

Invertebrate Response to Mine Reclamation (South-Central British Columbia): The Effects of Reclamation Age on Arthropod Assemblages

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Gervan, C.A., Gardner, W.C., Bottos, E.M., Van Hamme, J.D., Higgins, R.J. and Fraser, L.H. (2020): Invertebrate response to mine reclamation (south-central British Columbia): the effects of reclamation age on arthropod assemblages; *in* Geoscience BC Summary of Activities 2019: Minerals, Geoscience BC, Report 2020-01, p. 159–166.

Introduction

In Canada, regulations state that planning for mine closure must occur before mining companies begin production (Mining Association of Canada, 2019). Mine closure entails ecosystem reclamation: returning the land that has been altered by mining back to a functioning, self-sustaining ecosystem. More specifically, mine reclamation in British Columbia (BC) addresses terrestrial areas, water bodies and cultural resources (Government of British Columbia, 2019). Ecosystem reclamation research is required to reduce knowledge gaps and further understand the recovery trajectory of reclaimed sites to optimize reclamation practices.

Postmining landscapes undergo succession as it relates to flora and fauna (such as invertebrates). Currently, researchers call attention to the lack of information regarding the outcomes of mine reclamation (Bacher et al., 2018), especially addressing invertebrate-community recovery. Biotic diversity is identified as an important factor in achieving a functioning ecosystem. Biodiversity is defined as the variety of life in a given area with regard to genetic variance, trophic levels and taxonomy (Gaston and Spicer, 2004); it therefore follows that biodiversity can be used as a measure of ecological health.

Invertebrates comprise a significant portion of taxonomic biodiversity and provide essential ecosystem services (McGeoch et al., 2011). More specifically, arthropods play a fundamental role in ecological succession and soil formation in reclaimed areas as they contribute to nutrient turnover, decomposition, litter breakup, herbivory and pollination; they also act as dispersal agents (fungi and propagule) and are food resources for wildlife (Majer et al., 2002). Alternatively, invertebrates can play negatively perceived ecological roles, such as those of vectors for disease or pests in the context of agriculture and forestry. Recovery of species-rich invertebrate assemblages in reclaimed areas, particularly species correlated with ecosystem functions, is encouraged (Majer et al., 2002).

Invertebrates are sensitive to environmental change (Buchori et al., 2018). Their mobility allows them to relocate in response to environmental change and, despite their short generational times, they can produce large numbers of offspring, making them a good indicator of environmental change (Samways et al., 2010). Likewise, invertebrates provide a good study focus for measuring species richness (i.e., average species diversity within a site referred to as ‘alpha diversity’) and drawing comparisons between assemblage compositions found on different landscapes or treated mine-waste-contaminated sites (Gerlach et al., 2013). However, because of complexities associated with the taxonomic identification of invertebrates, they have not historically been used as a means of monitoring or assessing reclamation strategies. Recent progress in molecular identification techniques (deoxyribonucleic acid or DNA metabarcoding) has helped to overcome challenges in taxonomic identification of invertebrates. In this study, the

¹The lead author is a 2019 Geoscience BC Scholarship recipient.

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methods of assessing invertebrate-assemblage response to mine reclamation using DNA metabarcoding to identify invertebrates are examined.

Objectives

The aim of this study is to address research questions established on the basis of using parallel sequencing of invertebrate-community DNA metabarcodes as a new tool for examining reclamation trajectory. The first step entails assessing whether changes can be identified in invertebrate assemblages with different reclamation ages as a result of ongoing succession characterized by changes in biotic assemblages. Secondly, taxa will be examined to establish whether any display the effects of specific experimental factors (amendment, age).

Methodology

In 2017, four mines were sampled in BC: the Teck Resource Highland Valley Copper mine, the New Gold Inc. New Afton mine, the Imperial Metals Corporation Mount Polley mine and the Avino Silver & Gold Mines Ltd. Bralorne Gold mine (Figure 1). At each mine, two reclamation treatments were sampled: sites reclaimed within eight years ('new') and sites that were reclaimed more than eighteen years ago ('old'). A grassland 'control' site was also sampled at the Highland Valley Copper mine site. In this

paper, only data from the 'new' and 'old' Highland Valley Copper and New Afton sites, as well as the 'control' site, are examined. Site descriptions, including the year of reclamation, reclaimed materials and the methods used to conduct reclamation of the sampled areas, are presented in Table 1.

Vegetation data and invertebrate samples were collected from the Highland Valley Copper and New Afton sites. Vegetation was measured using canopy cover by species in 0.5 by 0.5 m quadrats. Invertebrate samples were collected using two types of traps: pitfall traps (Figure 2) and Malaise traps (Figure 3). Pitfall traps were used to collect primarily ground-dwelling invertebrates (Bassett and Fraser, 2015). At each site, a 100 m transect was laid out and pitfall traps were placed every 10 m. Pitfall traps were constructed using a 450 g container (Solo[®] cup) placed so that its top is level with the surface of the ground. The pitfall traps were filled with ethanol and a plastic plate, held up by nails, was placed over them to reduce the amount of ethanol evaporation. Malaise traps are tent-like structures, with bottles of ethanol attached to them and used to collect primarily flying invertebrates (Thomas, 2016). One Malaise trap was placed at each site. The invertebrate traps were left on site for five days once over the summer and samples recovered from them were stored at -20 °C until laboratory processing.

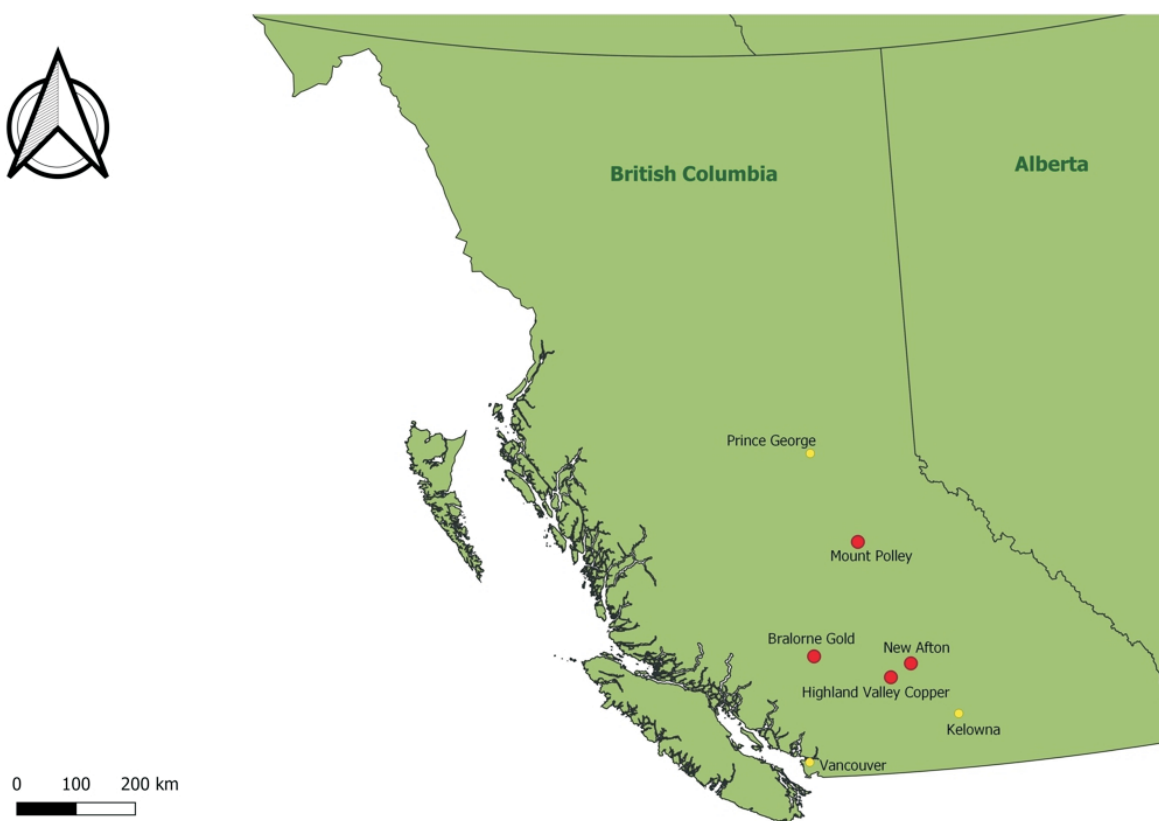


Figure 1. Location of mines in the study area sampled in July and August of 2017 and 2018, including Highland Valley Copper and New Afton, where vegetation and invertebrate data were collected for this paper.

In 2018, the New Afton and Highland Valley Copper sites were resampled. Vegetation and invertebrate sampling were conducted in the same manner as in 2017, except that it was conducted using a 40 m² grid instead of a 100 m transect.

Laboratory methods

Identification of the collected invertebrates was conducted using high-throughput DNA metabarcoding. This is done by homogenizing invertebrate tissue in liquid nitrogen using a mortar and pestle (Beng et al., 2016). The DNA was extracted from the homogenized tissue using a Mag-Bind[®] Blood and Tissue Kit from Omega Bio-tek Inc. A 450 base-pair region of the mitochondrial cytochrome c oxidase sub-unit 1 gene was amplified in two rounds of polymerase chain reaction (PCR) using the universal PCR primer pair MHeMf and dghHCO2198 (Beng et al., 2016). The amplicons were sequenced on an Ion S5™ sequencing platform using an Ion 520™ and Ion 530™ Chip Kit. The bioinformatic pipeline, AMPtk, was used to cluster sequences into operational taxonomic units (OTUs) at an identity threshold of 97% (Palmer et al., 2018); one OTU represented the sequence of one species (Ji et al., 2013). Taxonomies were assigned to each OTU using the Barcode of Life Data System (BOLD) downloaded at the time of analysis (Yu et al., 2012).

Statistical Analysis

The 2017 Highland Valley Copper and New Afton data were analyzed using three types of statistical analyses: one-way analysis of variance (ANOVA), analysis of similarity percentages (SIMPER; Clarke, 1993) and nonmetric multidimensional scaling (NMDS). Data used in the NMDS and SIMPER analyses were rarified data subsampled to 10 000 reads and converted into presence-absence data. The NMDS diagram was created using Euclidean distances among the five sites (Highland Valley Copper ‘control’, Highland Valley Copper ‘old’, Highland Valley Copper ‘new’, New Afton ‘old’, New Afton ‘new’) being compared. Calculations carried out using SIMPER were based on the Bray-Curtis dissimilarity index. One-way ANOVA was used to compare species richness between the sites. Log-transformed data were tested for normality, using the Shapiro-Wilk test, and for homogeneity, using the Bartlett’s test. There was a gap in New Afton ‘old’ data as a result of samples still being processed for sequencing; therefore, the mean of the five sites was calculated and applied to

Table 1. Site description (years since reclamation from 2019, reclaimed materials and reclamation methods) of the two mines (Highland Valley Copper and New Afton) sampled for invertebrates and vegetation data in summer of 2017.

Mine	Site	Year reclaimed	Years since reclamation	Reclaimed material	Reclamation method
Highland Valley Copper	New	2014	5	Waste rock and overburden	Biosolids
	Old	2000	19	Waste rock and overburden	Seeded (crested wheatgrass)
	Control	n/a	n/a	n/a	n/a
New Afton	New			Tailings	
	Old	2001	18	Tailings	Fertilizer and cattle manure



Figure 2. Pitfall trap set up in 2017 to collect epigeal invertebrates in the study area.



Figure 3. Malaise trap constructed in 2017 to capture flying invertebrates in the study area.

the sixth sample when performing the ANOVA test to measure species richness. All the statistical analyses were conducted in RStudio (RStudio, 2015), a free, open-source integrated development environment for the R software system for statistical computing.

Results

The NMDS diagram illustrates distinct clustering between sample sites; however, the Highland Valley Copper ‘control’ and New Afton ‘new’ sites show distinct variation in invertebrate assemblages (Figure 4).

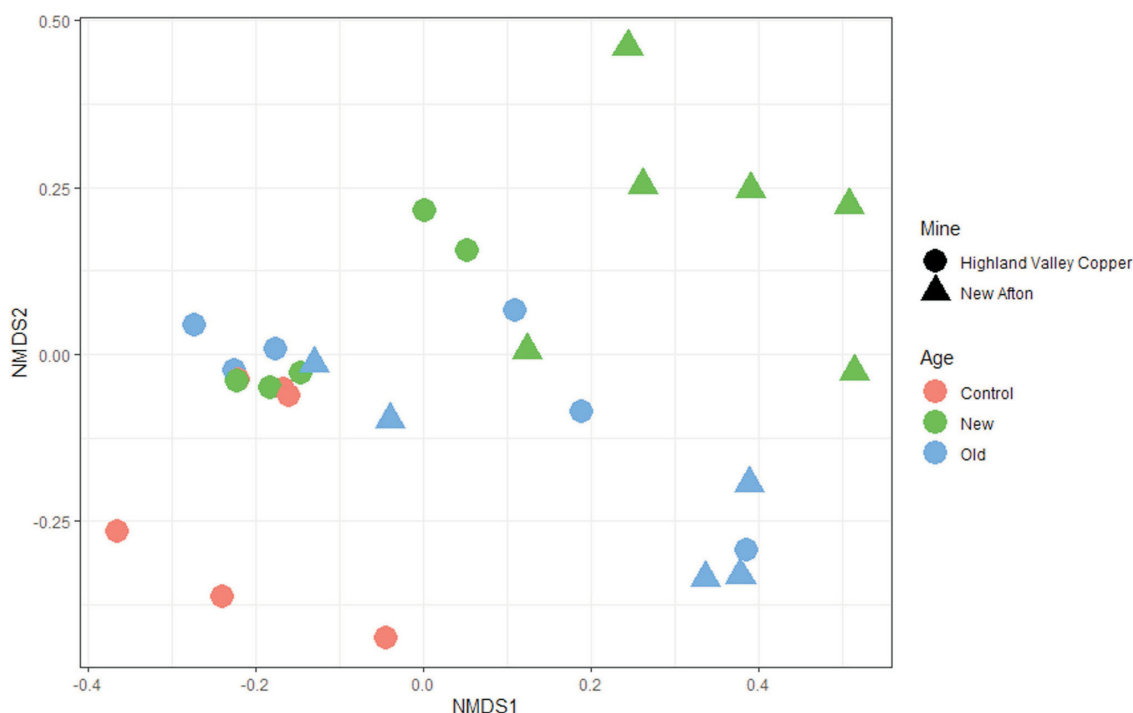


Figure 4. Nonmetric multidimensional scaling (NMDS) diagram created using Euclidian distance, illustrating invertebrate assemblages of different ages ('new', 'old', 'control') between 2017 samples from the Highland Valley Copper and New Afton study sites.

The SIMPER analysis describes the species that are responsible for the difference between the New Afton 'new' site and the Highland Valley Copper 'control' site. Figure 5 highlights the OTUs that define the cumulative top 20% contribution to the difference in invertebrate assemblage between sites. The invertebrate species that explain the distinction between sites are primarily *Hymenoptera* (order of insects that includes sawflies, wasps, bees and ants), *Trichoptera* (caddisfly) and *Phaonia apicalis* (Table 2). It should be noted that there are multiple OTUs annotated as *Phaonia apicalis*, which is a result of genetic variation within the species.

The results of an ANOVA test comparing species richness between sites revealed that there was no significant difference in species richness between sites ($p = 0.448$; Table 3). Figure 6 shows that the New Afton 'new' site had the lowest number of species, whereas the Highland Valley Copper 'old' site contained the highest number of species; however, the variability in species richness for each site was high.

Discussion

The effects of postmining reclamation on recovering invertebrate assemblages were examined in BC. Sequencing of invertebrate DNA metabarcodes showed differences in composition between mine sites and different age diagrams.

Invertebrate assemblage similarity of age diagrams

Understanding the reclamation trajectory of fauna assemblages postmine reclamation is an important objective on the road to achieving a successful end land use. In this study, a separation between age sites ('control', 'old', 'new') is visible in the NMDS diagram (Figure 4). Most notably, there is a difference between the Highland Valley Copper 'control' site and the New Afton 'new' site, indicating separate invertebrate assemblages between three age plots. This could, in part, be due to a body of water near the New Afton 'new' site, which is unique to that site. Furthermore, a small cluster of 'old' sites (both Highland Valley Copper and New Afton) formed together (Figure 4). It should be noted that the New Afton sites are also located on a historic mine-tailings storage facility. Comparatively, the Highland Valley Copper treatment sites are located on wasterock and overburden, whereas the Highland Valley Copper 'control' site is located near the road in a fenced enclosure.

Invertebrate taxa characterizing reclamation age diagrams

Invertebrate DNA metabarcode analysis can be used to generate a profile for all the site assemblages; however, the focus of this study is on the recognition of species that differentiate sites; more specifically, on the most noticeable difference between the Highland Valley Copper 'control'

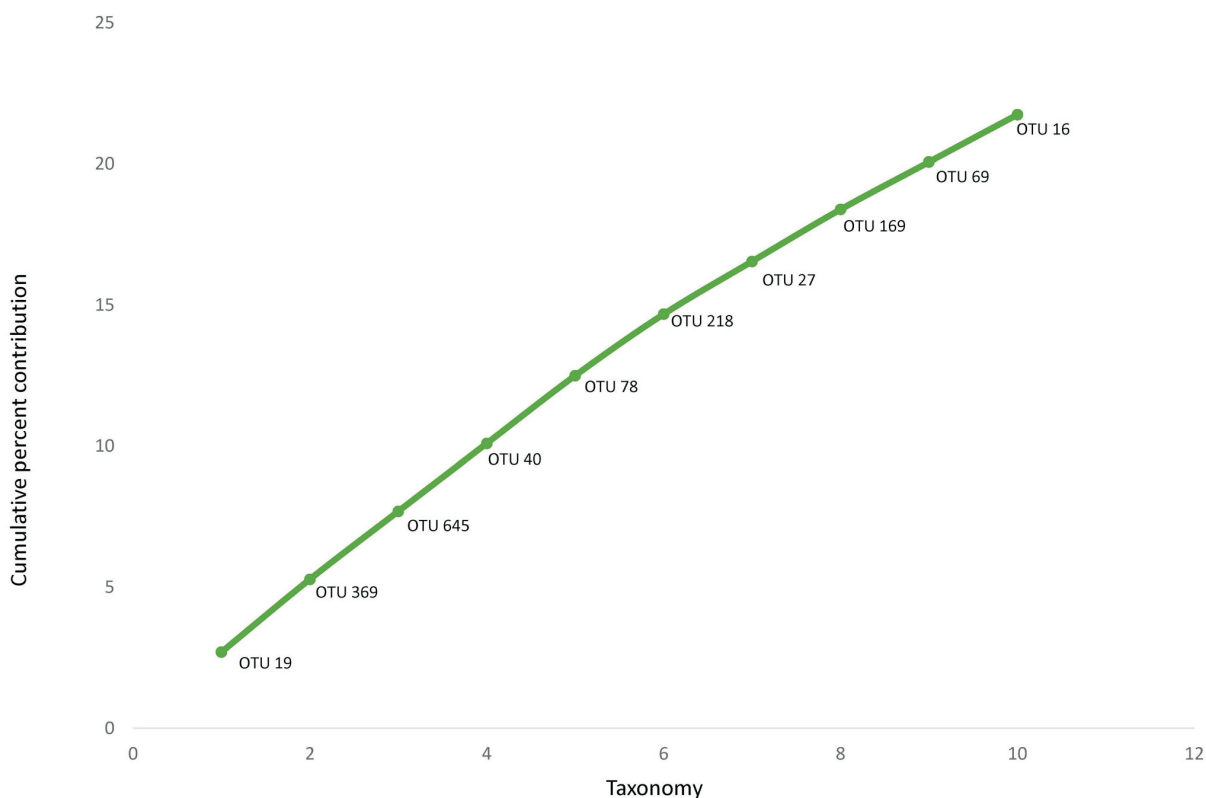


Figure 5. The top ten operational taxonomic units (OTU) explaining variation in community composition between the New Afton 'new' and Highland Valley Copper 'control' sites, as determined by SIMPER analysis based on the Bray-Curtis index of dissimilarity between samples. These top ten species account for 20% of the difference observed between the two sites.

site and the New Afton 'new' site. The taxon that was the most responsible for the difference between sites was *Hymenoptera* (OTU 19; Figure 5), which is an order of invertebrates that includes wasps, sawflies, ants and bees. In this case, the OTU 19 taxa were found predominantly on the New Afton 'new' site. Another species accountable for disparity between the two sites is *Trichoptera* (OTU 369, OTU 40, OTU 78, OTU 169). Four of the ten taxa responsible for the greatest cumulative percent contribution to assemblage difference between the two sites were *Trichoptera* taxa. The multiple OTUs may comprise multiple

species or genetic variations of caddisflies. The trichopteran taxa were found primarily in the New Afton 'new' site. Interestingly, *Trichoptera* are used as a bioindicator for good water quality (Pereira et al., 2012) and their presence on the New Afton 'new' site is likely explained by the aforementioned body of water in the vicinity.

Species richness characterizing age diagrams

Data showed no significant difference with regard to species richness between the sampled sites. Despite the New Afton 'new' site appearing to have fewer species detected on site, the variation between the replicates was too great to identify a significant result.

Table 2. List of species responsible for the top 20% of the difference in invertebrate assemblages recorded between the New Afton 'new' site and the Highland Valley Copper 'control' site.

OTU	Taxonomy
OTU 19	<i>Hymenoptera</i>
OTU 369	<i>Trichoptera</i>
OTU 645	<i>Phaonia apicalis</i>
OTU 40	<i>Trichoptera</i>
OTU 78	<i>Trichoptera</i>
OTU 218	<i>Phaonia apicalis</i>
OTU 27	<i>Phaonia apicalis</i>
OTU 169	<i>Trichoptera</i>
OTU 69	<i>Phloeostiba lapponica</i>
OTU 16	<i>Suillia nemorum</i>

Table 3. One-way analysis of variance results, comparing species richness defined by the number of operational taxonomic units between each of the five study sites: Highland Valley Copper 'new', 'old' and 'control' sites, and New Afton 'new' and 'old' sites, where 'f' represents the ratio of the two mean-square values and 'p' is the probability of finding the obtained results given that the null hypothesis is true. Abbreviation: d.f., degrees of freedom.

Species richness	d.f.	Mean square	F-value	P-value
Mine sites	4	0.7923	0.958	0.448

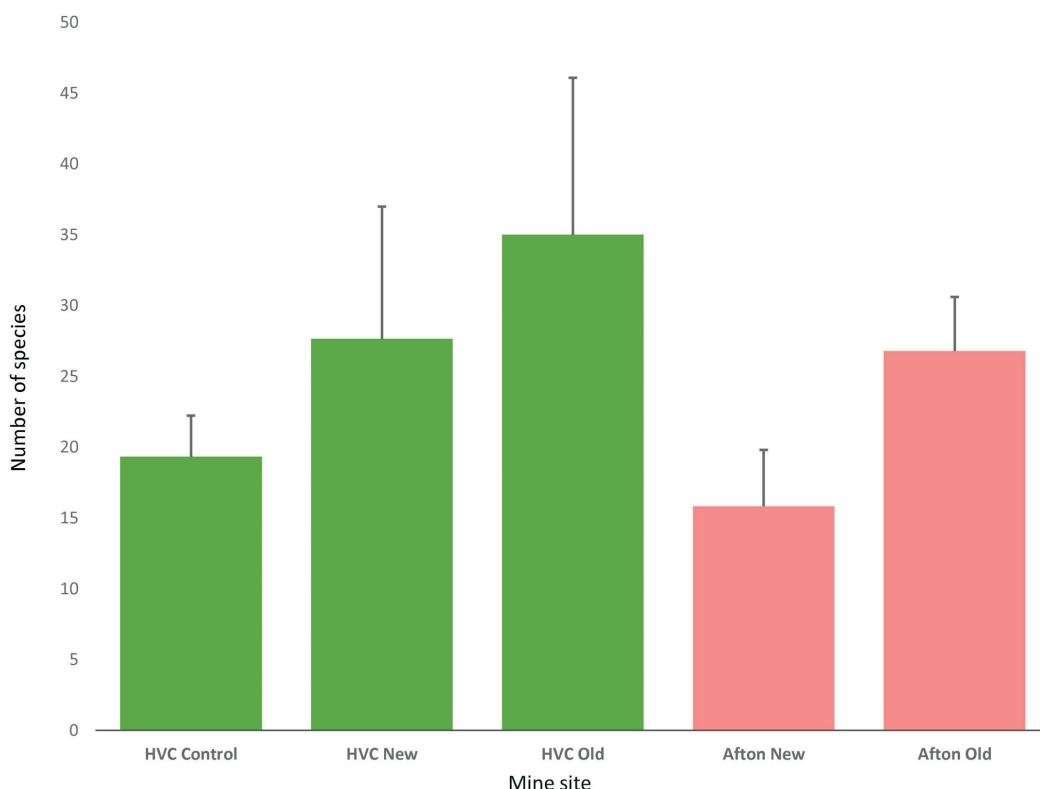


Figure 6. Distribution of species richness between the Highland Valley Copper (HVC) 'new', 'old', 'control' and New Afton (Afton) 'new', 'old' study sites.

Conclusions and Ongoing Work

The above results and correlations are based on data from 2017; additional analyses are underway as the remaining 2017 samples are currently being sequenced. The 2018 samples are also being sequenced and future papers will address the effects of biosolids as a soil amendment.

This study should significantly enhance the knowledge base guiding responsible development and reclamation of mineral extraction sites, factors that are important for the continued growth of the economy and the protection of the environment. Future studies identifying the environmental variables associated with invertebrate recovery may help land managers facilitate restoration through simulation of the relevant conditions. Further development of DNA metabarcoding will make monitoring reclamation via invertebrates more assessable.

Acknowledgments

Funding for this project is provided through Geoscience BC as well as through the Natural Sciences and Engineering Research Council of Canada Industrial Research Chair in Ecosystem Reclamation, with industry partners: Genome BC, Arrow Transportation Systems Inc., the Real Estate Foundation of BC, New Afton mine, Highland Valley Copper mine, Kinder Morgan Canada Limited, Metro Vancou-

ver and the British Columbia Cattlemen's Association. Special thanks to the student researchers who helped in the field and laboratory: J. Foster, B. McAmmond, C. Stephens, M. Coghill, J. Paulson and S. Vogel. The authors also thank peer reviewer A. Fischer.

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