Bow River Microbiome - Wastewater Derived and Endosymbiont Bacteria

***Wastewater Derived Bacteria***

Wastewater effluent bacteria/pathogens were filtered from the dataset based on common genera identified from the following papers (Cyprowski et al., 2018; Kristensen et al., 2020; Leight et al., 2018; Millar et al., 2022; Restivo et al., 2021)

effluent\_bac<-subset\_taxa(sample\_ps, Genus=="**Bifidobacterium**" | Genus=="**Clostridium**" | Genus=="**Propionibacterium**" | Genus=="**Peptostreptococcus**" | Genus=="**Actinomyces**" | Genus=="**Arcobacter**" | Genus=="**Ruminococcus**" | Genus=="**Escherichia coli**" | Genus=="**Campylobacter**" | Genus=="**Salmonella**" | Genus=="**Shigella**" | Genus=="**Trichococcus**" | Genus=="**Streptococcus**" | Genus=="**Blautia**" | Genus=="**Enterococcus**" | Genus=="**Vibrio vulnificus**" | Genus=="**Aeromonas**" | Genus=="**Legionella**" | Genus=="**Fusibacter**" | Genus=="**Bacillus**" | Genus=="**Romboutsia**" | Genus=="**Cloacibacterium**" | Genus=="**Pseudomonas**" | Genus=="**Acetoanaerobium**" | Genus=="**Acidaminococcus**" | Genus=="**Aquimonas**" | Genus=="**AUTHM297**" | Genus=="**Bact-08**" | Genus == "**BD1-7 clade**" | Genus=="**C1-B045**" | Genus=="**Candidatus Cloacimonas**" | Genus=="**Candidatus Paenicardinium**" | Genus=="**Candidatus Protochlamydia**" | Genus=="**Chiayiivirga**" | Genus=="**Desulfobacter**" | Genus=="**Flavitalea**" | Genus=="**Lelliottia**" | Genus=="**Leptotrichia**" | Genus=="**Mesotoga**" | Genus=="**Neochlamydia**" | Genus=="**Ottowia**" | Genus=="**Planctopirus**" | Genus=="**Prevotellaceae UCG-004**" | Genus=="**Proteiniclasticum**" | Genus=="**SC103**" | Genus=="**Steroidobacter**" | Genus=="**Succinivibrio**" | Genus=="**SWB02**" | Genus=="**Thermovirga**" | Genus=="**Turneriella**" | Genus=="**U29-B03**" | Genus=="**XBB1006**" | Genus==”**Shewanella**” Genus=="**Sphingobacterium**")

effluent\_bac #433 taxa

Relative Abundance of common effluent derived bacteria across sites:

Chart, bar chart

Description automatically generated

**Figure 1:** Mean relative abundance of common wastewater derived bacterial families in all invertebrate and spider samples.

* Policeman Flats has the highest mean relative abundance of wastewater derived bacteria, closely followed by Cushing Bridge and Cochrane. Does not seem to be influenced by the amount of wastewater exposure.
* PCR3 has a slightly higher relative abundance than PCR1 but both effluent input streams are higher than the control (BRR2).

Chart, bar chart

Description automatically generated

**Figure 2:** Mean relative abundance of common wastewater derived bacterial genera in all invertebrate and spider samples.

* High abundance of *Romboutsia* (common anaerobic gut bacteria found in wastewater)
* Increased abundance of *Trichococcus* at Cushing Bridge relative to other sites.
* Increased abundance of *Clostridium* at Policeman Flats

Timeline

Description automatically generated with medium confidence

**Figure 3:** Mean relative abundance of common wastewater derived bacterial genera in all invertebrate and spider samples separated by taxonomic order.

* Seems like Araneae are relatively robust to changes in abundance across sites (most amount of change at Graves Bridge potential due to an increase in nitrogen enrichment?) but most other taxa have effluent bacterial compositions that are very different among sites.

Top 5 most abundant effluent derived bacteria across sites:

Chart, bar chart

Description automatically generated

**Figure 4:** Mean relative abundance of top 5 most abundant common wastewater derived bacterial genera in all invertebrate and spider samples.

* Romboutsia was increased at Policeman Flats and PCR1/PCR3.
* Increased Enterococcus and Aeromonas at Policeman Flats
* Increased Trichococcus at Cushing Bridge
* Increased Candidatus Paenicardinium at Cochrane

***Endosymbiont bacteria***

ASVs were filtered for endosymbionts at the genus level based on Millar et al., 2022; Perrotta et al., 2022

endo<-subset\_taxa(sample\_ps, Genus=="**Buchnera**" | Genus=="**Candidatus**" | Genus=="**Hamiltonella**" | Genus=="**Rickettsia**" | Genus=="**Rickettsiella**" | Genus =="**Wolbachia**" | Genus=="**Spiroplasma**" | Genus=="**Candidatus\_Cardinium**" | Genus=="**Arsenophonus**")

endo #143 taxa, 265/340 samples

Chart, bar chart

Description automatically generated

**Figure 1:** Mean relative abundance of endosymbiont genera from the microbiome of all invertebrate and spider samples. Cochrane (n = 70), Sunalta (n = 47), Cushing Bridge (n = 66), Graves Bridge (n = 48), Policeman Flats (n = 72), BRR2 (n = 8), PCR1 (n = 24), PCR3 (n = 23).

* Cushing Bridge has the highest mean relative abundance of endosymbiont bacteria.
* Rickettsiella is only present at Cushing Bridge.
* Spiroplasma slightly increases at Policeman Flats but is not found in ACWA streams.
* Not much change in Wolbachia except none found in organisms from BRR2.
* Not much change in Rickettsia across sites.

Bar chart

Description automatically generated with medium confidence

**Figure 2:** Mean relative abundance of endosymbiont genera from the microbiome of all invertebrate and spider samples separated by taxonomic order. Araneae (n = 80), Diptera (n = 46), Ephemeroptera (n = 111), Tricoptera (n = 100). Araneae (n = 80), Diptera (n = 46), Ephemeroptera (n = 111), Tricoptera (n = 100).

* Decrease in Wolbachia in Diptera and Tricoptera
* Presence of Rickettsiella in Diptera and Tricoptera but not Ephemeroptera or Araneae
* No Buchnera in Tricoptera
* Increase in Rickettsia in Tricoptera
* Arsenophonus only found in Araneae

Chart, bar chart

Description automatically generated

**Figure 3:** Mean relative abundance of endosymbiont genera from the microbiome of all invertebrate and spider samples separated by taxonomic order and collection site. Araneae (n = 80), Diptera (n = 46), Ephemeroptera (n = 111), Tricoptera (n = 100).

* Arsenophonus only found (in very small percent) in spiders at Graves Bridge.
* Increase in Buchnera at Graves Bridge in spiders
* Presence of Rickettsiella and Buchnera in dipterans at Cushing Bridge
* Presence of Buchnera in ephemeroptera at Cushing Bridge
* Increase in Wolbachia in ephemeroptera at Cochrane and PCR3
* Presence of Spiroplasma and Rickettsiella in Tricoptera at Cushing Bridge