Stable Isotope Analysis Preliminary Results:

Use PERMaova (adonis) to see if there’s a statistical difference in carbon/nitrogen values between basal food source and primary consumer.

*How do you input the data into Adonis? What columns of data do you need?*

*Questions:*

*-Is the nitrogen enrichment at Graves Bridge enough to indicate that there is an influence of wastewater exposure on the food web structure in the Bow River?*

*Can the mixing model data and nitrogen enrichment trend answer the question of if it is possible for effects of wastewater exposure to be transferred to riparian ecosystems? I know this will depend on what we see in the microbiome data as well but the results don’t look very clear cut from the stable isotopes alone.*

1. **Checking for indication of carbonates (inorganic carbon)** in biofilm samples by plotting %C versus δ13C. If there is a positive trend, this would indicate carbonates.

HCl treated biofilm samples:

A picture containing graphical user interface

Description automatically generated

Untreated biofilm samples:

Chart

Description automatically generated with low confidence

Trend is negative for both HCl treated and untreated biofilm samples, indicating no effect of carbonates.

1. **Checking for indication of effect of lipids** in samples by plotting C:N ratio versus δ13C. If there is a negative trend, this indicates influence of lipids.

C : N ratio versus δ13C of all invertebrate samples

Chart, scatter chart

Description automatically generated

There is a negative trend between C:N ratio at most sites indicating that there is a possibility of a lipid effect.

Also plot the Carbon : Nitrogen ratio of each taxa. This ratio should not exceed 3.5, otherwise this may indicate an effect of lipids.

Chart, box and whisker chart

Description automatically generated

Almost every taxon’s C:N ratio exceeds the 3.5 threshold (red line). Could indicate an effect of lipids. Correcting for this would not make much of a difference since they would all be corrected mostly by the same amount.

1. **Checking for effect of weigh paper/homogenizing method.** Duplicates were taken by crushing half the insect with an acid washed glass rod in a clean microcentrifuge tube and the other half in the weigh paper to see whether the weigh paper effect the carbon or nitrogen values of the insects.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample | Crushing method | d13C | RPD (%) | D15N | RPD (%) |
| SUNAL\_Araneid\_SI\_3\_V | Glass Rod (Vial) | -27.7 | 0 | 7.9 | 2.5 |
| SUNAL\_Araneid\_SI\_3\_WP | Weigh Paper | -27.7 | 8.1 |
| POLFLT\_Lar\_Stonefly\_1\_SI\_1\_V | Glass Rod (Vial) | -24 | 1.2 | 7.6 | 1.3 |
| POLFLT\_Lar\_Stonefly\_1\_SI\_1\_WP | Weigh Paper | -24.3 | 7.7 |

Does not look like there’s an effect of weigh paper (homogenizing method) on the carbon or nitrogen values since the relative percent differences between weigh paper and glass rod homogenizing methods are less than 2.5%.

1. **Checking raw data biplots**

Plotting δ13C versus δ15N with all inverts included in this plot. Seems like basal food sources separate out and insects are relying more on terrestrial derived sources (shrubs) than aquatic (biofilm) at most sites except Policeman Flats. Chart, scatter chart

Description automatically generated

1. **Checking averaged data biplots**

Plotting mean ± SD of δ13C versus δ15N at each site, coloured by taxa and shape of points are the functional feeding group. All inverts included in this plot. Same trend is seen here with all sites except PMF with inverts closer to the terrestrial carbon signal. Higher error at Graves Bridge and Policeman Flats.

Chart, scatter chart

Description automatically generated

Seems like inverts from Cochrane, Sunalta, and Cushing Bridge are feeding on more terrestrial derived food sources whereas inverts from Graves Bridge and Policeman Flats are feeding on both terrestrial and aquatic. Could this have something to do with higher wastewater exposure at the downstream locations? There is also greater error in the isotope values at Policeman Flats which may cause more overlapping on the biplot.

Another source of error could be the biofilm collected at Cochrane, Sunalta, and Cushing bridge versus Graves bridge and Policeman flats. The two latter sites had extremely green and stringy (filamentous) biofilm whereas the prior 3 sites had sludgy muddy grainy biofilm. We could potentially be missing a food source if this is the case.

I thought gatherer-collector and scraper-grazer inverts would be lower in δ15N than predators, but it does not look like there is much of a difference.

Separating out Graves Bridge:

Chart, box and whisker chart

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Separating out Policeman Flats:

Chart, box and whisker chart

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1. **Checking for Nitrogen Enrichment Across Sites (Wastewater Exposure)**

All inverts across all sites:

Chart, box and whisker chart

Description automatically generated

Looks like Graves Bridge has an increase in d15N compared to the other sites. This is interesting because that site was right below the wastewater outfall from Bonnybrook (how far exactly in km?). This could indicate a shift in food web structure due to municipal wastewater exposure. However, Policeman flats dips down again. Potentially from dissipation of wastewater effluent?

An ANOVA of d15N~Site indicated that Graves Bridge is significantly different (enriched in d15N) compared to all other sites.

Does this trend hold true when filtering out several invert taxa individually?

Larval Chloroperlidae: Larval Heptageniidae:

Chart, box and whisker chart

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Araneidae: Tetragnathidae:

Chart, box and whisker chart

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Yes, this trend does hold with most individual taxa.

1. **Running MixSIAR model**

*1st model: Using larval inverts as consumers and Biofilm/Shrubs as basal food sources.*

**Mix data** = consumers. Included the δ13C and δ15N values for each larval invertebrate taxa at all 5 sites.

Site and Taxa were both random factors and Taxa was nested within Site.

*Can Taxa (family of invert) be a random factor here, or is it already incorporated into the model? I’ve seen people use ‘Individual’ to capture individual variation in organisms but the variable would have to be SAMPLE\_ID instead of family in that case I think.*

*What other variable could I use in the model to help explain variation in diet source contribution?*

**Source data** = Sources. Included the mean and standard deviation δ13C and δ15N values for basal food sources (biofilm and terrestrial shrubs) with Site as a random factor. I only included the non-treated biofilm since there was not much of an effect of HCl treated biofilm on the carbon values. I excluded the HCl treated biofilm from the source because there was no effect on the d13C value between treated and untreated biofilm.

**Discrimination factor** = Trophic Enrichment Factor. Used mean, SD of δ13C as 1.3, 0.4 and mean, SD of δ15N as 3.4, 1. Based on Post, D. (2002). Ecology.

Variable Diagnostics:

# source$data\_type: means

# source$by\_factor: 1

# random effects: 2

# fixed effects: 0

# nested factors: FALSE FALSE

# factors: Site Taxa

# continuous effects: 0

# error structure: Residual \* Process

# source$conc\_dep: FALSE

**Isospace plot for larvae consumers (stoneflies included):**

Chart

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**Model characteristics:**

I used the ‘normal’ run settings:

run<-list(chainLength=100000, burn=50000, thin=50, chains=3, calcDIC=TRUE) with a non-informative prior (alpha.prior=1).

**Diagnostics/summary statistics:**

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Shows the variation in source contributions. Blue = shrub, red = biofilm. Shows that variation in biofilm is much greater than shrub and shrub consists of a much larger percent source contribution.

**Gelman-Rubin (looks good here)**

Generally the Gelman diagnostic should be < 1.05

Out of **72** variables: 10 > 1.01, 1 > 1.05, 1 > 1.1

**Heidelberger and Welch Diagnostic (looks okay here)**

Chain 1 Chain 2 Chain 3

Stationarity 0 2 0

Half-width 26 29 26

**Geweke (out of 72), Looks okay here**

Chain 1 Chain 2 Chain 3

Geweke 2 4 5

**Summary statistics:**

Relative influence of Site and Taxa on the variance in consumer diet:

Mean (SD) for Site variable: 11.7 (4.97)

Mean (SD) for Taxa variable: 3.0 (4.13)

Indicates that the Site that organisms were collected at has more of an influence on the variation in diet of consumers than the family identity of the consumer.

Because stoneflies shift from feeding on algae/detritus in early instars to being carnivorous predators at later instars, I ran models with them both included and excluded.

Mean resource use of larval consumers (%), excluding stoneflies (all sites combined):

|  |  |  |  |
| --- | --- | --- | --- |
| Family | Mean Biofilm | Mean Shrub | Standard Deviation |
| Chironomidae | 32.0 | 68.0 | 33.6 |
| Hydropsychidae | 27.5 | 72.5 | 31.5 |
| Heptageniidae | 33.5 | 66.5 | 34.0 |
| Ephemerellidae | 36.7 | 63.3 | 34.6 |

|  |  |  |  |
| --- | --- | --- | --- |
| Site | Mean Biofilm | Mean Shrub | Standard Deviation |
| Cochrane | 2.8 | 97.2 | 13.3 |
| Sunalta | 2.2 | 97.8 | 11.7 |
| Cushing Bridge | 3.9 | 96.1 | 16.0 |
| Graves Bridge | 1.4 | 98.6 | 8.8 |
| Policeman Flats | 65.3 | 34.7 | 29.9 |

Looks like most larvae are feeding on terrestrial derived food sources at most sites except Policeman Flats where there is a mixed source contribution of consumers. The errors (SDs) are very high though, so I am not very confident in this analysis.

Mean resource use of larval consumers (%), including stoneflies (all sites combined):

|  |  |  |  |
| --- | --- | --- | --- |
| Family | Mean Biofilm | Mean Shrub | Standard Deviation |
| Chironomidae | 35.3 | 64.7 | 31.9 |
| Hydropsychidae | 32.6 | 67.4 | 31.1 |
| Heptageniidae | 44.8 | 55.2 | 33.4 |
| Ephemerellidae | 45.3 | 54.7 | 33.3 |
| Chloroperlidae | 35.0 | 65.0 | 33.3 |
| Perlidae | 18.0 | 82.0 | 23.6 |

|  |  |  |  |
| --- | --- | --- | --- |
| Site | Mean Biofilm | Mean Shrub | Standard Deviation |
| Cochrane | 0.7 | 99.3 | 5.1 |
| Sunalta | 0.3 | 99.7 | 3.4 |
| Cushing Bridge | 0.4 | 99.6 | 4.2 |
| Graves Bridge | 0.4 | 99.6 | 3.8 |
| Policeman Flats | 40.7 | 59.3 | 27.1 |

Seems like there is even less of a contribution of biofilm when stonefly are included in analysis.

*What are the next steps for this analysis? Should I run another one with aquatic adult inverts as one source, terrestrial inverts as a second source, and the two spider families as the consumers?*