Microbiome preliminary analysis

**Relative Abundance:**

All test samples together

*Phylum level*

Chart, bar chart

Description automatically generated

**Figure 1:** Mean relative abundance (%) of bacterial phyla composing more than 5% of total ASVs within aquatic macroinvertebrate and spider subjects at each sampling site. Sites are ordered based on flow of the wastewater treatment plants with Cochrane being the most upstream site on the river and Policeman Flats being the farthest downstream. Number of samples for aquatic macroinvertebrates are as follows: Cochrane (n = 54), Sunalta (n = 31), Cushing Bridge (n = 50), Graves Bridge (n = 32), Policeman Flats (n = 56), BRR2 (n = 8), PCR1 (n = 24), PCR3 (n = 23). Spiders had a consistent sample size of 16 across all sites.

‘Other’ category is at the bottom:

Chart, bar chart

Description automatically generated

**Figure 2:** Mean relative abundance (%) of the top four bacterial phyla within all aquatic macroinvertebrates and spiders collected from each sampling site. Number of samples for aquatic macroinvertebrates are as follows: Cochrane (n = 54), Sunalta (n = 31), Cushing Bridge (n = 50), Graves Bridge (n = 32), Policeman Flats (n = 56), BRR2 (n = 8), PCR1 (n = 24), PCR3 (n = 23). Spiders had a consistent sample size of 16 across all sites.

‘Other’ category is in the middle allowing a baseline for comparing Proteobacteria:

Chart, bar chart

Description automatically generated

Other

*Proteobacteria*

**Figure 3:** Mean relative abundance (%) of the top four bacterial phyla within all aquatic macroinvertebrates and spiders collected from each sampling site. Number of samples for aquatic macroinvertebrates are as follows: Cochrane (n = 54), Sunalta (n = 31), Cushing Bridge (n = 50), Graves Bridge (n = 32), Policeman Flats (n = 56), BRR2 (n = 8), PCR1 (n = 24), PCR3 (n = 23). Spiders had a consistent sample size of 16 across all sites.

*Family level*

Chart

Description automatically generated

**Figure 4:** Mean relative abundance (%) of bacterial families that comprise more than 10% of total ASVs within aquatic macroinvertebrates and spiders across all sampling locations. Number of samples for aquatic macroinvertebrates are as follows: Cochrane (n = 54), Sunalta (n = 31), Cushing Bridge (n = 50), Graves Bridge (n = 32), Policeman Flats (n = 56), BRR2 (n = 8), PCR1 (n = 24), PCR3 (n = 23). Spiders had a consistent sample size of 16 across all sites.

Chart, bar chart

Description automatically generated

**Figure 5:** Mean relative abundance (%) of the top 12 bacterial families within aquatic macroinvertebrates and spiders across all sampling locations. Number of samples for aquatic macroinvertebrates are as follows: Cochrane (n = 54), Sunalta (n = 31), Cushing Bridge (n = 50), Graves Bridge (n = 32), Policeman Flats (n = 56), BRR2 (n = 8), PCR1 (n = 24), PCR3 (n = 23). Spiders had a consistent sample size of 16 across all sites.

Blank Samples

*Phylum Level*

Chart, bar chart

Description automatically generated

**Figure 6:** Mean relative abundance (%) of bacterial phyla comprising more than 2% of total ASVs from water, mesh, and sheet blanks. Cushing Bridge (n = 3), PCR1 (n = 3 water blank, 3 mesh blank), Cochrane (n = 2), Policeman Flats (n = 2).

Chart, bar chart

Description automatically generated

**Figure 7:** Mean relative abundance (%) of the top four most abundant bacterial phyla from water, mesh, and sheet blanks. Cushing Bridge (n = 3), PCR1 (n = 3 water blank, 3 mesh blank), Cochrane (n = 2), Policeman Flats (n = 2).

*Family Level*

Chart, bar chart

Description automatically generated

**Figure 8:** Mean relative abundance (%) of the top five most abundant bacterial families from water, mesh, and sheet blanks. Cushing Bridge (n = 3), PCR1 (n = 3 water blank, 3 mesh blank), Cochrane (n = 2), Policeman Flats (n = 2).

Larval Hydropsychids

*Phylum Level*

Chart, bar chart

Description automatically generated

**Figure 9:** Mean relative abundance (%) of the top five most abundant bacterial phyla from larval Hydropsychidae at each sampling site. Cochrane (n = 8), Sunalta (n = 7), Cushing Bridge (n = 5), Graves Bridge (n = 8), Policeman Flats (n = 8), BRR2 (n = 8), PCR1 (n = 8), PCR3 (n = 8).

Larval Heptageniidae

Chart, bar chart

Description automatically generated

**Figure 10:** Mean relative abundance (%) of the top four most abundant bacterial phyla from larval Heptageniidae at each sampling site (n = 8 larvae/site).

Chironomidae (adults + larvae)

Chart, bar chart

Description automatically generated

**Figure 11:** Mean relative abundance (%) of the top four most abundant bacterial phyla from Chironomidae at each sampling site (Cochrane: n = 14, Cushing Bridge: n = 16, Policeman Flats: n = 16).

Perlidae (larvae)

Chart, bar chart

Description automatically generated

**Figure 12:** Mean relative abundance (%) of the top four most abundant bacterial phyla from larval Perlidae individuals at each sampling site (Cochrane: n = 8, Cushing Bridge: n = 5, Policeman Flats: n = 8).