensen. 2018. An upgraded comprehensive multilocus phylogeny of the Tardigrada tree of life. Zoologica Scripta 48:120-137.

Haefke, B. J., A. I. Spiers, W. R. Miller, and M. D. Lowman. 2014. Tardigrades of the canopy: *Doryphoribius elleneddiei* nov. sp. (Eutardigrada, Parachela, Hypsibiidae), a new species from eastern Kansas, USA. Transactions of the Kansas Academy of Science 117:299-304.

Harrison, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ 2:e616.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978.

Kaczmarek, Ł., B. Gołdyn, S. J. McInnes, and Ł. Michalczyk. 2016a. Diversity of limno-terrestrial tardigrades of the Americas in relation to the Great American Biotic Interchange hypothesis (GABI). Zoological Journal of the Linnean Society 178:737-746.

Kaczmarek, Ł, Ł. Michalczyk, and S. J. McInnes. 2016b. Annotated zoogeography of non-marine Tardigrada. Part III: North America and Greenland. Zootaxa 4203:1-249.

Kaczmarek, Ł., K. Zawierucha, J. Buda, D. Stec, M. Gawlak, L. Michalczyk, and M. Roszkowska. 2018. An integrative redescription of the nominal taxon for the *Mesobiotus harmsworthi* group (Tardigrada: Macrobiotidae) leads to descriptions of two new *Mesobiotus* species from Arctic. PLOS One 13:e0204756.

Kelsey, R. G., E. D. Forsman, and J. K. Swingle. 2009. Terpenoid resin distribution in conifer needles with implications for red tree vole, *Arborimus longicaudus*, foraging. The Canadian Field-Naturalist 123:12-18.

Kinchin, I. M. 1994. The Biology of Tardigrades. Portland Press, London.

McInnes, S. J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. Journal of Natural History 28:257-352.

Maser, C.O. 1966. Life histories and ecology of *Phenacomys albipes*, *Phenacomys longicaudus*, *Phenacomys silvicola*. M.S. Thesis, Oregon State University, Corvallis, OR.

McInnes, S. J., and P. J. A. Pugh. 2007. An attempt to revisit the global biography of limno-terrestrial Tardigrada. Journal of Limnology 66:90-96.

Merrifield, K., and R. E. Ingham. 1998. Nematodes and other aquatic invertebrates in *Eurhynchium oreganum* from Mary's Peak, Oregon Coast Range. The Bryologist 101:505-511.

Meyer, H. A. 2013. Terrestrial and freshwater Tardigrades of the Americas. Zootaxa 3747:1-71.

Miller, W. R. 1997. Tardigrades: bears of the moss. The Kansas School Naturalist 43:1-15.

Miller, W. R. 2004. Tardigrades: bears of the canopy. *In* M. D Lowman and H. B. Rinkner (editors), Forest Canopies, Elsevier Academic Press, San Diego. Pp. 251-258.

Miller, W. R., L. Gallardo, and T. Clark. 2013. Do water bears climb trees too? *In* M. D. Lowman (editor), Tree Tops at Risk: the Challenges of Global Canopy Ecology and Conservation, Springer, New York. Pp. 307-311.

Mogle, M. J., S. A. Kimball, W. R. Miller, and R. D. McKown. 2018. Tardigrades in bird plumage support avian-mediated long distance dispersal. PeerJ 6:e5035.

Pilato, G., and M. G. Binda. 2010. Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. Zootaxa 2404:1-54.

Porazińska, D. L., D. H. Wall, and R. A. Virginia. 2002. Invertebrates in ornithogenic soils on Ross Island, Antarctica. Polar Biology 25:569-574.

Ramazzotti, G., and W. Mauchi. 1983. Il Philum Tardigrada (3rd edition), Memorie dell'Istituto italiao id Idrobiologia Marco Marchi 41:1-1012.

Salmon, J. T. 1951. Polyvinyl alcohol as a mounting medium in microscopy. The Microscope 8:139-142.

Schlabach, S., E. Donaldson, K. Hobelman, W. R. Miller, and M. D. Lowman. 2018. Tardigrades of the Canopy: *Milnesium burgessi* nov. sp. (Eutardigrada: Apochela: Milnesiidae) a new species from Kansas, USA. Transactions of the Kansas Academy of Science 121:39-48.

Schram, M. D., and P. G. Davidson. 2012. A history and method of constructing homemade loops. Transactions of the Kansas Academy of Science 115:35-40.

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

Spiers, A. I., B. J. Haefke, W. R. Miller, and M. D. Lowman. 2013. Tardigrades in the Canopy: *Doryphoribius dawkinsi* Michalczyk and Kaczmarek, 2010—New records from eastern Kansas, USA. Transactions of the Kansas Academy of Science 116:125-128.

Tibbs, L., A. Emanuels, and W. R. Miller. 2016. Tardigrades of the Canopy: Argentine species *Milnesium beatae* Roszkowska, Ostrowska and Kaczmarek, 2015 (Eutardigrada, Milnesidae) discovered in the trees of Kansas, USA. Transactions of the Kansas Academy of Science 119:173-178.

Voegtlin, D. J. 1982. Invertebrates of the H. J. Andrews Experimental Forest, western Cascade mountains, Oregon: a survey of arthropods associated with the canopy of old-growth *Pseudotsuga menziesii*. Special Publication 4. USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, Corvallis, OR.

Wright, J. C. 1991. The significance of four xeric parameters in the ecology of terrestrial Tardigrada. Journal of Zoology 224:59-77.

Young, A. R., B. Chappell, W. R. Miller, and M. D. Lowman. 2016. Tardigrades of the Canopy: *Milnesium swansoni* nov. sp. (Eutardigrada: Apochela: Milnesiidae) a new species from Kansas. Zootaxa 4072:559-568.

Young, A. R., J. E. D. Miller, J. Villella, G. Carey, and W. R. Miller. 2018. Epiphyte type and sampling height impact mesofauna communities in Douglas-fir trees. PeerJ 6:e5699.

Zawierucha K., K. Zmudczyńska-Skarbek, Ł. Kaczmarek, and K. Wojczulanis-Jakubas. 2016. The influence of a seabird colony on abundance and species composition of water bears (Tardigrada) in Hornsund (Spitsbergen, Arctic). Polar Biology 39:713-723.

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Zawierucha, K., J. Buda, D. Fontaneto, R. Ambrosini, A. Franzetti, M. Wierzgoń, and M. Bogdziewicz. 2019. Fine-scale spatial heterogeneity of invertebrates within cryoconite holes. Aquatic Ecology 53:179-190.