

**Effects of traffic-derived Cu pollution and climate change on arboreal  
Collembola in Western Washington, USA**

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**Abstract**

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Dr. Patrick C. Tobin

Increased demand for travel in the Pacific Northwest has led to higher inputs of heavy metal pollutants in the region, many of which have been shown to increase in toxicity for various organisms at higher temperatures. I investigated the effects of Cu and supraoptimal temperatures on a moss-dwelling and soil-dwelling species of Collembola. I reared *Folsomia candida* Willem under laboratory conditions and collected *Choreutinula americana* sp. nov. from two sites representing high (Seattle) and low (Hoh Rainforest) background Cu levels in Western Washington. I first exposed Collembola to aqueous Cu solutions ranging from 0.1 to 10,000 ppm to establish LC<sub>25</sub> and LC<sub>50</sub> values. I then exposed each population to their respective LC<sub>25</sub> and LC<sub>50</sub> concentrations at three temperature regimes based on ambient temperatures, B1 (+1.8°C), and A2 (+3.5°C) IPCC climate change scenarios. I found *C. americana* to be more sensitive to Cu than lab-reared *F. candida*, and increased temperatures greatly lowered both species tolerance to Cu. The results of this study highlight the impact that interactions between climate change and pollutants have on ecosystem health.

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## Chapter 1: Introduction

Seattle is currently ranked as the 10<sup>th</sup> most traffic-congested city in the United States (INRIX 2016). Despite its reputation as an environmentally conscious city, Seattle has more cars per person (637 per 1,000 people) than the other nine densest large US cities, including Los Angeles (Balk 2017). As cities become denser, most will begin to add people at a faster rate than they add vehicles (Balk 2017). However, when Seattle's population increased by 12% from 2010 to 2015, the number of vehicles also increased by 12% over the same time period (Balk 2017), bringing the citywide average traffic count to >1 million vehicles per day (WSDOT 2015). With this increase in vehicles comes an inevitable increase in traffic-derived heavy metal pollutants, including Iron, Copper, Zinc, Cadmium, and Titanium (Pearson et al. 2000, Bakirdere and Yaman 2008, Thorpe and Harrison 2008, Apeageyi et al. 2011, Wesley 2012). Copper is of particular concern to the State of Washington because it negatively affects endangered Salmonid populations, even at relatively low concentrations in aqueous environments (Boxall and Maltby 1997, Baldwin et al. 2003, Sandahl et al. 2004, Linbo et al. 2006, Sandahl et al. 2007).

Despite the high impact of Cu in aquatic ecosystems, there has been scant attention given to the effects of increasing Cu pollution on PNW forest ecosystems (Bidwell 2017). Western Washington forests are defined by heavy loads of epiphytic bryophytes, which provide ecosystem services in the form of water retention, nutrient cycling, and habitat creation (Binkley and Graham 1981, Nadkarni 1984, Pypker et al. 2006, Cornelissen et al. 2007, Lindo and Gonzalez 2010). There is a history of using bryophytes, such as moss, and lichen as indicators of air quality due

to their sensitivity to a range of environmental pollutants (Rühling and Tyler 1973, Bengston et al. 1982, Gjengedal and Steinnes 1990, Tyler 1990, Bates 1992, Pott and Turpin 1996, Pearson et al. 2000, Cornelissen et al. 2007, Cesa et al. 2008, Harmens et al. 2008, Zvereva and Kozlov 2010, Baceva et al. 2012, Gonzalez and Pokrovsky 2014, Donovan et al. 2016, Bidwell 2017). Along with providing a snapshot of background Cu levels across Western Washington, epiphytic mosses also support diverse microarthropod communities that can be studied in the context of that pollution (Nadkarni 1984, Lindo and Winchester 2006, Lindo and Gonzalez 2010, Lindo et al. 2013). One of the most abundant organisms living within these epiphyte communities are Collembola, which hold several functional roles within the ecosystem of their own, including stimulation of hyphal growth through fungal grazing, pollination of mosses, and decomposition of organic matter (Fountain and Hopkin 2005, Rosenstiel et al. 2012, Lindo et al. 2013, Shortlidge 2014). Collembola have also been used extensively in ecotoxicology studies examining the effects of heavy metals (Crommentuijn et al. 1995, Sandifer and Hopkin 1996, Pedersen et al. 1997, Scott-Fordsmand et al. 1997, Smit and Van Gestel 1997, Pedersen et al. 1999, Filser et al. 2000, Fountain and Hopkin 2001, 2005, Sorensen and Holmstrup 2005, Creamer et al. 2008, Krogh 2009, OECD 2009, Pfeffer et al. 2010, Kim and An 2014, Son et al. 2017). Despite the rapid increase in traffic-derived heavy metal pollution in Western Washington, there have been no studies to date that examine the sensitivity of forest ecosystems, and specifically epiphytic communities of these forests, to Cu pollution. The first objective of my thesis was to develop a dose-response relationship between Cu and Collembola mortality. I use both lab-reared

and wild-collected Collembola populations to quantify the effect of Cu pollution on Collembola across Western Washington. The results of this study are presented in Chapter 2, and provide important insight into the resiliency of PNW forest ecosystems in the face of increasing traffic-derived heavy metal pollutants.

Looking to the future, PNW ecosystems will experience stress from not only increased deposition of heavy-metal pollutants, but also from a warming climate. According to climate change projections, Washington State will see an increase in temperatures of 1.8°C by the 2040s and 3.0°C by the 2080s (IPCC 2007, Adair and Reeder 2009, Miles et al. 2010, Abatzoglou et al. 2014, TNC 2016). These changes in temperature will also be accompanied by shifts in precipitation, with ~22% less rain in the summer months by 2050 and a ~38-46% decrease in April 1<sup>st</sup> snow water equivalent (Adair and Reeder 2009, Miles et al. 2010, Luce et al. 2016). On a large scale these changes will likely lead to increased tree mortality, changes in insect populations and community structure, and larger areas burned by wildfire (Miles et al. 2010, Stavros et al. 2014, Clark et al. 2016, Halofsky and Peterson 2016, Hicke et al. 2016, Kolb et al. 2016, Luce et al. 2016, Halofsky et al. 2017). No studies to date have examined the combined effects of increased temperatures and heavy metal pollutants on PNW forest ecosystems. Thus, the second objective of my thesis was to measure the interacting effect of supraoptimal temperatures and sub-lethal doses of Cu on Collembola from both lab-reared and wild-collected populations. These results are presented in Chapter 3, and provide insight into the relationship between Cu pollution and climate change for microarthropod communities in PNW forest ecosystems. This information is important for predicting the resiliency of

these ecosystems under the combined stress of heavy-metal pollution and a warming climate.

I wrote Chapters 2 and 3 of this thesis to be combined in manuscript form. The results of Chapter 2 form the basis for the experimental design in Chapter 3. In Chapter 2, I established the initial values of Cu that cause 25% and 50% mortality ( $LC_{25}$  and  $LC_{50}$ ) in lab-reared and wild-collected Collembolan populations, while in Chapter 3 I used those initial values and 5-year temperature averages for Seattle, which I increased based on IPCC climate change scenarios (IPCC 2007) to quantify the interactive effects of Cu pollution and temperature on lab-reared and wild-collected Collembolan populations; I also established new Cu  $LC_{25}$  and  $LC_{50}$  values for each population under three temperature regimes. The results of this work further our understanding of how increasing heavy metal pollutants and a warming climate will affect forest ecosystems in the PNW, which should inform adaptive planning to protect these ecosystems into the future.

- Abatzoglou, J. T., D. E. Rupp, and P. W. Mote. 2014. Seasonal Climate Variability and Change in the Pacific Northwest of the United States. *Journal of Climate* **27**:2125-2142.
- Adair, J., and S. Reeder. 2009. Focus on Impacts of Climate Change in Washington State. *in* W. S. D. o. Ecology, Community, Trade, and Economic Development.
- Apeageyi, E., M. S. Bank, and J. D. Spengler. 2011. Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts. *Atmospheric Environment* **45**:2310-2323.
- Baceva, K., T. Stafilov, R. Sajn, and C. Tanaselia. 2012. Moss biomonitoring of air pollution with heavy metals in the vicinity of a ferronickel smelter plant. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **47**:645-656.
- Bakirdere, S., and M. Yaman. 2008. Determination of lead, cadmium and copper in roadside soil and plants in Elazig, Turkey. *Environ Monit Assess* **136**:401-410.
- Baldwin, D. H., J. F. Sandahl, J. S. Labenia, and N. L. Scholz. 2003. Sublethal effects of copper on coho salmon: impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environ Toxicol Chem* **22**:2266-2274.
- Balk, G. 2017. Booming Seattle is adding cars just as fast as people. *Seattle Times*, Online.
- Bates, J. W. 1992. Mineral nutrient acquisition and retention by bryophytes. *Journal of Bryology* **17**:223-240.

- Bengston, C., L. Folkesson, and A. Göransson. 1982. Growth reduction and branching frequency in *Hylocomium splendens* near a foundry emitting copper and zinc. *Lindbergia* **8**:129-138.
- Bidwell, A. 2017. Urbanization impacts on epiphytic nitrogen and metal cycling in *Acer macrophyllum* stands in Western Washington, USA. University of Washington.
- Binkley, D., and R. L. Graham. 1981. Biomass, Production, and Nutrient Cycling of Mosses in an Old-Growth Douglas-Fir Forest. *Ecology* **62**:1387-1389.
- Boxall, A. B. A., and L. Maltby. 1997. The Effects of Motorway Runoff on Freshwater Ecosystems: 3. Toxicant Confirmation. *Archives of Environmental Contamination and Toxicology* **33**:9-16.
- Cesa, M., B. Campisi, A. Bizzotto, C. Ferraro, F. Fumagalli, and P. L. Nimis. 2008. A factor influence study of trace element bioaccumulation in moss bags. *Arch Environ Contam Toxicol* **55**:386-396.
- Clark, J. S., L. Iverson, C. W. Woodall, C. D. Allen, D. M. Bell, D. C. Bragg, A. W. D'Amato, F. W. Davis, M. H. Hersh, I. Ibanez, S. T. Jackson, S. Matthews, N. Pederson, M. Peters, M. W. Schwartz, K. M. Waring, and N. E. Zimmermann. 2016. The impacts of increasing drought on forest dynamics, structure, and biodiversity in the United States. *Glob Chang Biol* **22**:2329-2352.
- Cornelissen, J. H., S. I. Lang, N. A. Soudzilovskaia, and H. J. During. 2007. Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Ann Bot* **99**:987-1001.

- Creamer, R. E., D. L. Rimmer, and H. I. J. Black. 2008. Do elevated soil concentrations of metals affect the diversity and activity of soil invertebrates in the long-term? *Soil Use and Management* **24**:37-46.
- Crommentuijn, T., C. J. A. M. Doodeman, J. J. C. Vanderpol, A. Doornekamp, M. C. J. Rademaker, and C. A. M. Vangestel. 1995. Sublethal Sensitivity Index as an Ecotoxicity Parameter Measuring Energy Allocation under Toxicant Stress: Application to Cadmium in Soil Arthropods. *Ecotoxicology and Environmental Safety* **31**:192-200.
- Donovan, G. H., S. E. Jovan, D. Gatzolis, I. Burstyn, Y. L. Michael, M. C. Amacher, and V. J. Monleon. 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. *Sci Total Environ* **559**:84-93.
- Filser, J., R. Wittmann, and A. Lang. 2000. Response types in Collembola towards copper in the microenvironment. *Environmental Pollution* **107**:71-78.
- Fountain, M. T., and S. P. Hopkin. 2001. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. *Ecotoxicol Environ Saf* **48**:275-286.
- Fountain, M. T., and S. P. Hopkin. 2005. *Folsomia candida* (Collembola): a "standard" soil arthropod. *Annu Rev Entomol* **50**:201-222.
- Gjengedal, E., and E. Steinnes. 1990. Uptake of metal ions in moss from artificial precipitation. *Environmental Monitoring and Assessment* **14**:77-87.
- Gonzalez, A. G., and O. S. Pokrovsky. 2014. Metal adsorption on mosses: Toward a universal adsorption model. *J Colloid Interface Sci* **415**:169-178.

- Halofsky, J., and D. Peterson. 2016. Climate Change Vulnerabilities and Adaptation Options for Forest Vegetation Management in the Northwestern USA. *Atmosphere* **7**.
- Halofsky, J. E., D. L. Peterson, and H. R. Prendeville. 2017. Assessing vulnerabilities and adapting to climate change in northwestern U.S. forests. *Climatic Change*.
- Harmens, H., D. A. Norris, G. R. Koerber, A. Buse, E. Steinnes, and A. Ruhling. 2008. Temporal trends (1990-2000) in the concentration of cadmium, lead and mercury in mosses across Europe. *Environ Pollut* **151**:368-376.
- Hicke, J. A., A. J. H. Meddens, and C. A. Kolden. 2016. Recent Tree Mortality in the Western United States from Bark Beetles and Forest Fires. *Forest Science* **62**:141-153.
- INRIX. 2016. Traffic Scorecard.
- IPCC. 2007. Climate change 2007: An Assessment of the Intergovernmental Panel on Climate Change. *Weather* **57**:267-269.
- Kim, S. W., and Y.-J. An. 2014. Jumping behavior of the springtail *Folsomia candida* as a novel soil quality indicator in metal-contaminated soils. *Ecological Indicators* **38**:67-71.
- Kolb, T. E., C. J. Fettig, M. P. Ayres, B. J. Bentz, J. A. Hicke, R. Mathiasen, J. E. Stewart, and A. S. Weed. 2016. Observed and anticipated impacts of drought on forest insects and diseases in the United States. *Forest Ecology and Management* **380**:321-334.

- Krogh, P. H. 2009. Toxicity testing with the collembolans *Folsomia fimentaria* and *Folsomia candida* and the results of a ringtest. Danish Ministry of the Environment, Environmental Project No. 1256.
- Linbo, T. L., C. M. Stehr, J. P. Incardona, and N. L. Scholz. 2006. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. *Environ Toxicol Chem* **25**:597-603.
- Lindo, Z., and A. Gonzalez. 2010. The Bryosphere: An Integral and Influential Component of the Earth's Biosphere. *Ecosystems* **13**:612-627.
- Lindo, Z., N. Winchester, R. Didham, and Y. Basset. 2013. Out on a limb: microarthropod and microclimate variation in coastal temperate rainforest canopies. *Insect Conservation and Diversity* **6**:513-521.
- Lindo, Z., and N. N. Winchester. 2006. A comparison of microarthropod assemblages with emphasis on oribatid mites in canopy suspended soils and forest floors associated with ancient western redcedar trees. *Pedobiologia* **50**:31-41.
- Luce, C. H., J. M. Vose, N. Pederson, J. Campbell, C. Millar, P. Kormos, and R. Woods. 2016. Contributing factors for drought in United States forest ecosystems under projected future climates and their uncertainty. *Forest Ecology and Management* **380**:299-308.
- Miles, E. L., M. M. Elsner, J. S. Littell, L. W. Binder, and D. P. Lettenmaier. 2010. Assessing regional impacts and adaptation strategies for climate change: the Washington Climate Change Impacts Assessment. *Climatic Change* **102**:9-27.

- Nadkarni, N. M. 1984. Biomass and mineral capital of epiphytes in an *Acer macrophyllum* community of a temperate moist coniferous forest, Olympic Peninsula, Washington State. *Canadian Journal of Botany* **62**:2223-2228.
- OECD. 2009. Test No. 232: Collembolan Reproduction Test in Soil. OECD Publishing.
- Pearson, J., D. M. Wells, K. J. Sellar, A. Bennett, A. Soares, J. Woodall, and M. J. Ingrouille. 2000. Traffic exposure increases natural <sup>15</sup>N and heavy metal concentrations in mosses. *New Phytologist* **147**:317-326.
- Pedersen, M. B., J. r. A. Axelsen, B. Strandberg, J. Jensen, and M. J. Attrill. 1999. The Impact of a Copper Gradient on a Microarthropod Field Community. *Ecotoxicology* **8**:467-483.
- Pedersen, M. B., E. J. M. Temminghoff, M. P. J. C. Marinussen, N. Elmegaard, and C. A. M. van Gestel. 1997. Copper accumulation and fitness of *Folsomia candida* Willem in a copper contaminated sandy soil as affected by pH and soil moisture. *Applied Soil Ecology* **6**:135-146.
- Pfeffer, S. P., H. Khalili, and J. Filser. 2010. Food choice and reproductive success of *Folsomia candida* feeding on copper-contaminated mycelium of the soil fungus *Alternaria alternata*. *Pedobiologia* **54**:19-23.
- Pott, U., and D. H. Turpin. 1996. Changes in atmospheric trace element deposition in the Fraser Valley, BC, Canada from 1960 to 1993 measured by moss monitoring with *Isothecium stoloniferum*. *Canadian Journal of Botany* **74**:1345-1353.

- Pypker, T. G., M. H. Unsworth, and B. J. Bond. 2006. The role of epiphytes in rainfall interception by forests in the Pacific Northwest. I. Laboratory measurements of water storage. *Canadian Journal of Forest Research* **36**:809-818.
- Rosenstiel, T. N., E. E. Shortlidge, A. N. Melnychenko, J. F. Pankow, and S. M. Eppley. 2012. Sex-specific volatile compounds influence microarthropod-mediated fertilization of moss. *Nature* **489**:431-433.
- Rühling, A., and G. Tyler. 1973. Heavy Metal Deposition in Scandanavia. *Water, Air, and Soil Polution* **2**:445-455.
- Sandahl, J. F., D. H. Baldwin, J. J. Jenkins, and N. L. Scholz. 2004. Odor-evoked field potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. *Canadian Journal of Fisheries and Aquatic Sciences* **61**:404-413.
- Sandahl, J. F., D. H. Baldwin, J. J. Jenkins, and N. L. Scholz. 2007. A Sensory System at the Interface between Urban Stormwater Runoff and Salmon Survival. *Environmental Science & Technology* **41**:2998-3004.
- Sandifer, R. D., and S. P. Hopkin. 1996. Effects of pH on the toxicity of cadmium, copper, lead and zinc to *Folsomia candida* Willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere* **33**:2475-2486.
- Scott-Fordsmand, J. J., P. H. Krogh, and J. M. Weeks. 1997. Sublethal toxicity of copper to a soil-dwelling springtail (*Folsomia fimetaria*) (Collembola: Isotomidae). *Environmental Toxicology and Chemistry* **16**:2538-2542.
- Shortlidge, E. 2014. Testing the Ecological and Physiological Factors Influencing Reproductive Success in Mosses. Portland State University.

- Smit, C. E., and C. A. Van Gestel. 1997. Influence of temperature on the regulation and toxicity of zinc in *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf* **37**:213-222.
- Son, J., Y. S. Lee, S. E. Lee, K. I. Shin, and K. Cho. 2017. Bioavailability and Toxicity of Copper, Manganese, and Nickel in *Paronychiurus kimi* (Collembola), and Biomarker Discovery for Their Exposure. *Arch Environ Contam Toxicol* **72**:142-152.
- Sorensen, T. S., and M. Holmstrup. 2005. A comparative analysis of the toxicity of eight common soil contaminants and their effects on drought tolerance in the collembolan *Folsomia candida*. *Ecotoxicol Environ Saf* **60**:132-139.
- Stavros, E. N., J. T. Abatzoglou, D. McKenzie, and N. K. Larkin. 2014. Regional projections of the likelihood of very large wildland fires under a changing climate in the contiguous Western United States. *Climatic Change* **126**:455-468.
- Thorpe, A., and R. M. Harrison. 2008. Sources and properties of non-exhaust particulate matter from road traffic: a review. *Sci Total Environ* **400**:270-282.
- TNC. 2016. *Adapting to Change: Climate Impacts and Innovation in Puget Sound*. Puget Sound Conservation.
- Tyler, G. 1990. Bryophytes and heavy metals: a literature review. *Botanical Journal of the Linnean Society* **104**:231-253.
- Wesley, I. 2012. Better Brakes Rule. Pages 173-901 *in* W. S. D. o. Ecology, editor.
- WSDOT. 2015. Traffic Report. Washington State Department of Transportation.

Zvereva, E. L., and M. V. Kozlov. 2010. Impacts of Industrial Polluters on Bryophytes:  
a Meta-analysis of Observational Studies. *Water, Air, & Soil Pollution*  
**218**:573-586.

## Chapter 2. Effects of Vehicle-Derived Cu on Collembola In Western Washington, USA

### Abstract

In this study, I investigated the effects of Cu pollution on two species of Collembola. I reared the Collembolan species *Folsomia candida* Willem, under laboratory conditions and collected *Choreutinula americana* from two field sites representing high (Seattle) and low (Hoh Rainforest) background Cu levels in Western Washington. I exposed Collembola to aqueous Cu solutions ranging from 0.1 to 10,000 ppm for 10 days at 20°C. I found *Choreutinula americana* to be more sensitive to Cu than *F. candida*, with the LC<sub>25</sub> and LC<sub>50</sub> values being 19 and 131 ppm for *C. americana*, and 77 and 2,460 ppm for *F. candida* respectively. A follow up experiment revealed that the extraction procedure that wild-collected Collembola were subjected to resulted in much higher mortality in the lab-reared population when individuals were subsequently exposed to Cu. The results of this study reveal that Collembola in the Pacific Northwest are sensitive to Cu pollution, and may become more sensitive when exposed to other stressors.

**Keywords:** Collembola; heavy metals; traffic pollution; Pacific Northwest

## **Introduction**

The Pacific Northwest (PNW) is currently experiencing some of the fastest population growth within the United States (2017). Seattle and the surrounding Puget Sound Region is home to 4.25 million people, and estimates project that there will be an increase of ~1.5 million new residents by 2040 (PSRC 2014). This increase in population will in turn increase travel demand throughout the region by 40% (PSRC 2014), adding to Seattle's current citywide average traffic count of >1 million vehicles per day (WSDOT 2015). Traffic pollution leads to increased levels of heavy metal pollutants such as iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and titanium (Ti), and increased nitrogen (N) deposition (Pearson et al. 2000, Bakirdere and Yaman 2008, Thorpe and Harrison 2008, Apeagyei et al. 2011, Wesley 2012). Seattle's infamous stop-and-go traffic along the major transportation corridors I-5 and I-90 results in heavily increased deposition of the metals Cu, Zn, and Ti through brake and tire attrition (Bidwell 2017). With such increases in transportation demand on the horizon and many vehicles continuing to arrive from out of state, more efforts will be necessary to understand and address the effects of heavy metal inputs into the ecosystems of the PNW.

The State of Washington has already made efforts to reduce Cu pollution through regulations on brake pad composition, which will reduce the allowable composition of Cu by weight in brake pads sold throughout the state. The Washington Department of Ecology adopted the Better Brake Rule in October of 2012, but the benefits of the law will be slow to take effect (Wesley 2012). As of 1 January 2015, the manufacture of brake pads containing asbestos, lead, and other

pollutants (excluding Cu) was prohibited, but under the law distributors can continue to sell existing inventory until 2025. Copper is to be reduced to a maximum of 5% by weight for pads manufactured starting in 2021, and by 2025 brake pads containing more than 0.5% Cu by weight may not be sold in Washington. The impetus for this law was the discovery that even small amounts of Cu in aquatic systems can be detrimental to Salmonid populations, many of which are currently listed as threatened or endangered under the Endangered Species Act (Sandahl et al. 2007). Copper acts as a neurobehavioral toxin in fish by triggering the death of their olfactory epithelial cells and neurons in the lateral line system (Baldwin et al. 2003, Sandahl et al. 2004, Linbo et al. 2006, Sandahl et al. 2007). The implications of damage to these neurobehavioral systems are profound as they are used to detect movement and chemical signals in their environment that range from avoiding predators and finding food to returning to their natal freshwaters and assessing the reproductive status of potential mates (Baldwin et al. 2003, Sandahl et al. 2004, Linbo et al. 2006, Sandahl et al. 2007). With increasing concern over the status of fisheries around the country, the Better Brakes Law is being put forward as a model to improve the health of aquatic ecosystems around the nation. With the expanding population in the PNW and the accompanied increase in traffic-derived heavy metal pollutants, however, there is a need to develop a better understanding of how these environmental toxins are affecting other ecosystems in the PNW.

PNW forests are characterized by large epiphytic bryophyte colonies, which play an important functional role in water retention, nutrient cycling (including nitrogen fixation), microclimate, and habitat creation (Binkley and Graham 1981,

Nadkarni 1984, Pypker et al. 2006, Cornelissen et al. 2007, Burger et al. 2010, Lindo and Gonzalez 2010). Bryophytes (mosses, liverworts and hornworts) are non-vascular plants that lack roots, have leaves that are one cell-layer thick, and lack a protective epidermis. These qualities make bryophytes sensitive to a range of environmental pollutants including heavy metals; consequently, they have been used historically as bioindicators of air quality (Rühling and Tyler 1973, Bengston et al. 1982, Gjengedal and Steinnes 1990, Tyler 1990, Bates 1992, Pott and Turpin 1996, Pearson et al. 2000, Cornelissen et al. 2007, Cesa et al. 2008, Harmens et al. 2008, Zvereva and Kozlov 2010, Baceva et al. 2012, Gonzalez and Pokrovsky 2014, Donovan et al. 2016, Bidwell 2017). Since mosses readily absorb pollutants via dry or wet deposition (Gjengedal and Steinnes 1990, Bates 1992, Gonzalez and Pokrovsky 2014), they can provide a snapshot of background metal levels within an area and be used to compare pollution levels between sites across the PNW region. Epiphytic mosses are also home to a myriad of other life forms, including bacteria, nematodes, fungi, insects, and microarthropod communities (Nadkarni 1984, Lindo and Winchester 2006, Lindo and Gonzalez 2010, Lindo et al. 2013). Collembola are particularly abundant in epiphytic mosses (Hopkin 1997, Fountain and Hopkin 2005, Lindo and Winchester 2006, Lindo et al. 2013), and play a vital role in decomposition, stimulation of fungal growth, and pollination of mosses (Fountain and Hopkin 2005, Rosenstiel et al. 2012, Shortlidge 2014). Collembola are used frequently as a representative organism in ecotoxicology studies due to their small size, abundance, and sensitivity to a range of environmental toxins (Crommentuijn et al. 1995, Sandifer and Hopkin 1996, Pedersen et al. 1997, Scott-Fordsmand et al.

1997, Smit and Van Gestel 1997, Pedersen et al. 1999, Filser et al. 2000, Fountain and Hopkin 2001, 2005, Sorensen and Holmstrup 2005, Creamer et al. 2008, Krogh 2009, OECD 2009, Pfeffer et al. 2010, Kim and An 2014, Son et al. 2017).

This combination of abundance, sensitivity to heavy metal pollutants, and important functional role within epiphyte communities make Collembola an ideal study organism to begin understanding how increased transportation pollution will affect PNW forest ecosystems. The standard toxicology testing procedure is designed for eudaphic (soil-dwelling) Collembola (Fountain and Hopkin 2005, Krogh 2009, OECD 2009); however, this protocol involves the need of an artificial soil for use in bioassays. In this paper, I introduce a new testing procedure that simplifies the test substrate, allows for continuous monitoring of the test individuals for mortality, and is appropriate for the testing of Collembola that live in non-soil environments. The main objective of this study was to use this method to develop a dose-response relationship between Cu and Collembola mortality in three distinct Collembola populations: a standard, lab-reared colony, a population collected from urban environments in the PNW, and a population collected from rural environments in the PNW. I hypothesized that Collembola from these three populations would differ in their sensitivity to concentrations of Cu, with the lab-reared population being the most sensitive due to their lack of previous exposure to Cu, and the rural population being more sensitive than the urban population due to the higher background levels of Cu found in the urban field site.

## Materials and Methods

### 2.1 Study Sites

The study area consisted of two field sites chosen to represent a less polluted (rural) area and a more polluted (urban) area in Western Washington. The rural site is located on the west side of the Olympic Peninsula in the Hoh Rainforest along the Hoh River (47.82°N, 124.20°W), and the urban site is located within Seward Park (47.55°N, 122.25°W) in the city of Seattle.

The rural stand was dominated by bigleaf maple (*Acer macrophyllum* Pursh), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka Spruce (*Picea sitchensis* (Bong.) Carriér), while the urban stand was dominated by Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), bigleaf maple, and western redcedar (*Thuja plicata* Donn ex D. Don). I chose to collect epiphytic moss samples from *A. macrophyllum* because it was the only tree occurring in Seattle city parks and the Olympic Peninsula on which enough epiphytes reliably grow to support Collembolan populations of sufficient size for experimentation. In the forests of the Hoh River Valley on the Olympic Peninsula, *A. macrophyllum* holds the largest epiphyte loads of all woody plant species, averaging ~ 35.5 kg dry weight per tree (Nadkarni 1984). In similar forests, total microarthropod abundance has been found to be 15-20 individuals per gram of dry weight of epiphyte, which would suggest >500,000 individuals live on one *A. macrophyllum* (Lindo and Winchester 2006). The dominant moss species found on the base of *A. macrophyllum* in the rural stand were *Kindbergia praelonga* (Hedw.) Ochyra and *Isothecium stoloniferum* (Brid.), while the dominant mosses found on the base of *A. macrophyllum* in the urban stand were *K. praelonga*, *I.*

*stoloniferum*, and *Orthotrichum lyelli* Hook & Taylor. I chose to collect *K. praelonga* for this study due to its availability in both rural and urban sites and tendency to form thick mats conducive to microarthropod habitat.

## 2.2 Moss Collection

At study sites, I removed *K. praelonga* from the bottom 2 meters of *A. macrophyllum* stems by hand and placed samples into a 6-liter Ziploc bag for transport back to the laboratory. I took care to remove small sections of moss (~15 cm diameter) from a large number of *A. macrophyllum* until the bag was full to minimize the impact to each tree's epiphytic moss and microarthropod communities. Samples from urban and rural sites were collected within one day of one another during the month of July 2016 and returned to the laboratory within 24 hours for processing.

## 2.3 Collembola Extraction

I placed *K. praelonga* samples from rural and urban sites into a Tullgren funnel array (Tullgren 1918) to live-extract the microarthropods within the moss. Tullgren funnels are a well-established technique for extracting microarthropods due to their sensitivity to desiccation and avoidance of heat and light sources, and have been found to be the most effective method for live-extracting Collembola (Hopkin 1997, Heneghan et al. 2004, Jones 2005, Lindo and Winchester 2006, Creamer et al. 2008, Yoshida and Hijii 2008, Kardol et al. 2011, Lindo et al. 2013, Bokhorst et al. 2014). The Tullgren funnel setup consisted of a plastic bucket with

its bottom removed to hold the moss, which was placed on top of hardware cloth over a Tupperware® container filled with deionized (DI) water to collect Collembola. A 15-watt light bulb and metal shield were placed over top of the bucket and moss to provide gentle heat and light that would facilitate Collembola to move downwards into the DI water without being hot enough to cause desiccation and mortality (Fig. 2.1). The extracted Collembola remained alive in the Tupperware® containers, as they lack sufficient mass to break the surface tension of the DI water. After 48 hours I removed the Tupperware® from underneath the Tullgren funnels and collected the extracted Collembola from the rural and urban sites into separate larger bins. I then observed the most common species for both sites and used individuals of that species in subsequent experiments. I identified the most common species for both rural and urban sites as *Choreutinula americana* (Collembola: Hypogastruridae) using online taxonomic keys provided through <http://www.collembola.org> (Bellinger et al. 1996-2017).

#### *2.4 Rearing Collembola in the Laboratory*

To establish a baseline sensitivity of Collembola to copper exposure, I reared *Folsomia candida* Willem (Collembola: Isotomidae) in the laboratory to pair with the field-extracted Collembola in experiments. *Folsomia candida* is a temperate species that has been used extensively in ecotoxicology experiments (Crommentuijn et al. 1995, Sandifer and Hopkin 1996, Holmstrup 1997, Hopkin 1997, Pedersen et al. 1997, Smit and Van Gestel 1997, Fountain and Hopkin 2001, Højer et al. 2001, Damgaard et al. 2002, Fountain and Hopkin 2005, Sorensen and Holmstrup 2005,

Krogh 2009, OECD 2009, Holmstrup et al. 2010, Pfeffer et al. 2010, Kim and An 2014); indeed, it is often referred to as the “white rat springtail” due to its nearly ubiquitous use in laboratory experiments involving Collembola (Hopkin 1997, Krogh 2009, OECD 2009). I reared *F. candida* in lidded plastic bins on a medium of charcoal and DI water. I added granulated active dry yeast to the bins every week as a food source and aerated the containers while adding new food. The Collembola were kept at 20°C in Percival Growth Chambers, and DI water was added regularly to ensure 100% humidity in the bins.

### *2.5 Copper Exposure*

To test the sensitivity of the rural, urban, and lab-reared Collembola populations to Cu, I created a range of aqueous Cu solutions along a  $\log_{10}$  scale from 0.1 to 10,000 ppm. I created the initial 10,000-ppm copper solution by dissolving cupric sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in DI water in a 100 mL analytical volumetric flask. I made the remaining range of solutions by pipetting small amounts of the 10,000 ppm solution into subsequent analytical volumetric flasks and diluting them with DI water to the desired concentration. I then pipetted 1 ml of each concentration of copper solution into 12 wells of a 24-well Falcon™ cell culture tray (Fisher Scientific, Hampton, NH). I also added 1 ml of DI water to 12 wells of three additional cell culture trays as a control for each population, leaving us with seven treatments for each population.

I then placed Collembola from rural, urban and lab-reared populations in separate cell culture trays for each Cu treatment and the control. I removed

Collembola from the plastic bins using a stainless steel scoopula and transferred them into all 12 wells of the cell culture trays for each treatment, aiming for approximately 10 individuals per well. I transferred the rural and urban populations immediately after they had been collected into the larger plastic bins, and the lab-reared population was removed from the growth chambers only long enough to transfer individuals into the cell culture trays. Once I had placed enough individuals from all populations into each treatment, I placed the cell culture trays in the growth chambers at 20°C for 10 days. I removed the trays every two days during this period and placed them under the microscope to count how many individuals remained alive at the time, ignoring individuals under 1.5 mm in size. Mortality was determined by looking for movement in each individual and prodding immobile individuals thoroughly to ensure that I could not coerce any movement before counting them as dead.

### *2.6 Tullgren Stress Experiment*

I ran a secondary experiment with individuals from the lab-reared *F. candida* population to measure the effect of heat and light exposure from the Tullgren funnels on mortality, and when exposed to Cu. I rewetted the same moss from which the wild populations were extracted and placed it in the Tullgren funnel array and then placed individuals from the lab-reared population into the moss and turned the funnel lights on for 48 hours (the same amount of time the wild populations were exposed for). I then placed the extracted individuals, which I will refer to as the stressed population, into cell culture trays with a control of DI water and Cu

concentrations of 1.0, 100, and 10,000 ppm, and placed a second set of lab-reared individuals that had not been subjected to the Tullgren funnels into the same range of concentrations for comparison. I will refer to this group as the unstressed population. I then placed the cell culture trays into the growth chambers at 20°C and removed after two and four days to assess mortality.

### *2.7 Statistical Analysis*

I used Abbott's formula to correct for control mortality using the DI water treatment for each respective population (Abbott 1925). I then pooled the mortality data over time to compare total mortality rates among the three populations. I subjected the pooled data to a logit analysis to determine the dose-response relationship between Cu and Collembola mortality for each population (Stokes et al. 2000). I used these dose-response curves for each population to assess slope and intercept heterogeneity among populations based upon the likelihood ratio  $\chi^2$  ( $G^2$ ) values. The results led me to pool the rural and urban populations into one "wild" population. I then used this final model to estimate  $LC_{50}$  and  $LC_{25}$  values for the wild and lab-reared populations (Stokes et al. 2000).

## **Results**

I found *C. americana* to be the dominant Collembolan species at both the rural (Hoh River Trust) and urban (Seward Park) sites. I observed that other urban sites did not have enough *C. americana* to detect in moss samples, and had lower numbers of Collembola in general. Seward Park did yield lower numbers than the

Hoh River Trust of *C. americana* for roughly the same amount of moss collected (Hoh River Trust: n = 542, Seward Park: n = 252), but they still remained the most dominant Collembolan species.

Mortality in Collembola from lab-reared, rural, and urban populations under control conditions (DI water) ranged from 0 to 1.3%. After correcting for mortality in each population and pooling the results over time, Cu concentration was a significant predictor variable of Collembola mortality ( $G^2 = 2,188.49$ ;  $df = 1$ ,  $P < 0.001$ ). I also detected significant differences in the intercepts ( $G^2 = 127.74$ ;  $df = 2$ ;  $P < 0.001$ ) and slopes ( $G^2 = 75.41$ ;  $df = 2$ ;  $P < 0.001$ ) of the population-specific dose-mortality curves. After excluding the lab-reared population in a comparison of rural and urban populations, I observed no significant difference in the estimates of the intercept ( $G^2 = 1.03$ ;  $df = 1$ ;  $P = 0.3106$ ) and slope ( $G^2 = 0.50$ ;  $df = 1$ ;  $P = 0.4778$ ) of their respective dose-response curves. Due to slope and intercept homogeneity, I combined the rural and urban populations into one wild population, which I then compared to the lab-reared population, and detected a significant difference in the estimates of the intercept ( $G^2 = 126.86$ ;  $df = 1$ ;  $P < 0.001$ ) and slope ( $G^2 = 74.76$ ;  $df = 1$ ;  $P < 0.001$ ) of their dose-response curves. Overall, the wild population, whether collected from urban or rural locations, was more susceptible to Cu exposure than the lab reared population (Fig. 2.2). The calculated  $LC_{25}$  and  $LC_{50}$  values for the wild population were 19 ppm and 131 ppm respectively, while the respective values for the lab population were 77 ppm and 2,460 ppm (Table 2.1).

In a follow-up study, I observed that *F. candida* (i.e., the lab-reared population) had higher mortality when exposed to Cu after being extracted through

the Tullgren funnels (Fig. 2.3). Individuals when extracted through this method (e.g., a stressed population) reached 100% mortality when exposed to the 10,000 ppm Cu solution after only 4 days. When individuals were not extracted through the use of Tullgren funnels (i.e., an unstressed population), mortality reached 37.9% mortality when exposed the 10,000 ppm Cu solution after 4 days, which was consistent with the lab-reared population mortality in the initial experiment at that dose and time. There was no mortality in the unstressed population when exposed to DI water, 1.0 ppm or 100 ppm Cu solutions after 4 days. This was also consistent with the data for the lab-reared population in the initial experiment. In contrast, the stressed population had 19.8% mortality when exposed to DI water control, 34.6% mortality for the 1 ppm Cu solution, and 98.2% mortality for the 100 ppm Cu solution after 4 days.

## **Discussion**

A recent study determined that the rural site (Hoh River Trust) had an average Cu concentration of  $3.52 \pm 1.98$  ppm while the urban site (Seward Park) had an average Cu concentration of  $11.49 \pm 3.80$  ppm (Bidwell 2017). The fact that the most abundant Collembolan species at both sites was *C. americana* reveals that the difference in background Cu levels between sites was not enough to severely affect the presence of this species; however, the other urban sites with only slightly higher background levels of Cu (Ravenna Park at  $11.95 \pm 4.04$  ppm and Interlaken Park at  $12.30 \pm 4.10$  ppm) had below detection limit numbers of *C. americana*. Along with Cu, Bidwell (2017) analyzed a suite of other metals for each site including Cd, Cr, Fe,

K, Mg, Mn, Ni, Pb, Sr, Ti, and Zn, many of which are known to negatively affect Collembola over a certain environmental threshold (Crommentuijn et al. 1995, Sandifer and Hopkin 1996, Smit and Van Gestel 1997, Fountain and Hopkin 2001, Sorensen and Holmstrup 2005, Creamer et al. 2008, Kim and An 2014, Son et al. 2017). Variations among these other metals are a potential explanation for why *C. americana* presence varied among sites without much change in Cu levels. Lead, for example, was found in concentrations up to 64.06 ppm at Ravenna Park, compared to mean background urban levels of 8.12 ppm (Bidwell 2017). Seward Park was also the largest and least disturbed forest of our urban sites, and supported noticeably more moss in the understory, a trait that has been identified as a strong indicator of the health and abundance of microarthropod communities (Bokhorst et al. 2014).

I found Cu concentration to be a significant predictor of Collembola mortality across all populations, though sensitivity to Cu varied among populations and between treatments in the Tullgren stress experiment. I initially hypothesized that the lab-reared population would be more susceptible to Cu pollution than the rural or urban population because *F. candida* is known to be one of the most sensitive Collembolan species to many chemicals (Fountain and Hopkin 2001, 2005). Also, this lab-reared population had no previous exposure to Cu or other stressors that select for hardier individuals. The Tullgren stress experiment demonstrated that there was a significant effect on *F. candida* mortality after exposure to the stress of being extracted through the Tullgren funnels. This additional stress that the wild populations were exposed to in the Tullgren funnels could explain why the lab-reared population, which I predicted would be more susceptible to Cu exposure, had

lower mortality than both the rural and urban populations. Other studies on *F. candida* have shown that the effect of drought increases their susceptibility to various pollutants, including Cu (Holmstrup 1997, Højer et al. 2001, Damgaard et al. 2002, Holmstrup et al. 2010). It would be beneficial for future toxicology experiments to design a test that allowed for more accurate comparison of mortality between field-collected and lab-reared Collembolan populations by correcting for the effect of the Tullgren funnel procedure on field-collected Collembolan mortality. Given the drastic increase in *F. candida* sensitivity to Cu after exposure to the Tullgren funnel procedure, it is possible that our predicted LC<sub>25</sub> and LC<sub>50</sub> values for *C. americana* overestimate the organism's sensitivity to Cu in the environment. Still, it has been shown that sub-lethal concentrations of many toxins, including Cu, detrimentally affect fitness of Collembola spp. (Crommentuijn et al. 1995, Scott-Fordsmand et al. 1997, Filser et al. 2000, Fountain and Hopkin 2001, Creamer et al. 2008, Pfeffer et al. 2010, Kim and An 2014). It should also be noted that the predicted LC<sub>25</sub> value for the wild population is within the range of background levels detected in our urban field sites.

The decision to run this experiment directly on the solution rather than in an artificial standard soil gave me the opportunity to assess Cu induced mortality more rapidly and continuously than the standard Organization for Economic Cooperation and Development (OECD) procedure allows. The standard procedure involves creating an artificial soil of the following materials by percentage of dry weight: 5% sphagnum peat, 20% kaolin clay, 74% sand, and 1% calcium carbonate (OECD 2009). This artificial soil is then dosed with the various concentrations of the

toxicant of interest and Collembola are placed into the treated soils for 28 days before mortality is assessed (OECD 2009). Others have also attempted to assess mortality in shorter intervals to determine non-mortality effects such as growth rate, food selection, and gut composition (Crommentuijn et al. 1995, Scott-Fordsmand et al. 1997, Fountain and Hopkin 2001). I chose not to use the standard procedure because the Collembola were collected from epiphytic moss rather than soil, and using moss rather than soil for the toxicology tests would have required the additional stress of re-extracting Collembola after the period of exposure to Cu. Re-extraction of live Collembola using Tullgren funnel or floating would likely have led to further mortality and unrecovered individuals remaining in the moss (Yoshida and Hijii 2008).

The mortality results from this experiment were comparable to studies that have used the OECD standard tests on *F. candida* (Krogh 2009). When multiple laboratories performed the standard OECD test to determine LC<sub>50</sub> values for *F. candida* when exposed to Cu, the average LC<sub>50</sub> value was 1,541 ppm with a 95% CI from 443 - 2,639 ppm (Krogh 2009) as compared to our result of 2,460 ppm with a 95% CI from 1,516 - 3,990 ppm. Several laboratories included in this study had LC<sub>50</sub> values that were higher than those I found, and several were not included in the statistical analysis, because either there were no effects or the results fell outside of the concentration range (Krogh 2009). Running this test directly on the Cu solution provided several advantages over the OECD standard test: (1) 100% humidity was guaranteed throughout the duration of the experiment; (2) Labor and time requirements were greatly reduced; (3) The Cu solution approach allowed for

comparison between an eudaphic species and a moss-dwelling species of Collembola; (4) Continuous monitoring of mortality was possible, and the total time necessary to estimate LC values was reduced; (5) Cell culture trays could be cleaned and reused between iterations.

This test is not suitable to assess certain ecotoxicology measures such as avoidance behavior, toxicity of food sources, or fecundity due to the crowding and limited movement within wells of the cell culture trays and the lack of a solid surface on which to present food. While additional studies would be necessary to determine the comparability of our test to the OECD standard test, the initial success, shorter time period required, reduction in labor, and reduction of variables presents a potential increase in the ease and efficiency of determining LC values and comparing different species and populations of Collembola over time.

2017. U.S. Census Bureau. <https://www.census.gov/en.html>.
- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**:265-267.
- Apeageyi, E., M. S. Bank, and J. D. Spengler. 2011. Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts. *Atmospheric Environment* **45**:2310-2323.
- Baceva, K., T. Stafilov, R. Sajn, and C. Tanaselia. 2012. Moss biomonitoring of air pollution with heavy metals in the vicinity of a ferronickel smelter plant. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **47**:645-656.
- Bakirdere, S., and M. Yaman. 2008. Determination of lead, cadmium and copper in roadside soil and plants in Elazig, Turkey. *Environ Monit Assess* **136**:401-410.
- Baldwin, D. H., J. F. Sandahl, J. S. Labenia, and N. L. Scholz. 2003. Sublethal effects of copper on coho salmon: impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environ Toxicol Chem* **22**:2266-2274.
- Bates, J. W. 1992. Mineral nutrient acquisition and retention by bryophytes. *Journal of Bryology* **17**:223-240.
- Bellinger, P. F., K. A. Christiansen, and F. Janssens. 1996-2017. Checklist of the Collembola of the World.
- Bengston, C., L. Folkeson, and A. Göransson. 1982. Growth reduction and branching frequency in *Hylocomium splendens* near a foundry emitting copper and zinc. *Lindbergia* **8**:129-138.
- Bidwell, A. 2017. Urbanization impacts on epiphytic nitrogen and metal cycling in

- Acer macrophyllum* stands in Western Washington, USA. University of Washington.
- Binkley, D., and R. L. Graham. 1981. Biomass, Production, and Nutrient Cycling of Mosses in an Old-Growth Douglas-Fir Forest. *Ecology* **62**:1387-1389.
- Bokhorst, S., D. A. Wardle, M.-C. Nilsson, and M. J. Gundale. 2014. Impact of understory mosses and dwarf shrubs on soil micro-arthropods in a boreal forest chronosequence. *Plant and Soil* **379**:121-133.
- Burger, A. E., R. A. Ronconi, M. P. Silvergieter, C. Conroy, V. Bahn, I. A. Manley, A. Cober, and D. B. Lank. 2010. Factors affecting the availability of thick epiphyte mats and other potential nest platforms for Marbled Murrelets in British Columbia. *Canadian Journal of Forest Research* **40**:727-746.
- Cesa, M., B. Campisi, A. Bizzotto, C. Ferraro, F. Fumagalli, and P. L. Nimis. 2008. A factor influence study of trace element bioaccumulation in moss bags. *Arch Environ Contam Toxicol* **55**:386-396.
- Cornelissen, J. H., S. I. Lang, N. A. Soudzilovskaia, and H. J. During. 2007. Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Ann Bot* **99**:987-1001.
- Creamer, R. E., D. L. Rimmer, and H. I. J. Black. 2008. Do elevated soil concentrations of metals affect the diversity and activity of soil invertebrates in the long-term? *Soil Use and Management* **24**:37-46.
- Crommentuijn, T., C. J. A. M. Doodeman, J. J. C. Vanderpol, A. Doornekamp, M. C. J. Rademaker, and C. A. M. Vangestel. 1995. Sublethal Sensitivity Index as an Ecotoxicity Parameter Measuring Energy Allocation under Toxicant Stress:

- Application to Cadmium in Soil Arthropods. *Ecotoxicology and Environmental Safety* **31**:192-200.
- Damgaard, C., R. Hojer, M. Bayley, J. J. Scott-Fordsmand, and M. Holmstrup. 2002. Dose-response curve modeling of excess mortality caused by two forms of stress. *Environmental and Ecological Statistics* **9**:195-200.
- Donovan, G. H., S. E. Jovan, D. Gatziolis, I. Burstyn, Y. L. Michael, M. C. Amacher, and V. J. Monleon. 2016. Using an epiphytic moss to identify previously unknown sources of atmospheric cadmium pollution. *Sci Total Environ* **559**:84-93.
- Filser, J., R. Wittmann, and A. Lang. 2000. Response types in Collembola towards copper in the microenvironment. *Environmental Pollution* **107**:71-78.
- Fountain, M. T., and S. P. Hopkin. 2001. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. *Ecotoxicol Environ Saf* **48**:275-286.
- Fountain, M. T., and S. P. Hopkin. 2005. *Folsomia candida* (Collembola): a "standard" soil arthropod. *Annu Rev Entomol* **50**:201-222.
- Gjengedal, E., and E. Steinnes. 1990. Uptake of metal ions in moss from artificial precipitation. *Environmental Monitoring and Assessment* **14**:77-87.
- Gonzalez, A. G., and O. S. Pokrovsky. 2014. Metal adsorption on mosses: Toward a universal adsorption model. *J Colloid Interface Sci* **415**:169-178.
- Harmens, H., D. A. Norris, G. R. Koerber, A. Buse, E. Steinnes, and A. Ruhling. 2008. Temporal trends (1990-2000) in the concentration of cadmium, lead and mercury in mosses across Europe. *Environ Pollut* **151**:368-376.
- Heneghan, L., A. Salmore, and D. A. Crossley. 2004. Recovery of decomposition and

- soil microarthropod communities in an Appalachian watershed two decades after a clearcut. *Forest Ecology and Management* **189**:353-362.
- Højer, R., M. Bayley, C. F. D. and, and M. Holmstrup. 2001. Stress synergy between drought and a common environmental contaminant: studies with the collembolan *Folsomia candida*. *Global Change Biology* **7**:485-494.
- Holmstrup, M. 1997. Drought tolerance in *Folsomia Candida* Willem (*Collembola*) after exposure to sublethal concentrations of three soil polluting chemicals. *Pedobiologia* **41**:361-368.
- Holmstrup, M., A. M. Bindsbol, G. J. Oostingh, A. Duschl, V. Scheil, H. R. Kohler, S. Loureiro, A. M. Soares, A. L. Ferreira, C. Kienle, A. Gerhardt, R. Laskowski, P. E. Kramarz, M. Bayley, C. Svendsen, and D. J. Spurgeon. 2010. Interactions between effects of environmental chemicals and natural stressors: a review. *Sci Total Environ* **408**:3746-3762.
- Hopkin, S. P. 1997. *Biology of the Springtails — (Insecta: Collembola)*. Oxford University Press.
- Jones, C. B. 2005. *Arthropods Inhabiting Epiphyte Mats in an Old-growth Redwood Forest Canopy*. Humboldt State University.
- Kardol, P., W. N. Reynolds, R. J. Norby, and A. T. Classen. 2011. Climate change effects on soil microarthropod abundance and community structure. *Applied Soil Ecology* **47**:37-44.
- Kim, S. W., and Y.-J. An. 2014. Jumping behavior of the springtail *Folsomia candida* as a novel soil quality indicator in metal-contaminated soils. *Ecological Indicators* **38**:67-71.

- Krogh, P. H. 2009. Toxicity testing with the collembolans *Folsomia fimentaria* and *Folsomia candida* and the results of a ringtest. Danish Ministry of the Environment, Environmental Project No. 1256.
- Linbo, T. L., C. M. Stehr, J. P. Incardona, and N. L. Scholz. 2006. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. *Environ Toxicol Chem* **25**:597-603.
- Lindo, Z., and A. Gonzalez. 2010. The Bryosphere: An Integral and Influential Component of the Earth's Biosphere. *Ecosystems* **13**:612-627.
- Lindo, Z., N. Winchester, R. Didham, and Y. Basset. 2013. Out on a limb: microarthropod and microclimate variation in coastal temperate rainforest canopies. *Insect Conservation and Diversity* **6**:513-521.
- Lindo, Z., and N. N. Winchester. 2006. A comparison of microarthropod assemblages with emphasis on oribatid mites in canopy suspended soils and forest floors associated with ancient western redcedar trees. *Pedobiologia* **50**:31-41.
- Nadkarni, N. M. 1984. Biomass and mineral capital of epiphytes in an *Acer* macrophyllum community of a temperate moist coniferous forest, Olympic Peninsula, Washington State. *Canadian Journal of Botany* **62**:2223-2228.
- OECD. 2009. Test No. 232: Collembolan Reproduction Test in Soil. OECD Publishing.
- Pearson, J., D. M. Wells, K. J. Sellar, A. Bennett, A. Soares, J. Woodall, and M. J. Ingrouille. 2000. Traffic exposure increases natural <sup>15</sup>N and heavy metal concentrations in mosses. *New Phytologist* **147**:317-326.
- Pedersen, M. B., J. r. A. Axelsen, B. Strandberg, J. Jensen, and M. J. Attrill. 1999. The Impact of a Copper Gradient on a Microarthropod Field Community.

- Ecotoxicology **8**:467-483.
- Pedersen, M. B., E. J. M. Temminghoff, M. P. J. C. Marinussen, N. Elmegaard, and C. A. M. van Gestel. 1997. Copper accumulation and fitness of *Folsomia candida* Willem in a copper contaminated sandy soil as affected by pH and soil moisture. *Applied Soil Ecology* **6**:135-146.
- Pfeffer, S. P., H. Khalili, and J. Filser. 2010. Food choice and reproductive success of *Folsomia candida* feeding on copper-contaminated mycelium of the soil fungus *Alternaria alternata*. *Pedobiologia* **54**:19-23.
- Pott, U., and D. H. Turpin. 1996. Changes in atmospheric trace element deposition in the Fraser Valley, BC, Canada from 1960 to 1993 measured by moss monitoring with *Isothecium stoloniferum*. *Canadian Journal of Botany* **74**:1345-1353.
- PSRC. 2014. *Transportation 2040: Toward a sustainable transportation system*. in P. S. R. Council, editor., Seattle, WA.
- Pypker, T. G., M. H. Unsworth, and B. J. Bond. 2006. The role of epiphytes in rainfall interception by forests in the Pacific Northwest. I. Laboratory measurements of water storage. *Canadian Journal of Forest Research* **36**:809-818.
- Rosenstiel, T. N., E. E. Shortlidge, A. N. Melnychenko, J. F. Pankow, and S. M. Eppley. 2012. Sex-specific volatile compounds influence microarthropod-mediated fertilization of moss. *Nature* **489**:431-433.
- Rühling, A., and G. Tyler. 1973. Heavy Metal Deposition in Scandanavia. *Water, Air, and Soil Pollution* **2**:445-455.
- Sandahl, J. F., D. H. Baldwin, J. J. Jenkins, and N. L. Scholz. 2004. Odor-evoked field

- potentials as indicators of sublethal neurotoxicity in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to copper, chlorpyrifos, or esfenvalerate. *Canadian Journal of Fisheries and Aquatic Sciences* **61**:404-413.
- Sandahl, J. F., D. H. Baldwin, J. J. Jenkins, and N. L. Scholz. 2007. A Sensory System at the Interface between Urban Stormwater Runoff and Salmon Survival. *Environmental Science & Technology* **41**:2998-3004.
- Sandifer, R. D., and S. P. Hopkin. 1996. Effects of pH on the toxicity of cadmium, copper, lead and zinc to *Folsomia candida* Willem, 1902 (Collembola) in a standard laboratory test system. *Chemosphere* **33**:2475-2486.
- Scott-Fordsmand, J. J., P. H. Krogh, and J. M. Weeks. 1997. Sublethal toxicity of copper to a soil-dwelling springtail (*Folsomia fimetaria*) (Collembola: Isotomidae). *Environmental Toxicology and Chemistry* **16**:2538-2542.
- Shortlidge, E. 2014. Testing the Ecological and Physiological Factors Influencing Reproductive Success in Mosses. Portland State University.
- Smit, C. E., and C. A. Van Gestel. 1997. Influence of temperature on the regulation and toxicity of zinc in *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf* **37**:213-222.
- Son, J., Y. S. Lee, S. E. Lee, K. I. Shin, and K. Cho. 2017. Bioavailability and Toxicity of Copper, Manganese, and Nickel in *Paronychiurus kimi* (Collembola), and Biomarker Discovery for Their Exposure. *Arch Environ Contam Toxicol* **72**:142-152.
- Sorensen, T. S., and M. Holmstrup. 2005. A comparative analysis of the toxicity of eight common soil contaminants and their effects on drought tolerance in the

- collembolan *Folsomia candida*. *Ecotoxicol Environ Saf* **60**:132-139.
- Stokes, M. E., C. S. Davis, and G. C. Koch. 2000. *Categorical data analysis using the SAS system*, 2nd ed. SAS Institute, North Carolina.
- Thorpe, A., and R. M. Harrison. 2008. Sources and properties of non-exhaust particulate matter from road traffic: a review. *Science of the Total Environment* **400**:270-282.
- Tullgren, A. 1918. Ein Sehr einfacher Ausleseapparat für terricole Tierfaunen. *Journal of Applied Entomology* **4**:149-150.
- Tyler, G. 1990. Bryophytes and heavy metals: a literature review. *Botanical Journal of the Linnean Society* **104**:231-253.
- Wesley, I. 2012. Better Brakes Rule. Pages 173-901 *in* W. S. D. o. Ecology, editor.
- WSDOT. 2015. Traffic Report. Washington State Department of Transportation.
- Yoshida, T., and N. Hijii. 2008. Efficiency of extracting microarthropods from the canopy litter in a Japanese cedar (*Cryptomeria japonica* D. Don) plantation: a comparison between the washing and Tullgren methods. *Journal of Forest Research* **13**:68-72.
- Zvereva, E. L., and M. V. Kozlov. 2010. Impacts of Industrial Polluters on Bryophytes: a Meta-analysis of Observational Studies. *Water, Air, & Soil Pollution* **218**:573-586.

## Chapter 3:

### Interacting Effects of Cu and Supraoptimal Temperatures on Collembola in Western Washington, USA

#### Abstract

I exposed two species of Collembola to Cu at previously determined LC<sub>25</sub> and LC<sub>50</sub> concentrations under three temperature regimes. Temperature regimes were based on ambient (Seattle June averages), B1 (+1.8°C), and A2 (+3.5°C) IPCC climate change scenarios. *Folsomia candida* Willem were reared under laboratory conditions and *Choreutinula americana* sp.nov. were collected from two field sites representing high (Seattle) and low (Hoh Rainforest) background Cu levels in Western Washington. Increasing temperatures greatly lowered both species tolerance to Cu, with LC<sub>25</sub> and LC<sub>50</sub> values at the A2 temperature regime being 0.5 and 39.3 ppm for *C. americana* and 0.1 and 31.2 ppm for *F. candida*, respectively. *Folsomia candida* was more sensitive to temperature increases alone than *C. americana*, though the interaction of Cu and temperature resulted in similar LC values at the A2 temperature regime. The results of this study highlight the importance of understanding the interacting effects of pollutants and climate change on organism and ecosystem health.

**Keywords:** Collembola; climate change; heavy metals; traffic pollution; Pacific Northwest

## **Introduction**

The anthropogenic warming of the global climate is perhaps one of the most pressing ecological issues facing society today. Evidence of human-driven warming has been found in the form of higher ocean and surface temperatures, increased rates of Arctic and Antarctic ice melt, increased sea levels, and rapidly increasing rates of climate warming (IPCC 2007). This evidence has been all but fully accepted within the scientific community, with 97% agreement among publishing climate scientists that warming of our global climate is human-induced (Cook et al. 2013), and nearly 200 leading scientific organizations around the world issuing public statements supporting that position (NASA 2017). The mounting evidence of the reality of global climate change has spurred a vast amount of research into the potential impacts of these changes within ecosystems around the world (Cook et al. 2013). National governments around the world have put forth efforts to both mitigate and adapt to our inevitably changing climate through international agreements and, in the case of the United States, a combined effort to take responsibility from agencies of the federal government (Yurkovich 2012, UNFCCC 2014, Halofsky and Peterson 2016). There is a need for continued efforts from scientists across disciplines to incorporate climate change into their studies to better inform policymakers in their efforts to combat and adapt to the complex changes our ecosystems will face in the years to come.

Compared to temperatures from 1970-1999, climate change models predict Washington State will be 1.8°C warmer by the 2040s and 3.0°C warmer by the 2080s (IPCC 2007, Adair and Reeder 2009, Miles et al. 2010, Abatzoglou et al. 2014,

TNC 2016). In response to these findings the Washington State legislature mandated that an assessment of the impacts of climate change in the region be prepared in collaboration with the University of Washington Climate Impacts Group. Broad scale patterns from these assessments show slightly increased annual precipitation (~2% by 2040s) but increased periods of summer drought (~22% less rain by the 2050s) with larger extreme precipitation events in autumn (Adair and Reeder 2009, Miles et al. 2010, Luce et al. 2016, TNC 2016). Changes in precipitation patterns will also lead to less snowpack, with a reduction of 38-46% of April 1<sup>st</sup> snow water equivalent that will further limit hydrologic systems and increase water short years in the Yakima basin from 14% (current) to 36% by the 2040s (Mote 2006, Miles et al. 2010). Modeling efforts directed towards Pacific Northwest (PNW) forest ecosystems predict changes in tree growth and regeneration, an increase in bark beetle outbreaks, increased tree mortality, and a 100-250% increase in area burned by fire (Miles et al. 2010, Stavros et al. 2014, Clark et al. 2016, Halofsky and Peterson 2016, Hicke et al. 2016, Kolb et al. 2016, Luce et al. 2016, TNC 2016, Halofsky et al. 2017). Many of these studies have focused on broad impacts that will effect PNW forest ecosystems as changing climate patterns lead to large disturbance events such as insect and pathogen outbreaks and wildfires (Miles et al. 2010, Stavros et al. 2014, Clark et al. 2016, Halofsky and Peterson 2016, Hicke et al. 2016, Kolb et al. 2016, Luce et al. 2016, Halofsky et al. 2017). However, examinations at a smaller scale using sensitive organisms may provide insight into less visible pathways to change that are likely already underway and will continue to define change in undisturbed areas.

The combined effects of a warming climate and increased deposition of heavy metal pollutants from the transportation sector will further increase stress to PNW ecosystems, an effect that will be magnified as the human populations continue to grow (Pearson et al. 2000, Bakirdere and Yaman 2008, Thorpe and Harrison 2008, Apeageyi et al. 2011, Wesley 2012). Past studies have shown that increasing temperature stress makes several organisms present in the PNW less tolerant to various pollutants, including heavy metals (Holmstrup 1997, Højer et al. 2001, Sjørnsen et al. 2001, Damgaard et al. 2002, Holmstrup et al. 2008, Slotsbo et al. 2009, Holmstrup et al. 2010, Bandow et al. 2014a, Bandow et al. 2014b, Holmstrup et al. 2014, Jegede et al. 2017). Several studies have attempted to assess the potential impacts of increased temperatures and drought conditions on Collembola (Holmstrup et al. 2002, Kardol et al. 2011, Waagner et al. 2012, Xu et al. 2012, Holmstrup and Bayley 2013, Holmstrup et al. 2013, Krab et al. 2013, Krab et al. 2014, Vestergård et al. 2015, Daghighi et al. 2017, Greenslade and Slatyer 2017). Collembola is a subclass of Entognatha within the same subphylum as Insecta, and is a commonly used test animal in the field of environmental toxicology (Fountain and Hopkin 2001, 2005, Krogh 2009). However, there are no known prior studies that simultaneously assess the effects of temperature and heavy metal deposition on bryophyte derived Collembola of the Pacific Northwest (PNW). I used lethal concentration (LC) values from experiments conducted on the sensitivity of lab-reared and wild Collembola collected from the PNW to Cu pollution (Chapter 2) to test the interactive effects of increased temperature and sublethal doses of Cu on Collembola mortality to quantify the impacts of these two abiotic stressors on

Collembola. I used both a field-collected Collembolan species considered to be native to the PNW, and a laboratory-reared species commonly used in ecotoxicology studies.

## **Materials and Methods**

### *3.1 Study Site*

My study area is located in the Hoh Rainforest between Upper Hoh Road and the Hoh River (47.82°N, 124.20°W). The site is dominated by bigleaf maple (*Acer macrophyllum*), western hemlock (*Tsuga heterophylla*), and sitka spruce (*Picea sitchensis*). The dominant moss present on *A. macrophyllum* is *Kinbergia praelonga*, and due to its dominance I selected it for Collembola sampling (Chapter 2.1).

### *3.2 Moss Collection*

I collected approximately 15 cm diameter cores of *K. praelonga* from the bottom 2 meters of *A. macrophyllum* stems and placed all samples into 6-liter Ziploc bags to transport back to the laboratory (Chapter 2.2). Samples were collected during the month of June 2017 and returned to the laboratory within 24 hours.

### *3.3 Collembola Extraction*

I placed *K. praelonga* samples into a Tullgren funnel array to live-extract Collembola from the moss (Chapter 2.3). After 48 hours, I removed the extracted Collembola from under the Tullgren funnels and pooled them into a large plastic bin. I identified the dominant Collembolan species as *Choreutinula americana* sp. nov.

(Collembola: Hypogastruridae; Chapter 2.3).

### *3.4 Laboratory-Reared Collembola*

I reared the temperate springtail *Folsomia candida* Willem (Collembola: Isotomidae) under laboratory conditions in large plastic bins filled halfway with charcoal and a layer of deionized (DI) water. I fed *F. candida* granulated active dry yeast every week and maintained the bins in growth chambers (Percival Scientific, Perry, Iowa) at 20°C (Chapter 2.4).

### *3.5 Supraoptimal Temperatures*

I obtained data from the National Weather Service for the Puget Sound region from 2012-2016, from which I estimated the mean minimum and maximum daily temperatures for the month of June (NOAA 2014); these 5-year mean temperatures were 12.0°C and 22.5°C, respectively. Based on sunrise (~0500) and sunset (~2100) for Seattle in June, I used the growth chambers to maintain the appropriate photoperiod under different temperature conditions. Under an ambient scenario (e.g., 12.0°C and 22.5°C as the minimum and maximum temperature, respectively), temperature was increased by 1°C per hour from 0500 until 22.5°C was obtained at 1530; this maximum temperature was maintained until 1700, after which temperatures were decreased by 1.5°C per hour until reaching 12.0°C at 0000. I also used two additional temperature regimes based on the IPCC B1 and A2 climate change scenarios (IPCC 2007). To simulate the B1 and A2 scenarios, I increased temperatures by 1.8°C and 3.5°C, respectively, from ambient

temperatures, *caeteris paribus* (Fig. 3.1).

### 3.6 Exposure to Copper

I used LC<sub>25</sub> and LC<sub>50</sub> values estimated from the previous year's research (Chapter 2) to create aqueous Cu solutions by dissolving cupric sulfate pentahydrate (CuSO<sub>4</sub> · 5H<sub>2</sub>O) in DI water in a 100 mL analytical volumetric flask. I created four solutions to match the LC<sub>25</sub> and LC<sub>50</sub> values for both the wild-collected (19 and 131 ppm Cu, respectively) and lab-reared populations (77 and 2,460 ppm Cu, respectively). I then pipetted 1ml of each solution (LC<sub>25</sub> and LC<sub>50</sub>) into 12 wells of Falcon 24-well cell culture trays (Fisher Scientific, Pittsburgh, Pennsylvania) along with a control of DI water in two additional cell culture trays. I then randomly added ~10 Collembola individuals from the lab-reared and wild populations into each cell culture well using a stainless steel scoopula. Cell culture trays were then randomly assigned to one of the temperature treatments (ambient, B1, and A2) in growth chambers. I removed trays every 2 days and counted live and dead individuals under a microscope to quantify the interactive effects of Cu and supraoptimal temperatures on Collembola mortality. Mortality was assessed over 10 days. For *F. candida*, individuals <1.5 mm in size were not counted as they were not considered adults.

### 3.7 Statistical Analysis

I first pooled mortality over time and subjected the data to a logit analysis to compare the dose-response relationship for both populations (Stokes et al. 2000). I

conducted an analysis for each population and tested the significance of the parameter estimates of the main effects of temperature regime and Cu dose, and their interaction, based on the Z-values. I then used the results of these analyses to estimate LC<sub>25</sub> and LC<sub>50</sub> values, and their respective confidence intervals, for both populations at each temperature regime (Stokes et al. 2000). I also tested the effect of temperature regime for each population at each Cu dose using logistic regression (Stokes et al. 2000). Lastly, I examined the effect of temperature regime and time, and their interaction, for each population at their respective control, LC<sub>25</sub>, and LC<sub>50</sub> values using logistic regression. The significance of parameter estimates was based on Z-values (Stokes et al. 2000).

## Results

When pooling mortality across time, there were significant differences between Cu doses ( $Z = 5.69, P < 0.001$ ) and between the ambient and A2 temperature regimes ( $Z = -2.64, P = 0.008$ ). There was no significant difference between the lab and wild populations ( $Z = 1.62, P = 0.106$ ) or between the B1 and A2 temperature scenarios ( $Z = -1.47, P = 0.142$ ; Fig. 3.2). When examining the wild population separately, there were significant differences for Cu dose ( $Z = 5.69, P < 0.001$ ) and between the ambient and A2 temperature scenarios ( $Z = -2.64, P = 0.008$ ; Fig. 3.2). In considering only the lab population, there were significant differences for Cu dose ( $Z = 10.26, P < 0.001$ ), and between the A2 and ambient temperature scenarios ( $Z = -4.19, P < 0.001$ ), the A2 and B1 temperature scenarios ( $Z = -4.02, P < 0.001$ ), and the interaction of Cu dose and ambient ( $Z = 2.12, P = 0.034$ ) and Cu dose

and B1 temperature scenarios ( $Z = 2.33, P = 0.020$ ; Fig. 3.2).

At the control dose, only the ambient temperature scenario for the wild population was significantly different from the A2 scenario ( $Z = -2.75, P = 0.006$ ), while the ambient ( $Z = -5.00, P < 0.001$ ) and B1 ( $Z = -3.30, P < 0.001$ ) scenarios were significantly different from the A2 scenario for the lab population (Fig. 3.3 and 3.4). At the  $LC_{25}$  dose there was no significant difference between A2 and ambient ( $Z = -0.23, P = 0.816$ ), and A2 and B1 ( $Z = -0.35, P = 0.724$ ) temperature regimes for the wild population, while only the ambient scenario for the lab population was significantly different from the A2 scenario ( $Z = -2.96, P = 0.003$ ; Fig. 3.3 and 3.4). At the  $LC_{50}$  dose the ambient scenario was significantly different from the A2 scenario ( $Z = -2.13, P = 0.033$ ) for the wild population, while the B1 scenario was significantly different from the A2 scenario ( $Z = -2.72, P = 0.007$ ) for the lab population (Figs. 3.3 and 3.4).

For the analysis examining mortality over time, the effects of temperature regime for each population at their respective control,  $LC_{25}$  and  $LC_{50}$  values is presented in Table 3.1. At the control level there was no significant difference between the A2 and ambient ( $Z = -0.253, P = 0.8002$ ), and A2 and B1 ( $Z = 1.011, P = 0.3120$ ) temperature regimes for the wild population over the 10-day exposure period. At the  $LC_{25}$  level there was no significant difference between the A2 and ambient ( $Z = -1.486, P = 0.137$ ) and A2 and B1 ( $Z = -0.305, P = 0.760$ ) temperature regimes for the wild population over the 10-day exposure period. Time was a significant factor at control ( $Z = 2.392, P = 0.0167$ ),  $LC_{25}$  ( $Z = 4.505, P < 0.001$ ), and  $LC_{50}$  values ( $Z = 4.478, P < 0.001$ ) for the wild population (Fig. 3.5). Time was also a

significant factor at control ( $Z = 7.313, P < 0.001$ ),  $LC_{25}$  ( $Z = 11.49, P < 0.001$ ), and  $LC_{50}$  levels ( $Z = 9.23, P < 0.001$ ) for the lab population (Fig. 3.5). At the control Cu dose for the lab population there was a significant interaction effect between time and ambient ( $Z = -2.199, P = 0.02789$ ) and time and B1 temperature regimes ( $Z = -2.659, P = 0.00783$ ).

Based on data from this study, I estimated the  $LC_{25}$  and  $LC_{50}$  values for the lab and wild populations under ambient temperature conditions, and when simulating temperature conditions under the B1 and A2 climate change scenarios. These values are presented in Table 3.2.  $LC_{25}$  and  $LC_{50}$  values were lower for both populations at all temperature regimes than they were during the initial experiment run at 20.0°C (Ch. 2).

## **Discussion**

Increased temperature increased mortality in Collembola when exposed to their  $LC_{25}$  and  $LC_{50}$  values of Cu, and this effect was observed in both populations. The lab-reared population was more sensitive to temperature increases than the wild population, which was also observed in a Tullgren funnel stress experiment (Chapter 2). It is possible that because the lab population, which has been reared for many generations at nearly constant temperatures (Hopkin 1997, Fountain and Hopkin 2005, Krogh 2009), does not experience temperature fluctuations similar to those under natural conditions, that these individuals are therefore more sensitive to changes in temperature. Interestingly, mortality in the lab population was lowest at the B1 scenario for control and  $LC_{50}$  Cu doses (Fig. 4), which suggests that the B1

scenario could be closer to the optimal temperature for *F. candida*. Another study found that *F. candida* abundance increased when soil temperatures were raised from 15°C to 18°C, even though 15°C was used as the ambient treatment (A'Bear et al. 2012). However, this relationship did not hold for the LC<sub>25</sub> Cu treatment in which the ambient temperature scenario had lower mortality than the B1 and A2 scenarios, which were not significantly different (Fig. 4). The estimated LC<sub>25</sub> and LC<sub>50</sub> values for Cu were also very similar between the ambient and B1 scenarios for the lab population, with a large increase in sensitivity to Cu at the A2 scenario (Table 1). The estimated LC<sub>25</sub> and LC<sub>50</sub> values for the wild population were similar or identical to previously estimated values (Chapter 2, Table 1). It is important to note that the LC<sub>25</sub> values for the wild population at all temperature scenarios (0.5 – 4.2 ppm, Table 1) were equal to or lower than current environmental levels of Cu in epiphytic mosses in forests around Western Washington, which range from 3.5 - 12.3 ppm on average (Bidwell 2017).

The interaction of Cu exposure and temperature did not provide a consistent pattern of the effects of increased temperatures and Cu on Collembola mortality. However, Collembola mortality was higher at all temperature regimes and doses for both populations than mortality recorded from the initial experiment conducted at a constant temperature of 20°C (Chapter 2), suggesting that temperature fluctuations can add stress to Collembola in a laboratory setting. While the wild population tolerated the temperature fluctuations better than the lab population, mortality in the former still increased when they were exposed to Cu, even in the ambient scenario (i.e., temperatures at which Collembola normally experience in an average

June in Seattle). It should be noted that under natural conditions, surface-dwelling Collembola can behaviorally respond to adverse environmental conditions by seeking microhabitats that are wetter and cooler (Hopkin 1997, Kaersgaard et al. 2004, Holmstrup et al. 2013, Krab et al. 2013), which was a option not available to them in this experiment. However, with climate change models predicting longer periods of drought and more extreme temperature events, organisms could be approaching the limit of what is tolerable.

Variation in metal sensitivity of Collembola when tested in concert with interacting effects of temperature and other stressors has been previously reported. For example, Jegede et al. (2017) found that LC<sub>50</sub> values of dimethoate were lower for *F. candida* at 28°C than at 20°C, while the opposite was true for the pesticide deltamethrin. Holmstrup et al. (2008) reported that exposure to mercury (Hg) lowered the ability of *F. candida* to tolerate and survive cold shock treatments while Slotsbo et al. (2009) reported that Hg also reduced tolerance to increased temperatures in *F. candida*. Smit and Van Gestal (1997) found that *F. candida* exposed to Zn showed decreased growth and reproductive rates as temperature was lowered, but mortality rates did not differ, likely due to internal Zn regulation by individuals. Bandow et al. (2014) studied the toxicity of lambda-cyhalothrin on two Collembolan species (*F. Candida* and *Sinella curviseta* Brook (Collembola: Entomobryidae)) and observed increased toxicity with increased temperatures for *F. Candida* but the opposite effect in *S. curviseta*. This same study also found that increased soil moisture increased fecundity in both species, while also increasing sensitivity to the toxin (Bandow et al. 2014). Collectively, these prior studies

demonstrate the interacting effects of both suboptimal and supraoptimal temperatures, and various toxicants, on Collembola mortality are not always clear.

Multiple studies have also examined the effect of drought on Collembola in combination with other factors under laboratory conditions (Holmstrup 1997, Højer et al. 2001, Sjørnsen et al. 2001, Damgaard et al. 2002, Holmstrup et al. 2002, Holmstrup et al. 2010, Waagner et al. 2012, Bandow et al. 2014a, Bandow et al. 2014b, Holmstrup et al. 2014). These studies have largely shown a compounding effect between drought and pollutants, leading to increased mortality in Collembola. However, in some cases these studies do not explain large variation in mortality, while some have shown decreased sensitivity to pollutants under drought conditions (Bandow et al. 2014a). There are several possible reasons for the variation within these studies. For example, some results vary by species of Collembola (Kaersgaard et al. 2004, Holmstrup et al. 2010, Holmstrup and Bayley 2013, Bandow et al. 2014a, Bandow et al. 2014b), the pollutants tested (Holmstrup 1997, Sjørnsen et al. 2001, Holmstrup et al. 2010, Bandow et al. 2014a, Bandow et al. 2014b), and whether mortality or some measure of fitness (i.e., reproductive rate) was used as the response variable (Smit and Van Gestel 1997, Sørensen and Holmstrup 2005, Holmstrup et al. 2013, Bandow et al. 2014a, Bandow et al. 2014b).

In this study, I examined the effects of temperature without introducing drought, as Collembola were placed directly on aqueous Cu solutions. Based on prior work with Collembola and drought, it is likely that drought in conjunction with higher temperatures will be more detrimental to Collembola in the absence of moisture (Hopkin 1997). Physiological tolerance to drought varies among species,

and affects their tolerance to temperature stress (Kaersgaard et al. 2004, Holmstrup et al. 2010, Holmstrup and Bayley 2013, Bandow et al. 2014a, Bandow et al. 2014b). In particular, epedaphic (surface dwelling) Collembola rely most heavily on behavioral responses to survive drought and temperature extremes (Kaersgaard et al. 2004, Krab et al. 2013).

With climate change models predicting longer periods of drought and more extreme temperature events, it is important to understand how these species will adapt, and in the face of anthropogenically-derived heavy metal pollution. In the absence of pollution, prior field studies have shown that drought and extreme temperatures lead to changes in species richness rather than abundance (Kardol et al. 2011, Holmstrup et al. 2013, Krab et al. 2013, Vestergård et al. 2015, Daghighi et al. 2017). A primary concern is with endemic species inhabiting environments that do not allow for dispersal to higher elevations or latitudes (i.e. polar and montane environments) (Greenslade and Slatyer 2017). The results of shifts in Collembola species composition and richness poorly studied; however, given the important ecosystem services these species provide, it is possible that the loss of Collembola diversity will have implications for plant cover, moss pollination, fungal diversity and abundance, regulation of eudaphic pathogens and bacteria, nutrient cycling, and breakdown of organic matter (Hopkin 1997, Kardol et al. 2011, A'Bear et al. 2012, Holmstrup et al. 2013, Vestergård et al. 2015).

The interaction between temperature and exposure to heavy metal pollutants on Collembola mortality suggests that the effects of increased temperatures, and especially under conditions of drought, will have the most

detrimental effect on Collembola populations in our most polluted ecosystems, such as urbanized centers (Holmstrup 1997, Højer et al. 2001). This research adds to the evidence that the interacting effects of pollution and a warming climate will surpass the individual effects of each stressor independently, with a specific context for forest ecosystems in the PNW. This research highlighted the sensitivity of Collembola to Cu when exposed to increased temperatures, and the urgency to develop a better understanding of the impacts that climate change and pollution will have on Collembola communities. Further research is needed to determine the resiliency of microarthropod communities in Western Washington and their ability to continue providing ecosystem services as those communities change in the face of pollution and a warming climate.

2017. U.S. Census Bureau. <https://www.census.gov/en.html>.
- A'Bear, A. D., L. Boddy, and T. Hefin Jones. 2012. Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. *Global Change Biology* **18**:1823-1832.
- Abatzoglou, J. T., D. E. Rupp, and P. W. Mote. 2014. Seasonal Climate Variability and Change in the Pacific Northwest of the United States. *Journal of Climate* **27**:2125-2142.
- Adair, J., and S. Reeder. 2009. Focus on Impacts of Climate Change in Washington State. *in* W. S. D. o. Ecology, editor., *Community, Trade, and Economic Development*.
- Apeageyi, E., M. S. Bank, and J. D. Spengler. 2011. Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts. *Atmospheric Environment* **45**:2310-2323.
- Bakirdere, S., and M. Yaman. 2008. Determination of lead, cadmium and copper in roadside soil and plants in Elazig, Turkey. *Environ Monit Assess* **136**:401-410.
- Bandow, C., A. Coors, N. Karau, and J. Rombke. 2014a. Interactive effects of lambda-cyhalothrin, soil moisture, and temperature on *Folsomia candida* and *Sinella curviseta* (Collembola). *Environ Toxicol Chem* **33**:654-661.
- Bandow, C., N. Karau, and J. Römcke. 2014b. Interactive effects of pyrimethanil, soil moisture and temperature on *Folsomia candida* and *Sinella curviseta* (Collembola). *Applied Soil Ecology* **81**:22-29.
- Bidwell, A. 2017. Urbanization impacts on epiphytic nitrogen and metal cycling in

- Acer macrophyllum* stands in Western Washington, USA. University of Washington.
- Clark, J. S., L. Iverson, C. W. Woodall, C. D. Allen, D. M. Bell, D. C. Bragg, A. W. D'Amato, F. W. Davis, M. H. Hersh, I. Ibanez, S. T. Jackson, S. Matthews, N. Pederson, M. Peters, M. W. Schwartz, K. M. Waring, and N. E. Zimmermann. 2016. The impacts of increasing drought on forest dynamics, structure, and biodiversity in the United States. *Glob Chang Biol* **22**:2329-2352.
- Cook, J., D. Nuccitelli, S. A. Green, M. Richardson, B. Winkler, R. Painting, R. Way, P. Jacobs, and A. Skuce. 2013. Quantifying the consensus on anthropogenic global warming in the scientific literature. *Environmental Research Letters* **8**.
- Daghighi, E., H. Koehler, R. Kesel, and J. Filser. 2017. Long-term succession of Collembola communities in relation to climate change and vegetation. *Pedobiologia*.
- Damgaard, C., R. Hojer, M. Bayley, J. J. Scott-Fordsmand, and M. Holmstrup. 2002. Dose-response curve modeling of excess mortality caused by two forms of stress. *Environmental and Ecological Statistics* **9**:195-200.
- Fountain, M. T., and S. P. Hopkin. 2001. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. *Ecotoxicol Environ Saf* **48**:275-286.
- Fountain, M. T., and S. P. Hopkin. 2005. *Folsomia candida* (Collembola): a "standard" soil arthropod. *Annu Rev Entomol* **50**:201-222.
- Greenslade, P., and R. Slatyer. 2017. Montane Collembola at risk from climate change in Australia. *European Journal of Soil Biology* **80**:85-91.

- Halofsky, J., and D. Peterson. 2016. Climate Change Vulnerabilities and Adaptation Options for Forest Vegetation Management in the Northwestern USA. *Atmosphere* **7**.
- Halofsky, J. E., D. L. Peterson, and H. R. Prendeville. 2017. Assessing vulnerabilities and adapting to climate change in northwestern U.S. forests. *Climatic Change*.
- Hicke, J. A., A. J. H. Meddens, and C. A. Kolden. 2016. Recent Tree Mortality in the Western United States from Bark Beetles and Forest Fires. *Forest Science* **62**:141-153.
- Højer, R., M. Bayley, C. F. D. and, and M. Holmstrup. 2001. Stress synergy between drought and a common environmental contaminant: studies with the collembolan *Folsomia candida*. *Global Change Biology* **7**:485-494.
- Holmstrup, M. 1997. Drought tolerance in *Folsomia Candida* Willem (*Collembola*) after exposure to sublethal concentrations of three soil polluting chemicals. *Pedobiologia* **41**:361-368.
- Holmstrup, M., A. Aubail, and C. Damgaard. 2008. Exposure to mercury reduces cold tolerance in the springtail *Folsomia candida*. *Comp Biochem Physiol C Toxicol Pharmacol* **148**:172-177.
- Holmstrup, M., and M. Bayley. 2013. *Protaphorura tricampata*, a euedaphic and highly permeable springtail that can sustain activity by osmoregulation during extreme drought. *J Insect Physiol* **59**:1104-1110.
- Holmstrup, M., A. M. Bindsbol, G. J. Oostingh, A. Duschl, V. Scheil, H. R. Kohler, S. Loureiro, A. M. Soares, A. L. Ferreira, C. Kienle, A. Gerhardt, R. Laskowski, P. E. Kramarz, M. Bayley, C. Svendsen, and D. J. Spurgeon. 2010. Interactions

- between effects of environmental chemicals and natural stressors: a review. *Sci Total Environ* **408**:3746-3762.
- Holmstrup, M., K. Hedlund, and H. Boriss. 2002. Drought acclimation and lipid composition in *Folsomia candida*: implications for cold shock, heat shock and acute desiccation stress. *Journal of Insect Physiology* **48**:961-970.
- Holmstrup, M., S. Slotsbo, S. N. Schmidt, P. Mayer, C. Damgaard, and J. G. Sorensen. 2014. Physiological and molecular responses of springtails exposed to phenanthrene and drought. *Environ Pollut* **184**:370-376.
- Holmstrup, M., J. G. Sørensen, I. K. Schmidt, P. L. Nielsen, S. Mason, A. Tietema, A. R. Smith, T. Bataillon, C. Beier, and B. K. Ehlers. 2013. Soil microarthropods are only weakly impacted after 13 years of repeated drought treatment in wet and dry heathland soils. *Soil Biology and Biochemistry* **66**:110-118.
- Hopkin, S. P. 1997. *Biology of the Springtails — (Insecta: Collembola)*. Oxford University Press.
- IPCC. 2007. *Climate change 2007: An Assessment of the Intergovernmental Panel on Climate Change. Weather* **57**:267-269.
- Jegede, O. O., O. J. Owojori, and J. Rombke. 2017. Temperature influences the toxicity of deltamethrin, chlorpyrifos and dimethoate to the predatory mite *Hypoaspis aculeifer* (Acari) and the springtail *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf* **140**:214-221.
- Kaersgaard, C. W., M. Holmstrup, H. Malte, and M. Bayley. 2004. The importance of cuticular permeability, osmolyte production and body size for the desiccation resistance of nine species of Collembola. *J Insect Physiol* **50**:5-15.

- Kardol, P., W. N. Reynolds, R. J. Norby, and A. T. Classen. 2011. Climate change effects on soil microarthropod abundance and community structure. *Applied Soil Ecology* **47**:37-44.
- Kolb, T. E., C. J. Fettig, M. P. Ayres, B. J. Bentz, J. A. Hicke, R. Mathiasen, J. E. Stewart, and A. S. Weed. 2016. Observed and anticipated impacts of drought on forest insects and diseases in the United States. *Forest Ecology and Management* **380**:321-334.
- Krab, E. J., R. Aerts, M. P. Berg, J. van Hal, and F. Keuper. 2014. Northern peatland Collembola communities unaffected by three summers of simulated extreme precipitation. *Applied Soil Ecology* **79**:70-76.
- Krab, E. J., I. M. Van Schroyen Lantman, J. H. C. Cornelissen, and M. P. Berg. 2013. How extreme is an extreme climatic event to a subarctic peatland springtail community? *Soil Biology and Biochemistry* **59**:16-24.
- Krogh, P. H. 2009. Toxicity testing with the collembolans *Folsomia fimentaria* and *Folsomia candida* and the results of a ringtest. Danish Ministry of the Environment, Environmental Project No. 1256.
- Luce, C. H., J. M. Vose, N. Pederson, J. Campbell, C. Millar, P. Kormos, and R. Woods. 2016. Contributing factors for drought in United States forest ecosystems under projected future climates and their uncertainty. *Forest Ecology and Management* **380**:299-308.
- Miles, E. L., M. M. Elsner, J. S. Littell, L. W. Binder, and D. P. Lettenmaier. 2010. Assessing regional impacts and adaptation strategies for climate change: the Washington Climate Change Impacts Assessment. *Climatic Change* **102**:9-27.

- Mote, P. W. 2006. Climate-Driven Variability and Trends in Mountain Snowpack in Western North America\*. *Journal of Climate* **19**:6209-6220.
- NASA. 2017. Global Climate Change: Vital Signs of the Planet. *in* H. Shaftel, editor. NASA.
- NOAA. 2014. National Weather Service Forecast Office: Seattle, WA. NOAA, <http://w2.weather.gov/climate/index.php?wfo=sew>.
- Pearson, J., D. M. Wells, K. J. Seller, A. Bennett, A. Soares, J. Woodall, and M. J. Ingrouille. 2000. Traffic exposure increases natural <sup>15</sup>N and heavy metal concentrations in mosses. *New Phytologist* **147**:317-326.
- Sjursen, H., L. E. Sverdrup, and P. H. Krogh. 2001. Effects of polycyclic aromatic compounds on the drought tolerance of *Folsomia fimetaria* (Collembola, Isotomidae). *Environmental Toxicology and Chemistry* **20**:2899-2902.
- Slotsbo, S., L. H. Heckmann, C. Damgaard, D. Roelofs, T. de Boer, and M. Holmstrup. 2009. Exposure to mercury reduces heat tolerance and heat hardening ability of the springtail *Folsomia candida*. *Comp Biochem Physiol C Toxicol Pharmacol* **150**:118-123.
- Smit, C. E., and C. A. Van Gestel. 1997. Influence of temperature on the regulation and toxicity of zinc in *Folsomia candida* (Collembola). *Ecotoxicol Environ Saf* **37**:213-222.
- Sorensen, T. S., and M. Holmstrup. 2005. A comparative analysis of the toxicity of eight common soil contaminants and their effects on drought tolerance in the collembolan *Folsomia candida*. *Ecotoxicol Environ Saf* **60**:132-139.
- Stavros, E. N., J. T. Abatzoglou, D. McKenzie, and N. K. Larkin. 2014. Regional

- projections of the likelihood of very large wildland fires under a changing climate in the contiguous Western United States. *Climatic Change* **126**:455-468.
- Thorpe, A., and R. M. Harrison. 2008. Sources and properties of non-exhaust particulate matter from road traffic: a review. *Sci Total Environ* **400**:270-282.
- TNC. 2016. Adapting to Change: Climate Impacts and Innovation in Puget Sound. Puget Sound Conservation.
- Tullgren, A. 1918. Ein Sehr einfacher Ausleseapparat für terricole Tierfaunen. *Journal of Applied Entomology* **4**:149-150.
- UNFCCC. 2014. Focus: Mitigation - Nationally appropriate mitigation commitments or actions by developed country parties. UNFCCC, United Nations Framework Convention on Climate Change.
- Vestergård, M., K. Dyrnum, A. Michelsen, C. Damgaard, and M. Holmstrup. 2015. Long-term multifactorial climate change impacts on mesofaunal biomass and nitrogen content. *Applied Soil Ecology* **92**:54-63.
- Waagner, D., M. Bayley, J. Marien, M. Holmstrup, J. Ellers, and D. Roelofs. 2012. Ecological and molecular consequences of prolonged drought and subsequent rehydration in *Folsomia candida* (Collembola). *J Insect Physiol* **58**:130-137.
- Wesley, I. 2012. Better Brakes Rule. Pages 173-901 in W. S. D. o. Ecology, editor.

Xu, G. L., T. M. Kuster, M. S. Gunthardt-Goerg, M. Dobbertin, and M. H. Li. 2012.

Seasonal exposure to drought and air warming affects soil Collembola and mites. *PLoS One* 7:e43102.

Yurkovich, E. 2012. *Climate Change Adaptation: What Federal Agencies are Doing*. CFCAE Solutions, U.S. Policy.

**Table 2.1** Copper LC values in ppm (95% Confidence Interval) for lab-reared and wild-collected Collembola

<b>Lethal Concentration (LC)</b>	<b>Population</b>	
	<b>Lab (<i>F. candida</i>)</b>	<b>Wild (<i>C. americana</i>)</b>
<b>LC 25</b>	<b>77 (48-125)</b>	<b>19 (12-22)</b>
<b>LC 50</b>	<b>2,460 (1,516-3,990)</b>	<b>131 (112-154)</b>

**Table 3.1** Z and P values showing the significance of Ambient and B1 temperature regimes (compared to the A2 temperature regime), on lab-reared and wild-collected *Collembola* mortality at their respective Cu control, LC<sub>25</sub> and LC<sub>50</sub> values.

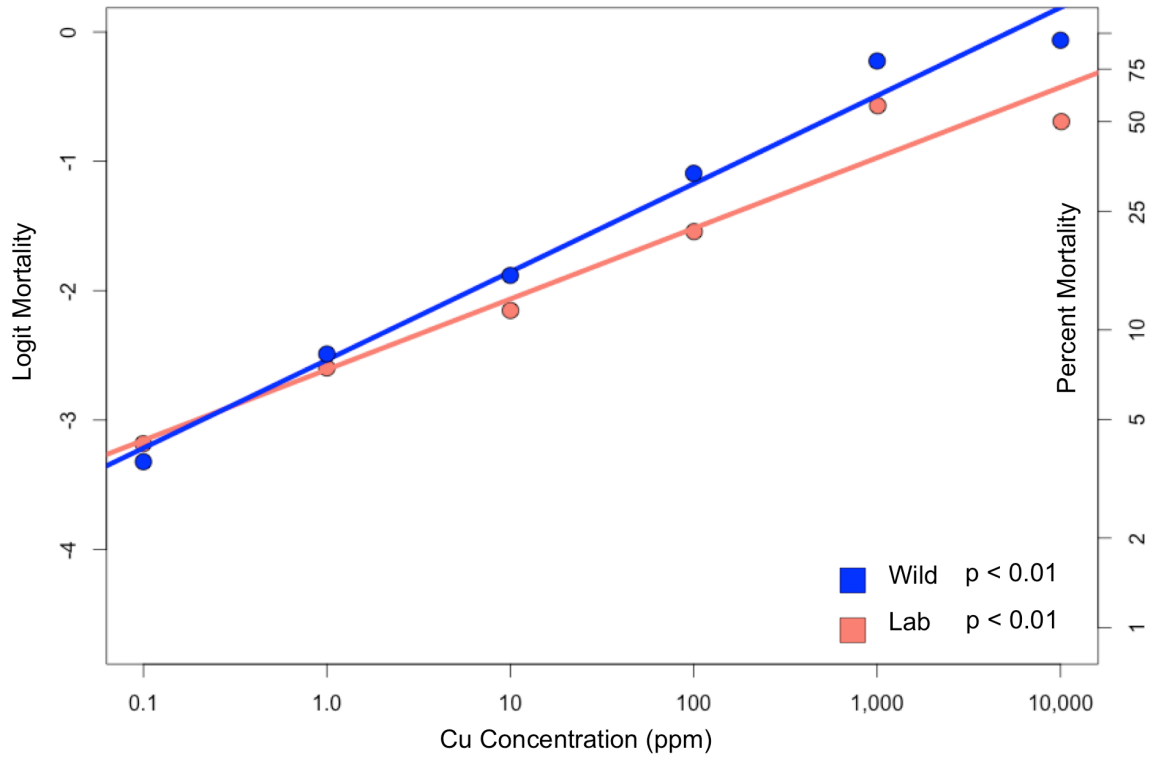
Population and Cu Concentration	Temperature Scenario			
	Ambient		B1	
	Z	P	Z	P
Lab (Control)	1.59	0.112	0.67	0.503
Lab (LC 25)	0.93	0.352	1.60	0.109
Lab (LC 50)	-0.30	0.767	-0.68	0.499
Wild (Control)	-0.25	0.800	1.01	0.312
Wild (LC 25)	-1.49	0.137	-0.31	0.760
Wild (LC 50)	-1.24	0.216	0.39	0.694

**Table 3.2** Lethal concentration (LC) values of Copper in ppm (+95% Confidence Intervals) for lab-reared and wild-collected Collembola at Ambient, B1, and A2 temperature scenarios

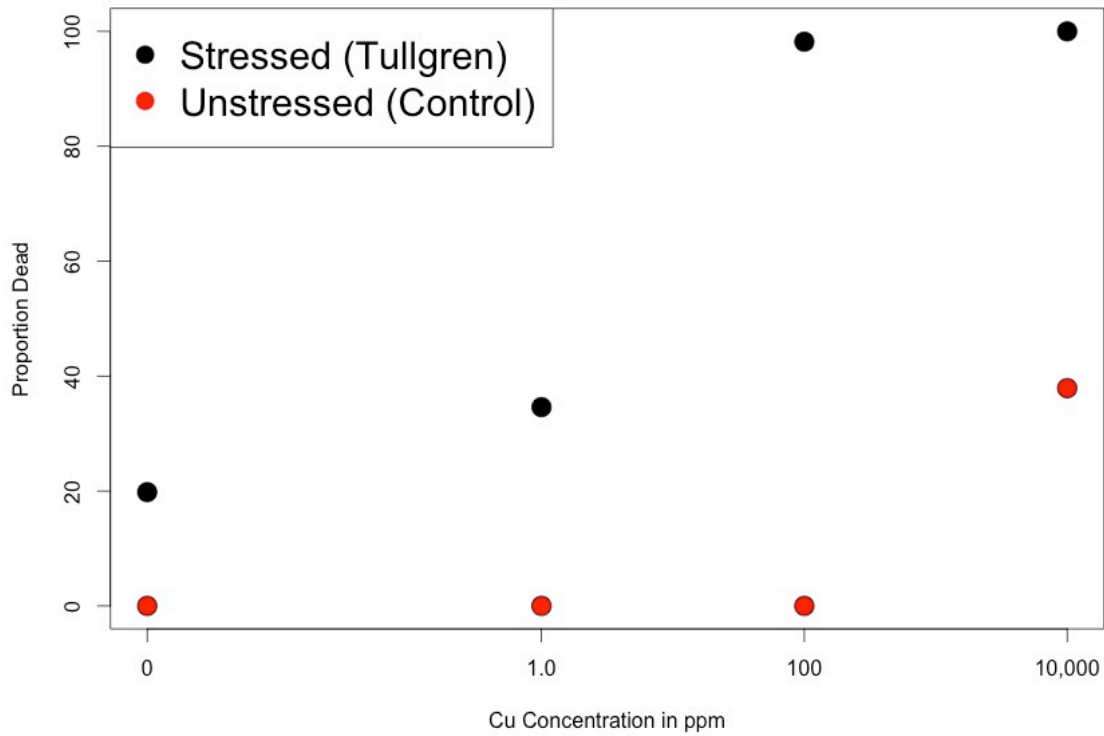
Lethal Concentration (LC) & Temperature Scenario	Population	
	Lab ( <i>F. candida</i> )	Wild ( <i>C. americana</i> )
LC 25 (Ambient)	1.6 (0.9 - 2.7)	4.2 (1.9 - 9.3)
LC 25 (B1)	1.6 (0.9 - 2.8)	1.7 (0.9 - 3.1)
LC 25 (A2)	0.06 (0.03 - 0.13)	0.5 (0.2 - 1.2)
LC 50 (Ambient)	182.4 (102.8 - 323.7)	109.9 (50.2 - 240.4)
LC 50 (B1)	162.1 (92.8 - 283.0)	38.45 (20.9 - 70.8)
LC 50 (A2)	31.2 (15.4 - 63.3)	39.3 (16.1 - 96.2)



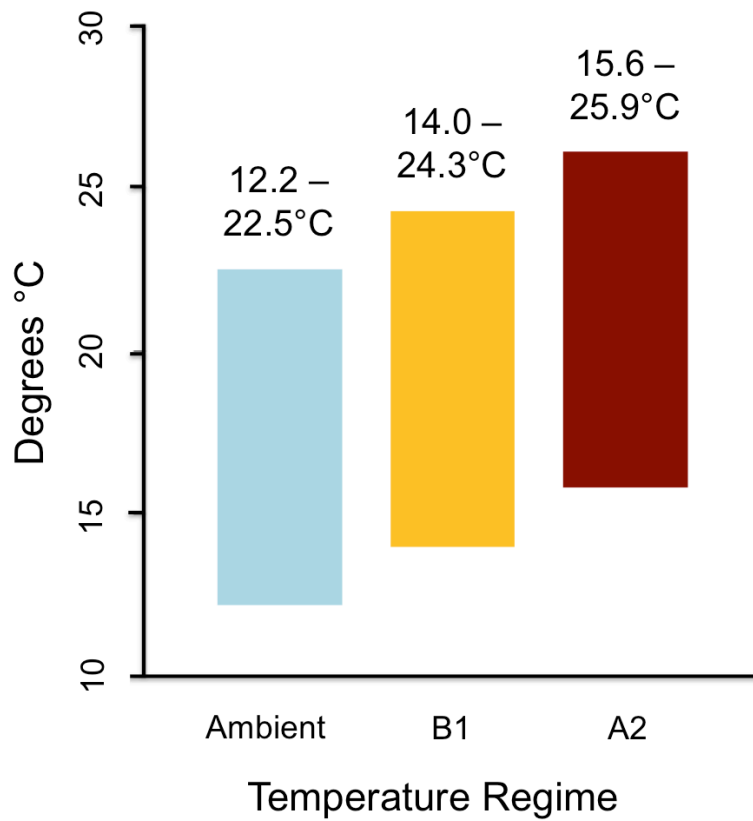
**Figure 2.1** Tullgren funnel setup showing overhead view of moss (left) and frontal view of whole system extracting microarthropods out of the moss into DI water in a Tupperware® container



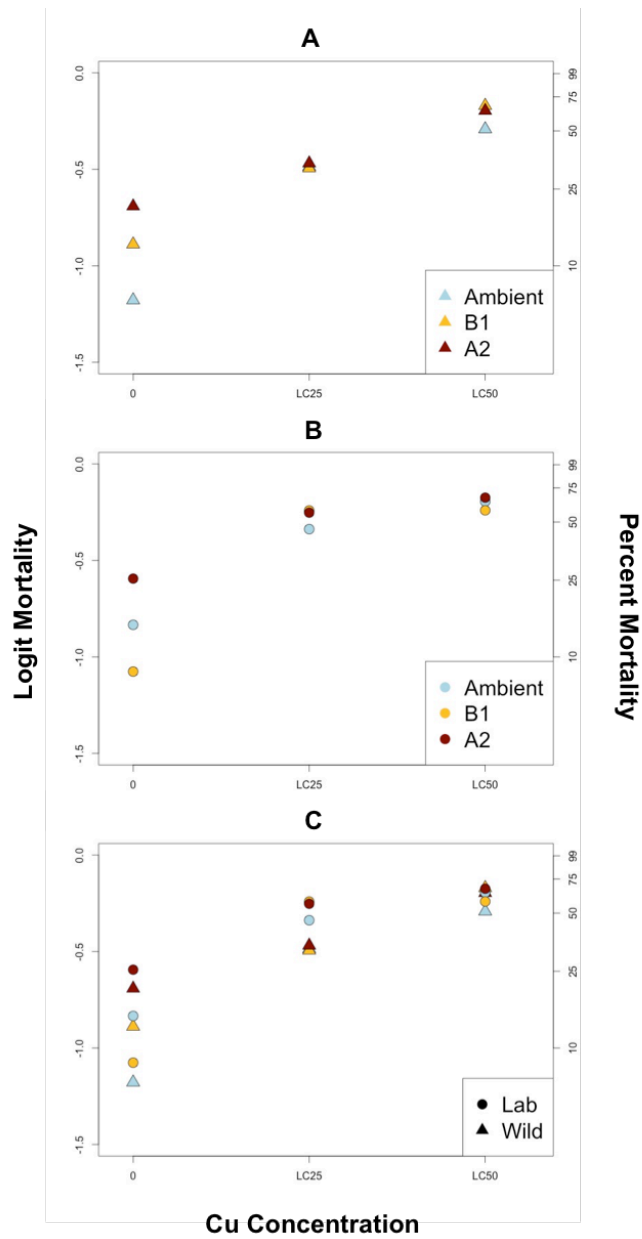
**Figure 2.2** Lab-reared and wild-collected (from epiphytic bryophytes in western Washington) Collembola mortality (pooled over time) after 10 days of exposure to Cu solutions



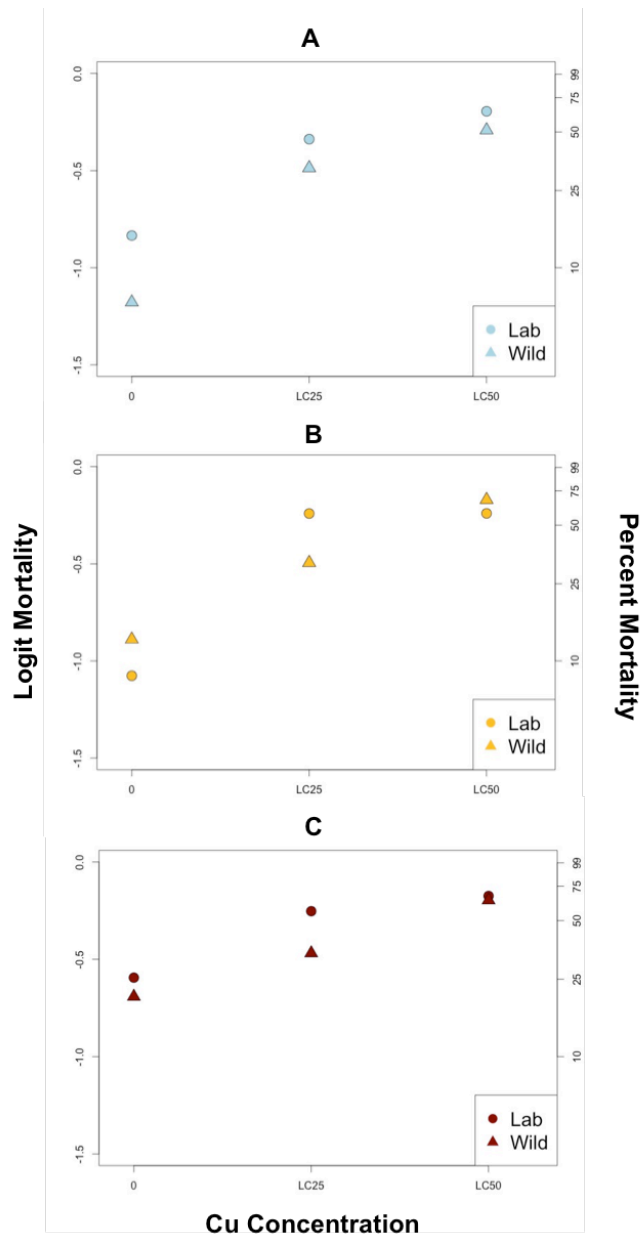
**Figure 2.3** Stressed (extracted through Tullgren funnel) and unstressed (control) *F. candida* mortality after four days of exposure to Cu solutions



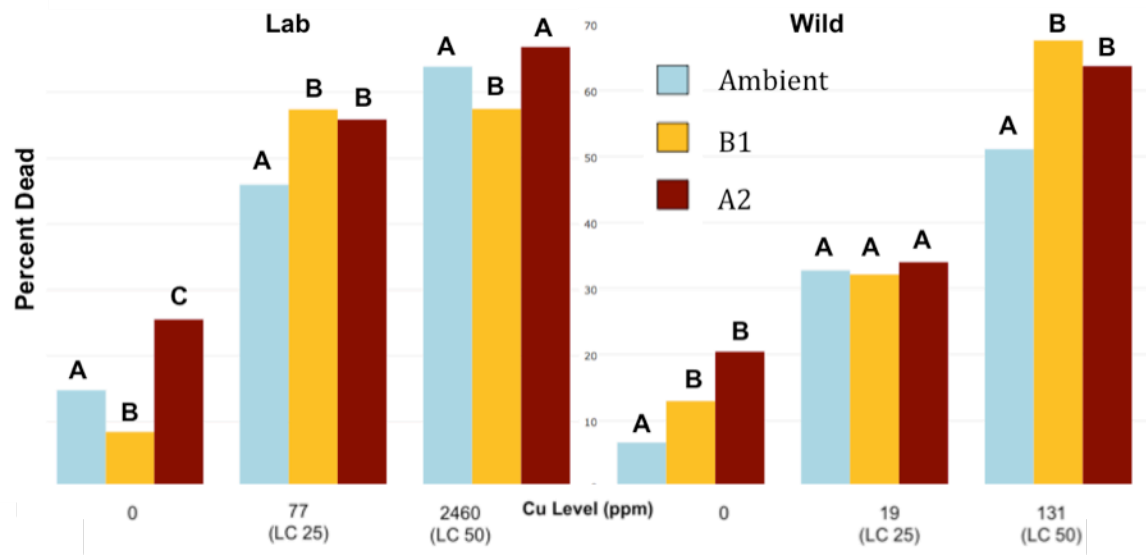
**Figure 3.1** Ranges of temperatures of the ambient conditions (5-year average from Seattle in June), and simulated temperatures under the B1 and A2 climate change scenarios used in growth chamber experiments



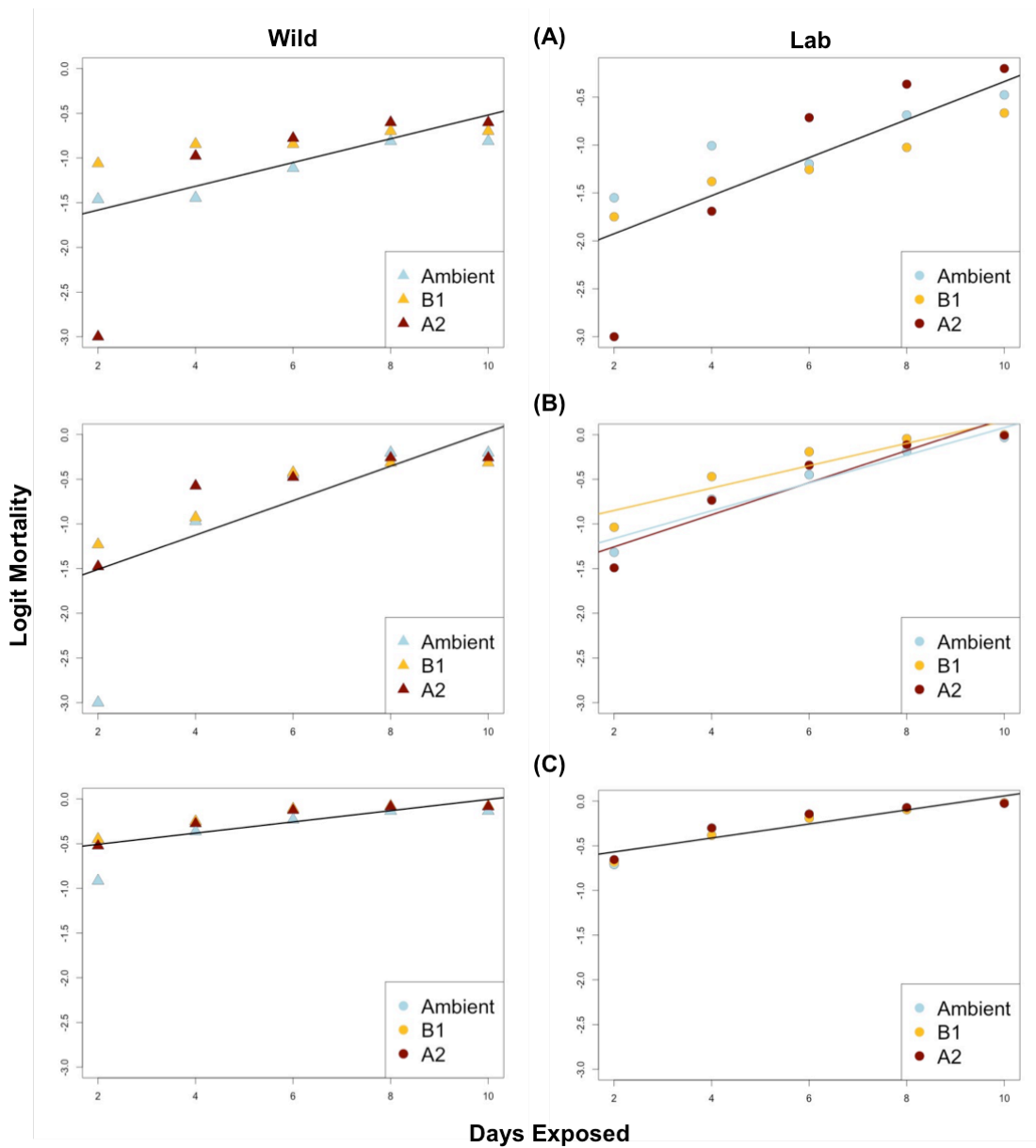
**Figure 3.2** Wild (A, field collected in western Washington), Lab (B, purchased), and all Collembola (C) mortality pooled over time in response to exposure to the Cu control, LC<sub>25</sub> and LC<sub>50</sub> at the ambient, B1, and A2 temperature regimes



**Figure 3.3** Collembola mortality at control, LC<sub>25</sub>, and LC<sub>50</sub> Cu concentrations under the ambient (A), B1 (B), and A2 (C) temperature regimes



**Figure 3.4** Lab-reared and wild-collected Collembola mortality at the control, and their respective LC<sub>25</sub>, and LC<sub>50</sub> Cu doses under the ambient, B1, and A2 temperature scenarios. Letters within a dose and population indicate significant difference ( $\alpha = 0.05$ )



**Figure 3.5** Dose- and time-response of wild-collected and lab-reared Collembola mortality under the ambient, B1, and A2 temperature regimes at the control (A) and their respective LC<sub>25</sub> (B), and LC<sub>50</sub> (C) Cu concentrations