Water Bears Don't Care –The Effects of Elevation on Tardigrade Class Distribution

Kelly Lau

Abstract

Previous studies aimed at elucidating the correlation between elevation and limno-terrestrial tardigrade distribution have been contradictory with positive, negative and no relationships being concluded. In particular, differences in the abundance of the two classes of tardigrades – Eutardigrada and Heterotardigrada – across various elevations has been observed but not studied. If elevation does affect tardigrade class distribution, one class of tardigrades should dominate over the other at different elevations. To better understand and clarify the relationship between tardigrade distribution and elevation, a minimum of three replicate samples of moss at three different elevations between 0m and 1010m were collected in Greater Vancouver for a total of 45 tardigrades identified by class. Data was analyzed by Two-way ANOVA to reveal no correlation between elevation and tardigrade class distribution with eutardigrades being dominant at every tested elevation. These results lend support to the idea that elevation may not be the key factor in determining the distribution of limno-terrestrial tardigrades.

Introduction

Tardigrades, commonly known as water bears, are microscopic animals typically measuring less than 1mm in length. The phylum Tardigrada is comprised of three classes – Heterotardigrada,

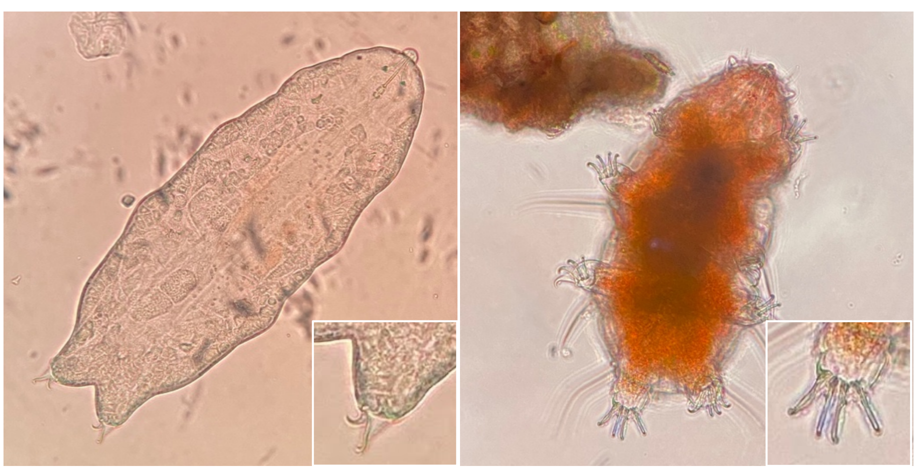

Figure 1. *Light microscope images of a eutardigrade (Left) and heterotardigrade (Right). Insets show the eutardigrade double claw and heterotardigrade individual claws.*

Eutardigrada and Mesotardigrada – and over 1,300 described species (Wright, n.d.; University of Florida, n.d.). However, because Mesotardigrada has only been described once in Japan, it will not be considered for the purpose of this study (Grothman et al., 2017). All tardigrades are bilaterally symmetrical, segmented and have 8 legs – three ventrolateral pairs and a posterior pair (Nelson, 2002; Møbjerg et al., 2018). Heterotardigrades and eutardigrades have distinct morphological differences important for their identification. For example, heterotardigrades are armoured with a thick cuticle while eutardigrades have smooth cuticles and are considered naked. Furthermore, heterotardigrades generally have four separate claws extending from each leg while eutardigrades have a pair of branched double claws (Miller, 1997; Møbjerg et al., 2018).

Tardigrades are ubiquitous across the globe and can be found in marine, freshwater, and terrestrial habitats (Nelson, 2002). Tardigrades are aquatic and need water to perform all their physiological functions such as breathing, feeding and reproduction (Miller, 1997). In terrestrial habitats, tardigrades are limno-terrestrial and can be found living in water films surrounding moss and lichen (Wright, n.d.; Schill, 2018). Eutardigrades are predominantly semiterrestrial or limnic while, in Heterotardigrada, only the Echiniscoididae family are found in terrestrial habitats (Møbjerg et al., 2018; University of Florida, n.d.).

Several attempts have been made to elucidate the relationship between elevation and tardigrade distribution, but independent findings have been conflicting. Many studies have reported a relationship between tardigrade distribution and elevation but whether species richness increases or decreases with elevation has been inconclusive (Dastych, 1985; Collins & Bateman, 2001; Boeckner & Proctor, 2005; Zawierucha et al., 2015). On the other hand, studies have also cited no dependence on elevation (Kathman & Cross, 1991). The same contradictory trend is also reflected in elevational studies of tardigrade class distribution. By studying 40 samples from elevations ranging between 150m and >1525m, data presented by Kathman and Cross (1991) suggest a greater number of eutardigrade across all elevations and samples compared to heterotardigrade on Vancouver Island, British Columbia. Studies conducted by Zawierucha et al. (2015) on the other hand showed that heterotardigrades of the Echiniscoididae family were two of the top three species dominating at higher elevations while eutardigrades dominated all three top spots at lower elevations. Due to contradictions presented by previous studies, here we aim to clarify the relationship between the class distribution of tardigrades and elevation. If elevation does affect the class distribution of tardigrades, we would expect to see one class of tardigrades being more dominant at one elevation compared to the other class.

Methods

Field Sampling

Samples of moss were collected from trees at three different elevations – high (>900m), moderate (300-600m) and low elevation (<100m) – at Mount Seymour (1010m), Burnaby Mountain (310m) and the University of British Columbia Vancouver Point Grey Campus (90m) respectively. A minimum of three replicate samples were collected from each elevation with at least 15 meters of distance between each sampling site to ensure independent sampling for a minimum of nine samples across all elevations. Roughly 5 x 5cm of moss was removed from trees with either a plastic butter knife or by hand into a plastic bag. Elevation was determined by an iPhone compass application. Samples that were not used within 48 hours were stored in the freezer (roughly -20°C) while those being used within the 48-hour period were kept at room temperature for further processing.

Sample Processing and Tardigrade Identification

Moss samples were added to cover the bottom of a small container and distilled water was used to submerge samples. Fresh samples were rehydrated in distilled water for at least 30 minutes before proceeding. Samples that were stored in the freezer were thawed then rehydrated for at least 24 hours. Rehydrated samples were then agitated briefly before the water was transferred to a clear watch glass using a transfer pipet. Absorbent moss samples were compressed to extract excess water. Large pieces of debris were not transferred between containers. A dissecting microscope at 40-60x total magnification was used to systematically look for tardigrades. Generally, tardigrades were found walking along the bottom of the watch glass or attached to a piece of debris. Once a tardigrade was found, a micropipette was used to carefully transfer the tardigrade to a microscope slide. Distilled water was used to prepare the microscope slide and viewed under a dissecting microscope at 200-400x total magnification. Photos were taken at 400x using an iPhone and tardigrades were identified as either class Eutardigrada or Heterotardigrada based on their characteristic morphological differences (Miller, 1997; Nelson, 2002; Møbjerg et al., 2018; "Tardigrades - Classification, Reproduction, Habitat and Survival," n.d.). Up to five tardigrades were identified per replicate sample for a total of 15 tardigrades per elevation.

Data Analysis

Data was analyzed by Two-Way ANOVA using GraphPad Prism 9 (Version 9.3.1) with a significance level of 0.05 to compare the class distribution of tardigrades to the elevation among and between samples.

Results

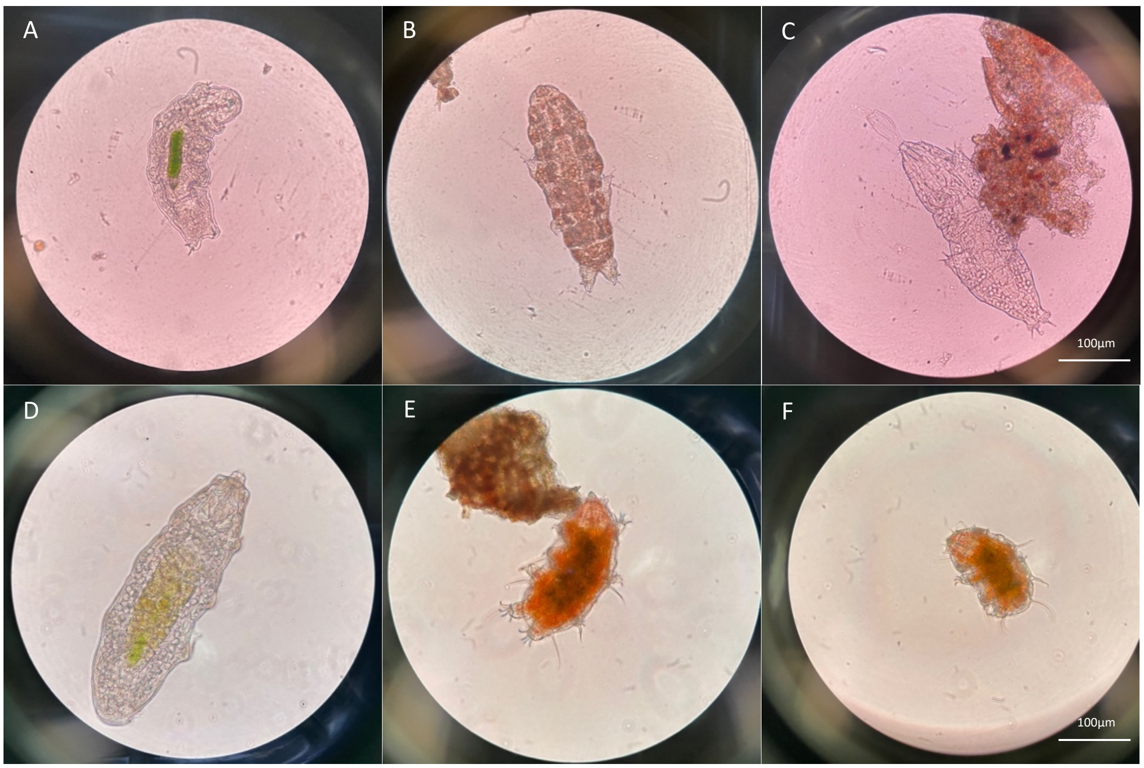

Figure 2. *Light microscope images of various eutardigrade (A-D) and heterotardigrade (E-F) identified. Total magnification, 400x. Scale bar, 100µm.*

Multiple families of Eutardigrada (Figure 2A-D) and the Echiniscoididae family of Heterotardigrada (Figure 2E-F) were observed. In general, eutardigrades were clear while heterotardigrades were reddish brown in colour. Observed eutardigrades had longer bodies and were more active than their heterotardigrade counterparts which were stubbier and more sluggish. Sizes of tardigrades varied between 100μm and 600μm. Tardigrades in samples that were frozen moved less than those from fresh samples. Tardigrades from frozen samples were often found inactive and floating on the surface of the water instead of walking along the bottom or attached to a piece of debris.

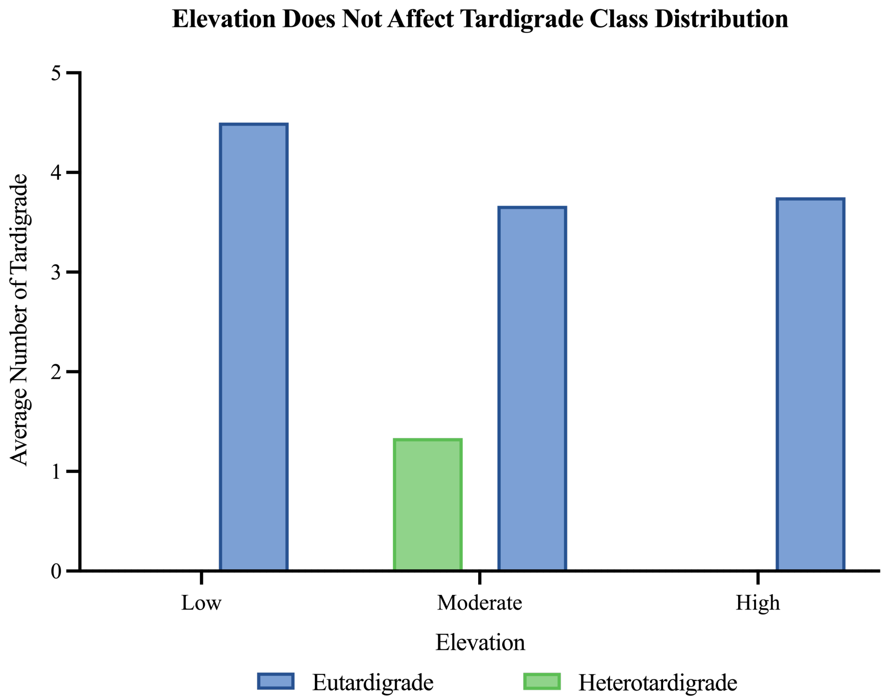

Figure 3. *A bar graph summarizing the average number of tardigrades of each class found at sampled elevations. A total of 15 tardigrades were identified per elevation. Low elevation samples were collected at 90m, moderate at 310m, and high at 1010m. The relationship between tardigrade class distribution and elevation was analyzed using a two-way ANOVA (P(0.7040)>0.05).*

15 tardigrades were identified per elevation for a total of 45 tardigrades across all samples (Figure 3). Five tardigrades were identified from each of the three replicate samples at low and moderate elevation while four replicate samples were observed for high elevation samples (Table S1). Eutardigrades were found to be more frequent across all elevations than heterotardigrades. At both low (90m) and high (1010m) elevation, no heterotardigrades were observed while four were identified at moderate elevation (310m). Heterotardigrades identified in the moderate elevation sample were all found within one replicate sample. Analysis using two-way ANOVA revealed no significant difference between the number of eu- and heterotardigrade across the three elevations.

Discussion

Given the contradictions presented by previous studies on the relationship between tardigrade distribution and elevation, the present study was conducted to investigate the matter by collecting samples from three different elevations, and identifying and quantifying tardigrades by class for further analysis. Analysis of data by two-way ANOVA revealed no correlation between tardigrade class distribution and elevation. Eutardigrades were found to be dominant at all three elevations lending support to previous findings by Kathman and Cross (1991).

Heterotardigrades were discovered at moderate elevation but were all found within one replicate sample. It is possible that heterotardigrades are only found at moderate elevation (relative to other sampled elevations) but given the small sample size of the current study, this cannot be concluded with confidence. On the other hand, heterotardigrades may have only been identified in a single replicate sample at one elevation due to patchiness in tardigrade abundance across the terrain of each sampling site. In both cases, more vigorous sample collection to increase sample size at each elevation will confirm whether heterotardigrades are truly only present at moderate elevations. As well, increasing the number of sampled elevation sites may help better understand the elevational range at which heterotardigrades are most abundant at. In addition to this, as tardigrades were identified based on the first five to be spotted, it is possible that the more commonly known eutardigrades were much easier to spot and identify than heterotardigrades because of observer bias. Bias can be minimized by performing a more rigorous extraction of tardigrades from samples by running samples through a cheese cloth or sieve as illustrated by

Kathman and Cross (1991) to remove larger pieces of debris from the sample making it easier to spot tardigrades.

Alternatively, because no relationship between elevation and tardigrade distribution was found, elevation may not the leading factor affecting tardigrade distribution but rather some other environmental factor such as substrate type, moisture, light exposure, or temperature. In the present study, no differences in substrate type were prominent among replicate samples but were different across samples collected at different elevations. However, previous analysis by Kathman and Cross (1991) revealed no significant effect of moss type on tardigrade distribution. To determine whether elevation is the main factor affecting tardigrade distribution, future studies may wish to keep the elevation the same across samples but vary the substrate type, moisture level or other environmental factors instead.

Of interest, tardigrades in samples that were frozen then thawed prior to viewing appeared to have more sluggish movements with many floating on the surface instead of moving along the bottom of the watch glass. Hengherr and colleagues (2009) suggest that limno-terrestrial tardigrade survival is linked to the cooling rate of the environment. Slower cooling rates allow tardigrades more time to synthesize and recruit temperature protecting agents leading to an increased survival rate (Hengherr et al., 2009). In this study, samples that were not processed immediately were kept at roughly -20^{o}C for several days before being thawed. The acute change in temperature from near zero to -20^{o}C may have been too sudden for the tardigrades to undergo proper preparation and, as a result, led to mortality.

Conclusion

Overall, data presented in this study indicate no correlation between tardigrade distribution and elevation, with eutardigrades being the major class across all three elevations. However, due to the small sample size and sampling sites, confidence would be increased by increasing both replicates and elevations sampled. Future studies would benefit from studying the effects of other environmental factors rather than elevation alone as elevation does not appear to be the main factor affecting tardigrade distribution.

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Appendix

Collection Site	University of British Columbia – Vancouver Point Grey		
Date of Collection	March 4, 2022		
Elevation (m)	90m		
Substrate Type	Furry green moss	Furry green moss	Furry green moss
Sample Code	L1	L2	L3
Number of Heterotardigrade	0	0	0
Number of Eutardigrade	5	5	5

Collection Site	Burnaby Mountain Park		
Date of Collection	March 9, 2022		
Elevation (m)	310m		
Substrate Type	Furry, green, fern-like moss	Furry, green, fern-like moss	Furry, green, fern-like moss
Sample Code	M1	M2	M3
Number of Heterotardigrade	0	0	4
Number of Eutardigrade	5	5	1

Collection Site	Mount Seymour			
Date of Collection	March 13, 2022			
Elevation (m)	1010m			
Substrate Type	Green cushion-like moss	Green cushion-like moss	Green cushion-like moss	Green cushion-like moss
Sample Code	H1	H2	H4	H6
Number of Heterotardigrade	0	0	0	0
Number of Eutardigrade	2	5	3	5

Table S1. *Details regarding the collection site, date, elevation, substrate type as well as the number of heterotardigrade and eutardigrade for each elevation.*