Hypothesis and Statistics

DIDER

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Contents

1 Research Question

1. Which macro invertebrates exhibit the same behavioral responses or traits?

2. How does the presence of ALAN and crayfish affect the distribution of macro invertebrate biological traits compared to the control group?

3. What alterations occur in the biological trait composition of macro invertebrates as a result of the combined presence of ALAN and crayfish, in comparison to the control group?

2 Hypothesis

1. The introduction of Artificial Light at Night (ALAN) significantly increases feeding and dispersal behavior of macroinvertebrates.

2. The presence of ALAN elicits a substantial reduction in the locomotion behavior of macroinvertebrates.

3. ALAN induces notable modifications in the voltinism and locomotion behavior of macroinvertebrates.

4. The presence of crayfish can significantly decrease feeding, dispersal and reproduction traits of macroinvertebrates compared to the control group.

5. The presence of crayfish can significantly alter the locomotion and voltinism traits of macroinvertebrates compared to the control group.

3 Statistical Method

3.1 Data preperation procedure to ease statistical analysis

3.2 Trait data generation by using species abundance data

The Tachet database, available at freshwaterecology.info website, was used Tachet et al. [8] to assign traits to macroinvertebrates. This database uses fuzzy coding to organize traits into 21 categories with 113 options. In the first step of statistical analysis, the trait data was collected for each species in the taxonomic datasheet using the `assign_traits` function in the `biomonitoR` package. The `aggregate_taxa` function was used to group taxa at different taxonomic levels Laini et al. [4].

N.B: *Elmis*(larvae), *Limnius*(larvae), *Elmis*(adult), *Liminus*(adult), *Hydraenidae*(adult) were excluded from the data table.

The `assign_traits` function creates a data frame that includes the names and taxonomic levels of the species in the taxonomic dataset, as well as the names and levels of related species in the trait dataset. It also shows the taxonomic distance between these species and their traits. Tachet et al. [8]

In the next step, `average_traits` function was used to calculate the average traits for each species, which will be used in further statistical analysis. Laini et al. [4]

In order to conduct further statistical analysis, the trait categories were standardized to ensure that the total sum within each trait (such as feeding) equaled 1. This standardization process ensured that all taxa (individual units of analysis) carried equal weight in the subsequent statistical analysis. Calapez et al. [1]

3.3 Calculating dissimilarity matrix

The function `gowdis` from the `FD` package was used to calculate the dissimilarity matrix of traits for macroinvertebrates, to answer research question 1. This function calculates Gower [2] similarity coefficient, which is accurately implemented using the procedure described by Podani [7]. The similarity value is then subtracted from 1 to create the dissimilarity coefficient (i.e., D = 1 - S), which is then used to compare two data sets. The function also includes variable weights according to the methodology described by Legendre and Legendre [5].

The feeding behavior of *Baetidae* and *Psychodidae* differs by 2.9% of the maximum possible difference. This indicates that these two species have similar feeding behaviors. This allows us to answer the first research question.

To examine and visualize the difference in traits among species, Principal Coordinate Analysis (PCoA) was performed. Before conducting the PCoA, the dissimilarity matrix underwent a square root transformation

```
biomonitor_data <- as_biomonitor(Final_data_NZ, traceB = TRUE)
biomonitor_data
#> $taxa_db
#>         Phylum      Class   Subclass           Order         Family Subfamily
#> 1  Arthropoda    Insecta             Ephemeroptera       Baetidae
#> 2  Arthropoda    Insecta                   Diptera Ceratopogonidae
#> 3  Arthropoda    Insecta                   Diptera    Chironomidae
#> 4  Arthropoda    Insecta             Ephemeroptera   Ephemerellidae
#> 5  Arthropoda    Insecta                   Diptera      Ephydridae
#> 6  Arthropoda   Crustacea                                Gammaridae
#> 7  Arthropoda    Insecta             Ephemeroptera    Heptageniidae
#> 8  Arthropoda    Insecta                Trichoptera   Hydropsychidae
#> 9  Arthropoda    Insecta             Ephemeroptera Leptophlebiidae
#> 10 Arthropoda    Insecta                   Diptera       Limoniidae
#> 11   Annelida Clitellata Oligochaeta
#> 12 Arthropoda    Insecta                   Diptera      Pediciidae
#> 13 Arthropoda    Insecta                   Diptera      Psychodidae
#> 14 Arthropoda    Insecta                Trichoptera   Rhyacophilidae
#> 15 Arthropoda    Insecta                   Diptera        Tipulidae
#>     Tribus   Genus Species Subspecies            Taxa Crayfish_ALAN Crayfish
#> 1                                          Baetidae          239      192
#> 2                                     Ceratopogonidae           15       15
#> 3                                        Chironomidae        36768    17810
#> 4                                       Ephemerellidae          36       30
#> 5                                         Ephydridae            4        4
#> 6            Gammarus                        Gammarus         3389     1472
#> 7                                       Heptageniidae            4        4
#> 8                                       Hydropsychidae           1        0
#> 9                                      Leptophlebiidae           1        1
#> 10                                        Limoniidae            6        5
#> 11                                        Oligochaeta          246      136
#> 12                                         Pediciidae            3        3
#> 13                                         Psychodidae           0        0
#> 14                                       Rhyacophilidae           0        0
#> 15                                          Tipulidae            9        7
```

Figure 1: Biomonitor data

```
taxa_aggregation <- aggregate_taxa(biomonitor_data)
taxa_aggregation
#> $Phylum
#>        Phylum Crayfish_ALAN Crayfish  ALAN Control
#> 1    Annelida           246      136   110     406
#> 2 Arthropoda         40475    19543 20932   19906
#>
#> $Class
#>        Class Crayfish_ALAN Crayfish  ALAN Control
#> 1 Clitellata           246      136   110     406
#> 2  Crustacea          3389     1472  1917    2038
#> 3    Insecta         37086    18071 19015   17868
#>
#> $Subclass
#>      Subclass Crayfish_ALAN Crayfish  ALAN Control
#> 1  unassigned         40475    19543 20932   19906
#> 2 Oligochaeta           246      136   110     406
#>
#> $Order
#>         Order Crayfish_ALAN Crayfish  ALAN Control
#> 1    unassigned         3635     1608  2027    2444
#> 2       Diptera        36805    17844 18961   17725
#> 3 Ephemeroptera          280      227    53     142
#> 4   Trichoptera            1        0     1       1
#>
#> $Family
#>           Family Crayfish_ALAN Crayfish  ALAN Control
#> 1      unassigned           246      136   110     406
#> 2        Baetidae           239      192    47     111
#> 3  Ceratopogonidae           15       15     0       7
#> 4     Chironomidae        36768    17810 18958   17710
#> 5    Ephemerellidae           36       30     6      28
#> 6       Ephydridae            4        4     0       0
#> 7       Gammaridae         3389     1472  1917    2038
#> 8     Heptageniidae            4        4     0       3
#> 9    Hydropsychidae            1        0     1       0
#> 10  Leptophlebiidae            1        1     0       0
#> 11       Limoniidae            6        5     1       1
#> 12       Pediciidae            3        3     0       0
#> 13      Psychodidae            0        0     0       4
#> 14    Rhyacophilidae            0        0     0       1
#> 15        Tipulidae            9        7     2       3
#>
```

Figure 2: Taxa aggregation 1

4

```
#> $Subfamily
#>    Subfamily Crayfish_ALAN Crayfish  ALAN Control
#> 1 unassigned         40721    19679 21042  20312
#>
#> $Tribus
#>       Tribus Crayfish_ALAN Crayfish  ALAN Control
#> 1 unassigned         40721    19679 21042  20312
#>
#> $Genus
#>        Genus Crayfish_ALAN Crayfish  ALAN Control
#> 1 unassigned         37332    18207 19125  18274
#> 2   Gammarus          3389     1472  1917   2038
#>
#> $Species
#>      Species Crayfish_ALAN Crayfish  ALAN Control
#> 1 unassigned         40721    19679 21042  20312
#>
#> $Subspecies
#>   Subspecies Crayfish_ALAN Crayfish  ALAN Control
#> 1 unassigned         40721    19679 21042  20312
#>
#> $Taxa
#>            Taxon Crayfish_ALAN Crayfish  ALAN Control
#> 1        Baetidae           239      192    47    111
#> 2  Ceratopogonidae           15       15     0      7
#> 3     Chironomidae        36768    17810 18958  17710
#> 4    Ephemerellidae          36       30     6     28
#> 5       Ephydridae           4        4     0      0
#> 6         Gammarus        3389     1472  1917   2038
#> 7    Heptageniidae           4        4     0      3
#> 8    Hydropsychidae          1        0     1      0
#> 9   Leptophlebiidae          1        1     0      0
#> 10      Limoniidae           6        5     1      1
#> 11     Oligochaeta         246      136   110    406
#> 12      Pediciidae           3        3     0      0
#> 13     Psychodidae           0        0     0      4
#> 14  Rhyacophilidae           0        0     0      1
#> 15       Tipulidae           9        7     2      3
#>
```

Figure 3: Taxa aggregation 2

```
#> $Tree
#>        Phylum      Class   Subclass         Order       Family Subfamily
#> 1  Arthropoda    Insecta              Ephemeroptera       Baetidae
#> 2  Arthropoda    Insecta                     Diptera Ceratopogonidae
#> 3  Arthropoda    Insecta                     Diptera    Chironomidae
#> 4  Arthropoda    Insecta              Ephemeroptera   Ephemerellidae
#> 5  Arthropoda    Insecta                     Diptera      Ephydridae
#> 6  Arthropoda  Crustacea                                  Gammaridae
#> 7  Arthropoda    Insecta              Ephemeroptera    Heptageniidae
#> 8  Arthropoda    Insecta                 Trichoptera   Hydropsychidae
#> 9  Arthropoda    Insecta              Ephemeroptera  Leptophlebiidae
#> 10 Arthropoda    Insecta                     Diptera      Limoniidae
#> 11    Annelida Clitellata Oligochaeta
#> 12 Arthropoda    Insecta                     Diptera      Pediciidae
#> 13 Arthropoda    Insecta                     Diptera      Psychodidae
#> 14 Arthropoda    Insecta                 Trichoptera   Rhyacophilidae
#> 15 Arthropoda    Insecta                     Diptera       Tipulidae
#>      Tribus  Genus Species Subspecies            Taxa Crayfish_ALAN Crayfish
#> 1                                            Baetidae           239      192
#> 2                                     Ceratopogonidae            15       15
#> 3                                        Chironomidae         36768    17810
#> 4                                      Ephemerellidae            36       30
#> 5                                          Ephydridae             4        4
#> 6           Gammarus                        Gammarus          3389     1472
#> 7                                       Heptageniidae             4        4
#> 8                                      Hydropsychidae             1        0
#> 9                                     Leptophlebiidae             1        1
#> 10                                        Limoniidae             6        5
#> 11                                       Oligochaeta           246      136
#> 12                                         Pediciidae             3        3
#> 13                                        Psychodidae             0        0
#> 14                                     Rhyacophilidae             0        0
#> 15                                          Tipulidae             9        7
#>      ALAN Control
#> 1      47     111
#> 2       0       7
#> 3   18958   17710
#> 4       6      28
#> 5       0       0
#> 6    1917    2038
#> 7       0       3
#> 8       1       0
#> 9       0       0
#> 10      1       1
#> 11    110     406
#> 12      0       0
#> 13      0       4
```

Figure 4: Taxa aggregation 3

```
traitscailing_data_av <- average_traits(traitscailing_data)
traitscailing_data_av
```

FEEDING_2 <dbl>	FEEDING_3 <dbl>	FEEDING_4 <dbl>	FEEDING_5 <dbl>	FEEDING_6 <dbl>	FEEDING_7 <dbl>	FEEDING_8 <dbl>	LOCOMOTION_1 <dbl>
0.2850340	0.09183673	0.5517007	0.0000000	0.0	0.07142857	0.00000000	0
0.3333333	0.25000000	0.4166667	0.0000000	0.0	0.00000000	0.00000000	0
0.1678571	0.06428571	0.3214286	0.1178571	0.0	0.24285714	0.08571429	0
0.3611111	0.22222222	0.3055556	0.0000000	0.0	0.11111111	0.00000000	0
0.1000000	0.10000000	0.3000000	0.3000000	0.1	0.00000000	0.10000000	0
0.0000000	0.75000000	0.2500000	0.0000000	0.0	0.00000000	0.00000000	0
0.1333333	0.14666667	0.7200000	0.0000000	0.0	0.00000000	0.00000000	0
0.0000000	0.00000000	0.0000000	0.7500000	0.0	0.25000000	0.00000000	0
0.3166667	0.43888889	0.2444444	0.0000000	0.0	0.00000000	0.00000000	0
0.2666667	0.26666667	0.2000000	0.0000000	0.0	0.26666667	0.00000000	0

1-10 of 14 rows | 62-69 of 114 columns   Previous **1** 2 Next

Figure 5: Average trait data

to enhance the properties of the dissimilarity. This transformation was carried out using the `dudi.pco` function, which facilitated the subsequent PCoA analysis and provided a more informative representation of trait variation across species. Gower [3]

The species which are closer in the figure have more similar traits (for example *Tipulidae*, *Limoniidae*, *Ephemerellidae*, *Ceratopogonidae* must be very similar between them on the basis of their feeding behavior). Please be noted that, the first two axes of the PCoA computed 56% variablity of the whole dataset.

3.4   Community Weighted Mean (CWM)

Prior to calculating the community weighted mean (CWM), the macroinvertebrate data was log transformed. This transformation was used to normalize abundance values for subsequent analysis. To calculate the CWM, the total abundance of macroinvertebrates was computed for each treatment(ALAN + crayfish, ALAN, crayfish, control). The relative abundance of each macroinvertebrate within each treatment was also calculated. Then, the abundance value of each macroinvertebrate in a given treatment group was divided by the total abundance of that treatment. Finally, the trait values were multiplied by the relative abundance of macroinvertebrates for each treatment. To perform this entire calculation process, the `functcomp` function from the `FD` package was used to ensure an efficient and standardized approach to CWM calculation and trait analysis. Miller, Damschen, and Ives [6]
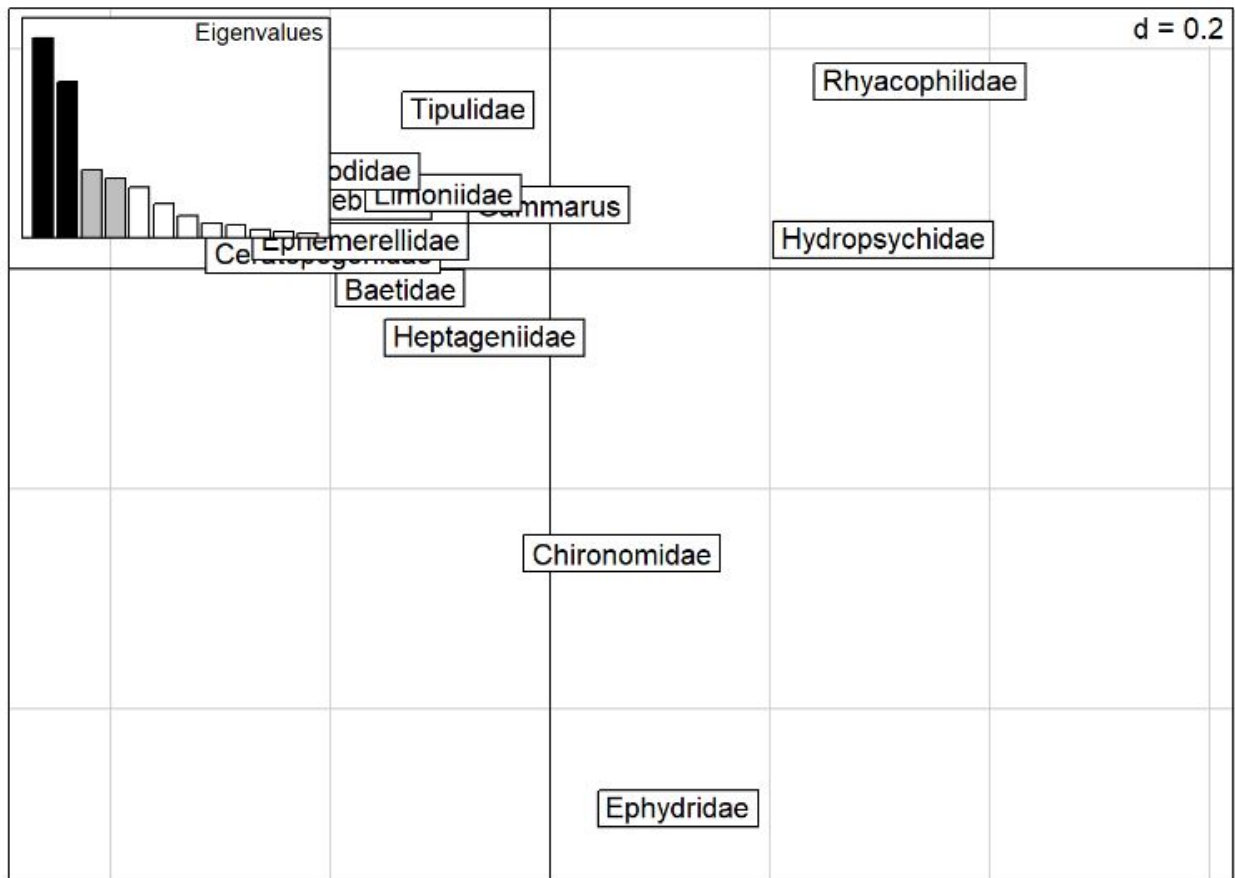
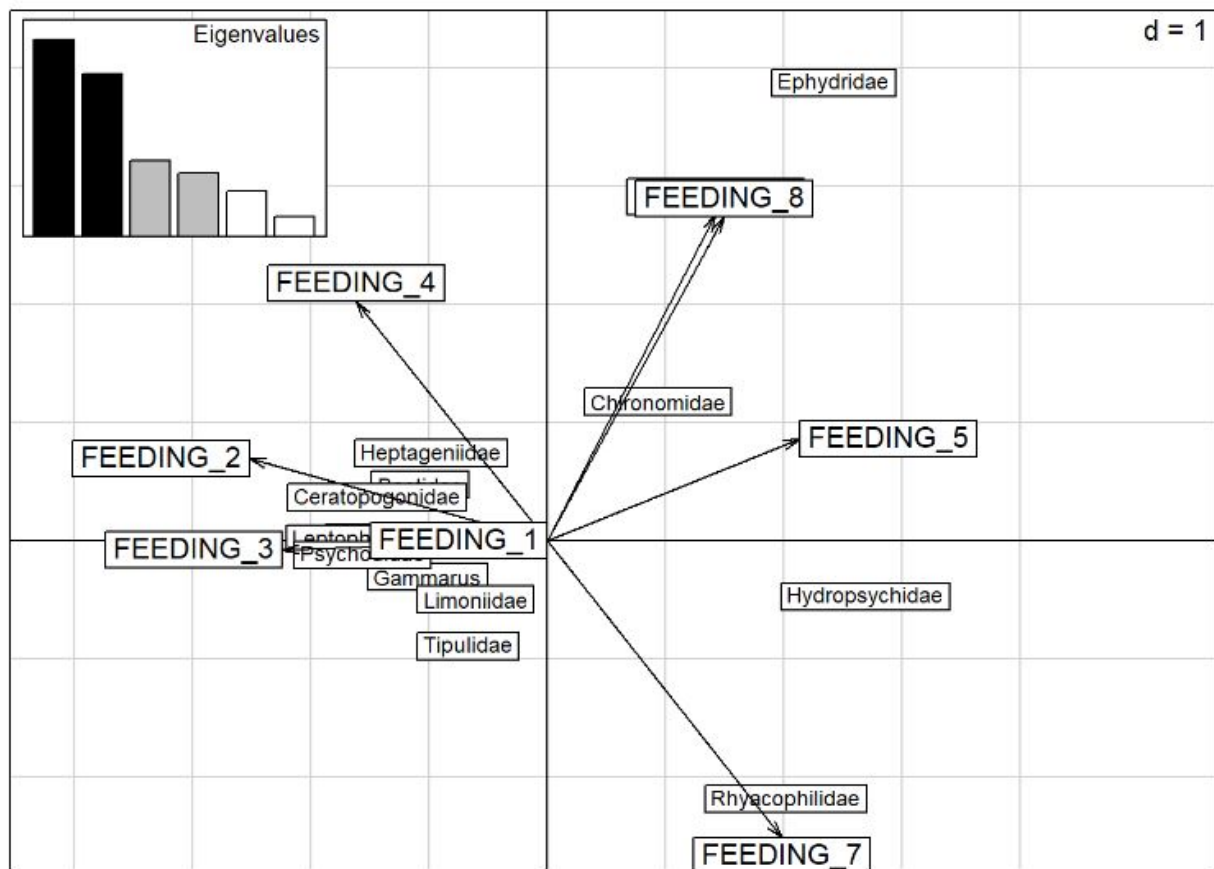Figure 6: Trait dissimilarity on the basis of feeding habit

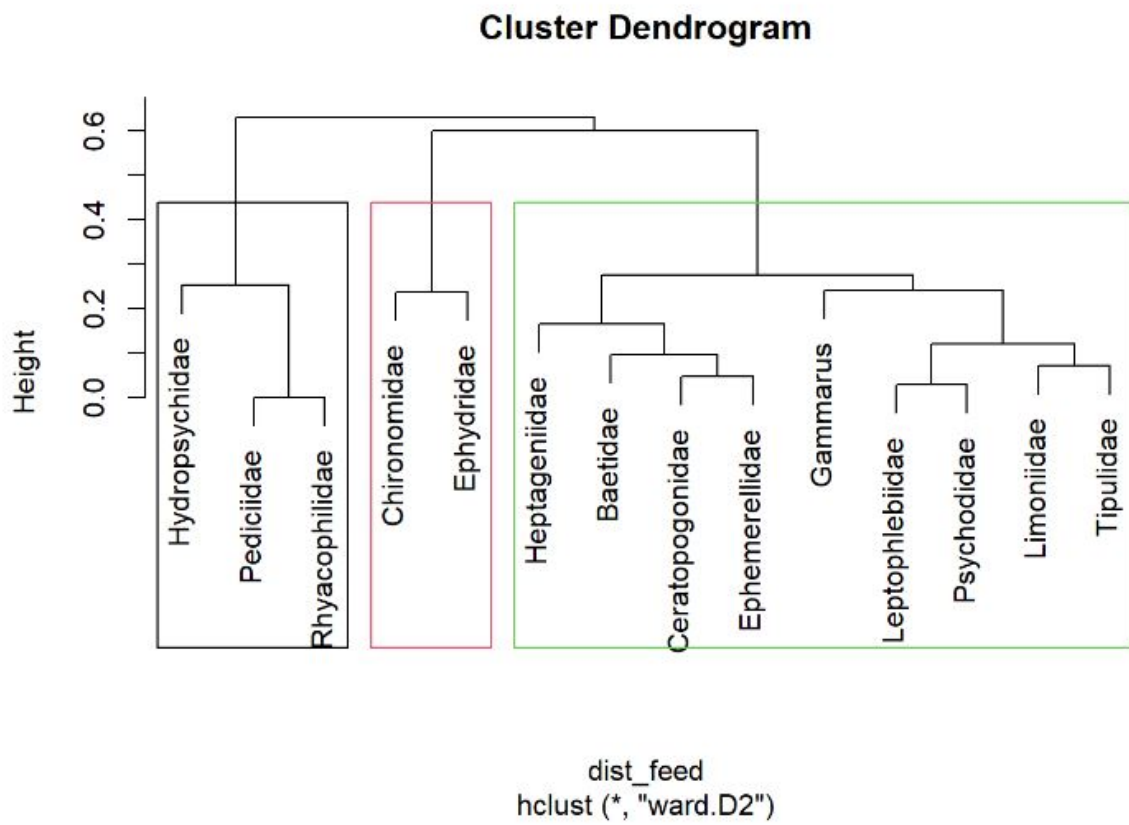Figure 7: Trait dissimilarity on the basis of feeding habit

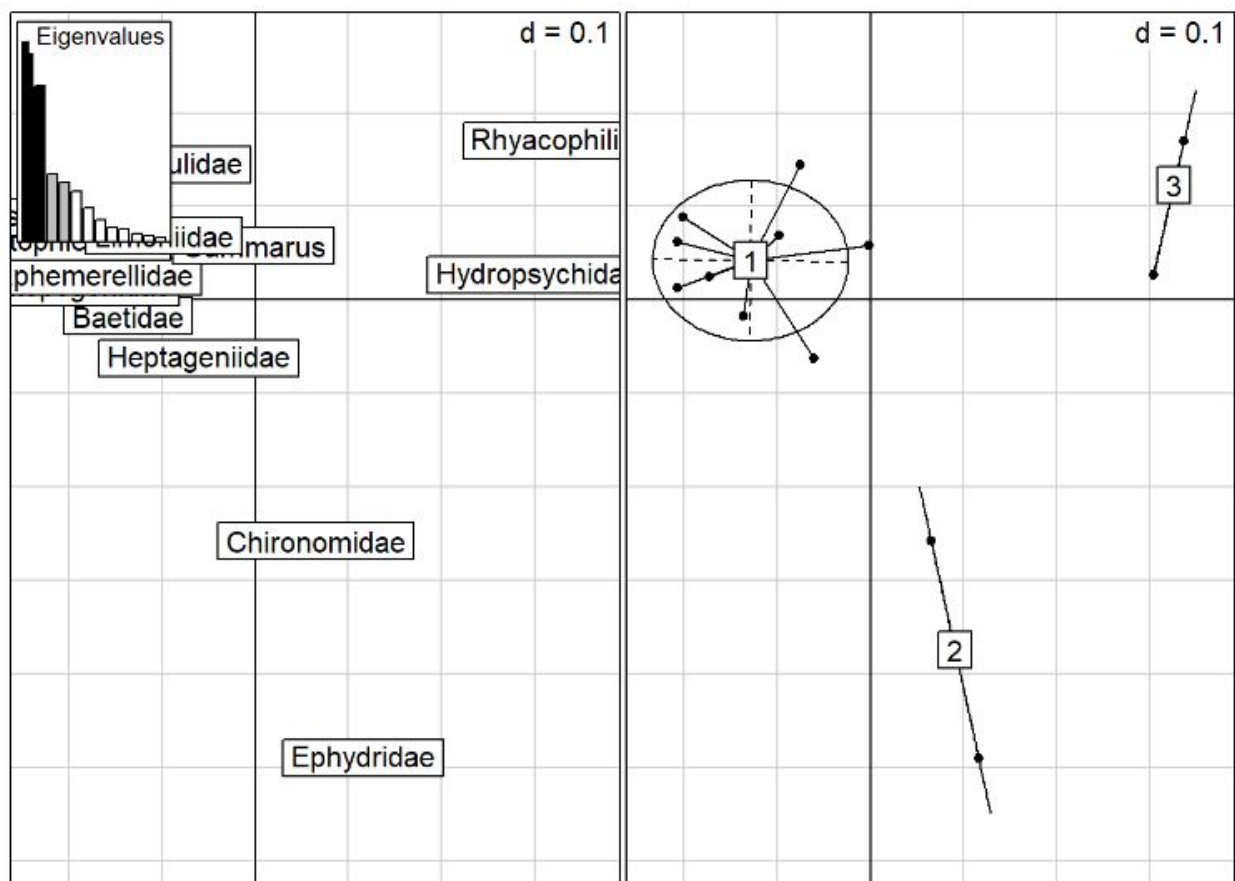## Cluster Dendrogram

Figure 8: Cluster dendogram on the basis of feeding habit

Figure 9: Classification of macroinvertebrates on the basis of feeding habit

Mean	ALAN	0.2500000
	Control	0.2500000
	Crayfish	0.2500000
	Crayfish_ALAN	0.2500000
Std. error mean	ALAN	0.1144833
	Control	0.1156088
	Crayfish	0.1146436
	Crayfish_ALAN	0.1142577
Median	ALAN	0.1461458
	Control	0.1583755
	Crayfish	0.1665743
	Crayfish_ALAN	0.1645448
Standard deviation	ALAN	0.3238077
	Control	0.3269911
	Crayfish	0.3242612
	Crayfish_ALAN	0.3231697
Variance	ALAN	0.1048514
	Control	0.1069232
	Crayfish	0.1051453
	Crayfish_ALAN	0.1044387

Figure 10: Descriptive statistics for feeding

Mean	ALAN	0.3750000
	Control	0.3750000
	Crayfish	0.3750000
	Crayfish_ALAN	0.3750000
Std. error mean	ALAN	0.1441583
	Control	0.1465960
	Crayfish	0.1461920
	Crayfish_ALAN	0.1458318
Median	ALAN	0.1977487
	Control	0.1754713
	Crayfish	0.1983959
	Crayfish_ALAN	0.1958617
Standard deviation	ALAN	0.4077412
	Control	0.4146361
	Crayfish	0.4134934
	Crayfish_ALAN	0.4124745
Variance	ALAN	0.1662529
	Control	0.1719231
	Crayfish	0.1709768
	Crayfish_ALAN	0.1701352

Figure 11: Descriptive statistics for locomotion

Mean	ALAN	0.4285714
	Control	0.4285714
	Crayfish	0.4285714
	Crayfish_ALAN	0.4285714
Std. error mean	ALAN	0.1577485
	Control	0.1582108
	Crayfish	0.1561325
	Crayfish_ALAN	0.1561674
Median	ALAN	0.2460989
	Control	0.2539430
	Crayfish	0.2606209
	Crayfish_ALAN	0.2612344
Standard deviation	ALAN	0.4173634
	Control	0.4185864
	Crayfish	0.4130878
	Crayfish_ALAN	0.4131801
Variance	ALAN	0.1741922
	Control	0.1752145
	Crayfish	0.1706415
	Crayfish_ALAN	0.1707178

Figure 12: Descriptive statistics for size

Mean	ALAN	0.2500000
	Control	0.2500000
	Crayfish	0.2500000
	Crayfish_ALAN	0.2500000
Std. error mean	ALAN	0.05387595
	Control	0.04782616
	Crayfish	0.04548066
	Crayfish_ALAN	0.04529542
Median	ALAN	0.2350439
	Control	0.2575094
	Crayfish	0.2827167
	Crayfish_ALAN	0.2810054
Standard deviation	ALAN	0.1077519
	Control	0.09565233
	Crayfish	0.09096131
	Crayfish_ALAN	0.09059084
Variance	ALAN	0.01161047
	Control	0.009149368
	Crayfish	0.008273960
	Crayfish_ALAN	0.008206701

Figure 13: Descriptive statistics for dispersal

3.5 Descriptive statistics

3.5.1 Descriptive statistics for feeding

3.5.2 Descriptive statistics for locomotion

3.5.3 Descriptive statistics for size

3.5.4 Descriptive statistics for dispersal

3.5.5 Descriptive statistics for voltinism

3.5.6 Descriptive statistics for reproduction

3.6 Nonparametric Test (Kruskal-Wallis test)

In order to fulfill the assumption of the significance test, a non-parametric test was employed since the weighted average trait values of macroinvertebrates did not exhibit a normal distribution. This non-parametric test was conducted to address the remaining research questions and determine whether our hypotheses were supported or not. The Kruskal-Wallis test was utilized to calculate the statistical significance of the differences among the median values of all traits for macroinvertebrates across the four different treatment groups (ALAN+Crayfish, Crayfish, Control).

Mean	ALAN	0.3333333
	Control	0.3333333
	Crayfish	0.3333333
	Crayfish_ALAN	0.3333333
Std. error mean	ALAN	0.1799921
	Control	0.1636274
	Crayfish	0.1580681
	Crayfish_ALAN	0.1575922
Median	ALAN	0.3561590
	Control	0.4183149
	Crayfish	0.4808876
	Crayfish_ALAN	0.4753677
Standard deviation	ALAN	0.3117554
	Control	0.2834109
	Crayfish	0.2737820
	Crayfish_ALAN	0.2729577
Variance	ALAN	0.09719145
	Control	0.08032173
	Crayfish	0.07495661
	Crayfish_ALAN	0.07450592

Figure 14: Descriptive statistics for voltinism

Mean	ALAN	0.2500000
	Control	0.2500000
	Crayfish	0.2500000
	Crayfish_ALAN	0.2500000
Std. error mean	ALAN	0.1128231
	Control	0.1119263
	Crayfish	0.1101700
	Crayfish_ALAN	0.1103802
Median	ALAN	0.1574041
	Control	0.1685779
	Crayfish	0.1625564
	Crayfish_ALAN	0.1579120
Standard deviation	ALAN	0.3191120
	Control	0.3165754
	Crayfish	0.3116079
	Crayfish_ALAN	0.3122022
Variance	ALAN	0.1018325
	Control	0.1002200
	Crayfish	0.09709950
	Crayfish_ALAN	0.09747024

Figure 15: Descriptive statistics for reproduction

3.6.1 Kruskal-Wallis test for feeding

3.6.2 Kruskal-Wallis test for locomotion

3.6.3 Kruskal-Wallis test for size

3.6.4 Kruskal-Wallis test for dispersal

3.6.5 Kruskal-Wallis test for voltinism

3.6.6 Kruskal-Wallis test for reproduction

3.7 Significance Test (Dunn's Test)

Dunn's test was performed to examine the statistical significance of the differences between each treatment group through pairwise comparisons. To visualize the results, data visualization was conducted using **RStudio**.

3.7.1 Significance test for feeding

![[Boxplot to visualize the significance levels of feeding habits across different treatments.
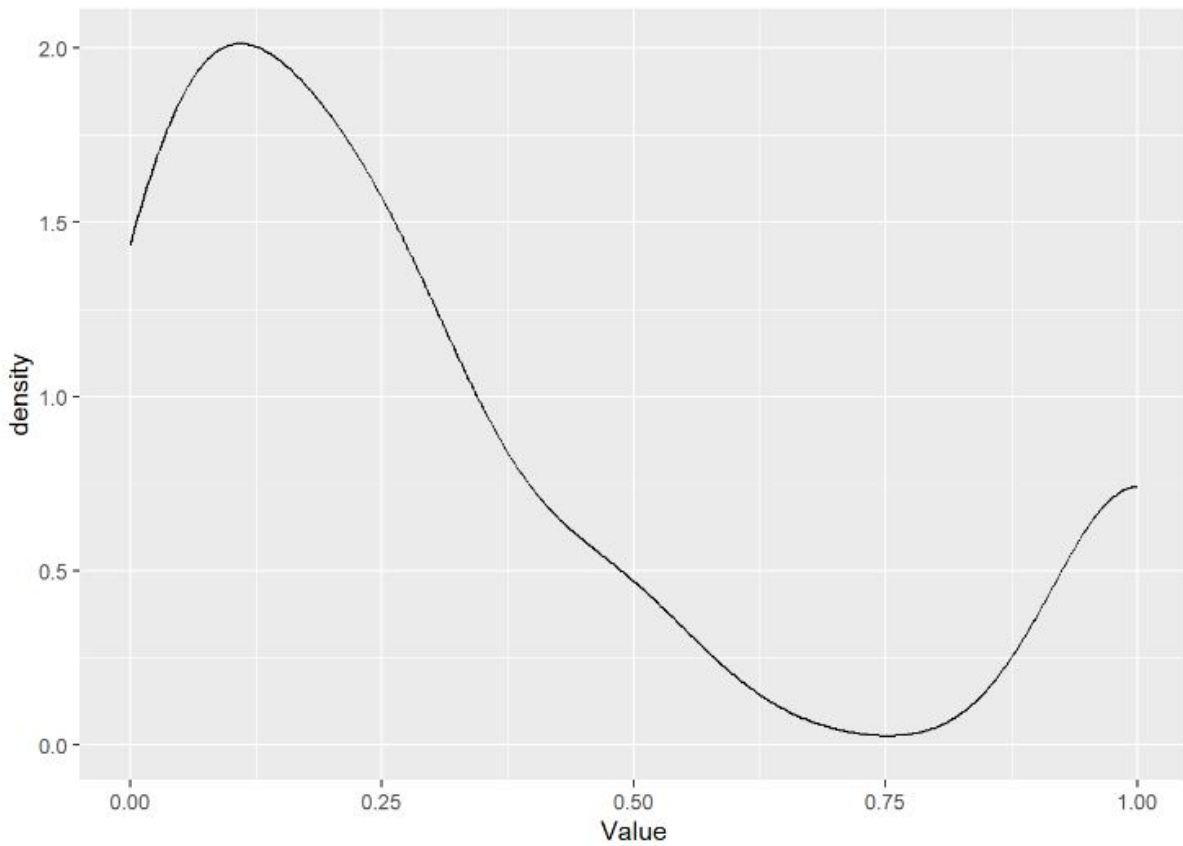
Figure 16: Density plot for weighted average trait values of macroinvertebrates

```
kruskal.test(Value ~ Treatment, data = FEEDING_trait)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.0092516, df = 3, p-value = 0.9998
```

Figure 17: Kruskal-Wallis test for feeding

```
kruskal.test(Value ~ Treatment, data = LOCOMOTION_trait)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.014427, df = 3, p-value = 0.9995
```

Figure 18: Kruskal-Wallis test for locomotion

```
kruskal.test(Value ~ Treatment, data = SIZE_trait)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.036735, df = 3, p-value = 0.9981
```

Figure 19: Kruskal-Wallis test for size

```
kruskal.test(Value ~ Treatment, data = DISPERSAL_trait)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.022059, df = 3, p-value = 0.9991
```

Figure 20: Kruskal-Wallis test for dispersal

```
kruskal.test(Value ~ Treatment, data = VOLTINISM_trait)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.12821, df = 3, p-value = 0.9883
```

Figure 21: Kruskal-Wallis test for voltinism

```
kruskal.test(Value ~ Treatment, data = REPRODUCTION_trait)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.046961, df = 3, p-value = 0.9973
```

Figure 22: Kruskal-Wallis test for reproduction

```
dunnTest(Value ~ Treatment,
         data=FEEDING_trait,
         method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>              Comparison          Z    P.unadj P.adj
#> 1        ALAN - Control  0.05335399 0.9574499     1
#> 2       ALAN - Crayfish -0.04001549 0.9680808     1
#> 3    Control - Crayfish -0.09336948 0.9256100     1
#> 4   ALAN - Crayfish_ALAN -0.01333850 0.9893577    1
#> 5 Control - Crayfish_ALAN -0.06669249 0.9468265    1
#> 6 Crayfish - Crayfish_ALAN  0.02667700 0.9787174  1

t.test1 <- FEEDING_trait %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test1
```

Figure 23: Significance test for feeding

```
dunnTest(Value ~ Treatment,
         data=LOCOMOTION_trait,
         method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>               Comparison          Z    P.unadj P.adj
#> 1          ALAN - Control 0.02685764 0.9785733     1
#> 2          ALAN - Crayfish 0.08057292 0.9357816    1
#> 3       Control - Crayfish 0.05371528 0.9571620    1
#> 4     ALAN - Crayfish_ALAN 0.10743056 0.9144474    1
#> 5  Control - Crayfish_ALAN 0.08057292 0.9357816    1
#> 6 Crayfish - Crayfish_ALAN 0.02685764 0.9785733    1

t.test2 <- LOCOMOTION_trait %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test2
```

Figure 24: Significance test for locomotion

3.7.2 Significance test for locomotion

![Boxplot to visualize the significance levels of locomotion trait across different treatments.

3.7.3 Significance test for size

```
dunnTest(Value ~ Treatment,
         data=SIZE_trait,
         method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>               Comparison           Z    P.unadj P.adj
#> 1          ALAN - Control   0.03286993 0.9737783     1
#> 2          ALAN - Crayfish -0.13147971 0.8953958     1
#> 3       Control - Crayfish -0.16434964 0.8694559     1
#> 4     ALAN - Crayfish_ALAN -0.09860978 0.9214481     1
#> 5  Control - Crayfish_ALAN -0.13147971 0.8953958     1
#> 6 Crayfish - Crayfish_ALAN  0.03286993 0.9737783     1

t.test3 <- SIZE_trait %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test3
```

Figure 25: Significance test for size

![Boxplot to visualize the significance levels of size trait across different treatments.

3.7.4 Significance test for dispersal

![Boxplot to visualize the significance levels of dispersal trait across different treatments.

```
dunnTest(Value ~ Treatment,
         data=DISPERSAL_trait,
         method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>              Comparison          Z   P.unadj P.adj
#> 1        ALAN - Control  0.07426107 0.9408027     1
#> 2        ALAN - Crayfish  0.14852213 0.8819307     1
#> 3     Control - Crayfish  0.07426107 0.9408027     1
#> 4   ALAN - Crayfish_ALAN  0.07426107 0.9408027     1
#> 5 Control - Crayfish_ALAN  0.00000000 1.0000000     1
#> 6 Crayfish - Crayfish_ALAN -0.07426107 0.9408027     1

t.test4 <- DISPERSAL_trait %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test4
```

Figure 26: Significance test for dispersal

3.7.5 Significance test for voltinism

```
dunnTest(Value ~ Treatment,
         data=VOLTINISM_trait,
         method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>               Comparison          Z   P.unadj P.adj
#> 1         ALAN - Control -0.1132277 0.9098500     1
#> 2        ALAN - Crayfish -0.2264554 0.8208472     1
#> 3     Control - Crayfish -0.1132277 0.9098500     1
#> 4   ALAN - Crayfish_ALAN -0.3396831 0.7340952     1
#> 5 Control - Crayfish_ALAN -0.2264554 0.8208472     1
#> 6 Crayfish - Crayfish_ALAN -0.1132277 0.9098500     1

t.test5 <- VOLTINISM_trait %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test5
```

Figure 27: Significance test for voltinism

![Boxplot to visualize the significance levels of voltinism trait across different treatments.

3.7.6 Significance test for reproduction

![Boxplot to visualize the significance levels of reproduction trait across different treatments.

3.7.7 Significance test for all traits together

![Boxplot to visualize the significance levels of all traits across different treatments.

```
dunnTest(Value ~ Treatment,
        data=REPRODUCTION_trait,
        method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>                Comparison           Z    P.unadj P.adj
#> 1          ALAN - Control -0.13337273 0.8938986     1
#> 2          ALAN - Crayfish -0.21339637 0.8310178    1
#> 3        Control - Crayfish -0.08002364 0.9362185   1
#> 4     ALAN - Crayfish_ALAN -0.13337273 0.8938986    1
#> 5  Control - Crayfish_ALAN  0.00000000 1.0000000    1
#> 6 Crayfish - Crayfish_ALAN  0.08002364 0.9362185    1

t.test6 <- REPRODUCTION_trait %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test6
```

Figure 28: Significance test for reproduction

```
kruskal.test(Value ~ Treatment, data = CWM_final)
#>
#>  Kruskal-Wallis rank sum test
#>
#> data:  Value by Treatment
#> Kruskal-Wallis chi-squared = 0.017804, df = 3, p-value = 0.9994
dunnTest(Value ~ Treatment,
        data=CWM_final,
        method="bonferroni")
#> Warning: Treatment was coerced to a factor.
#> Dunn (1964) Kruskal-Wallis multiple comparison
#>   p-values adjusted with the Bonferroni method.
#>                Comparison           Z    P.unadj P.adj
#> 1          ALAN - Control -0.02349716 0.9812537     1
#> 2          ALAN - Crayfish -0.11487499 0.9085442    1
#> 3        Control - Crayfish -0.09137784 0.9271924   1
#> 4     ALAN - Crayfish_ALAN -0.09137784 0.9271924    1
#> 5  Control - Crayfish_ALAN -0.06788068 0.9458806    1
#> 6 Crayfish - Crayfish_ALAN  0.02349716 0.9812537    1

t.test7 <- CWM_final %>%
  t_test(Value ~ Treatment) %>%
  adjust_pvalue(method = "bonferroni") %>%
  add_significance()
t.test7
```

Figure 29: Significance test for all traits together

4 Data Visualization

4.1 Treatment vs Weighted feeding average

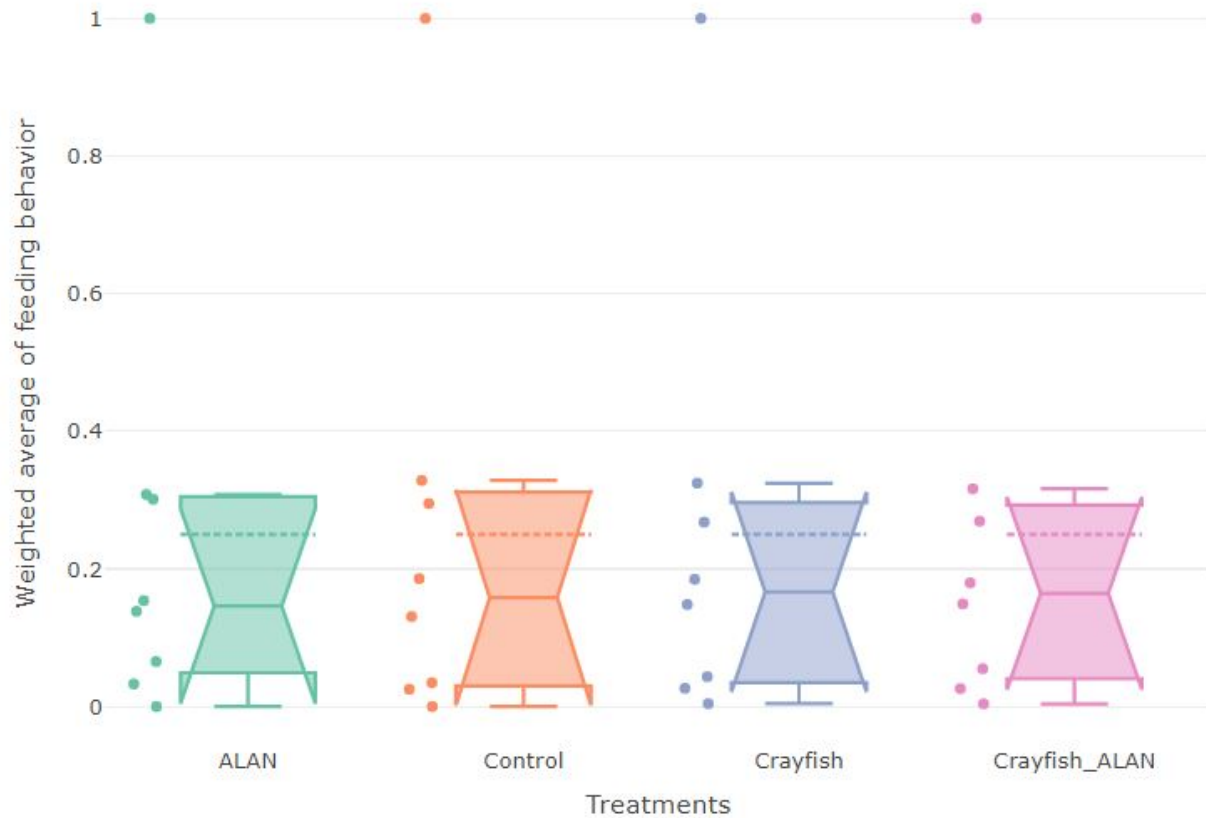
Figure 30: Weighted average of feeding behavior in different treatment groups

4.2 Treatment vs Weighted locomotion average

4.3 Treatment vs Weighted size average

4.4 Treatment vs Weighted dispersal average

4.5 Treatment vs Weighted voltinism average

4.6 Treatment vs Weighted reproduction average

N.B: Functional diversity metrics, including functional diversity, evenness, richness, and divergence, can be effectively computed and utilized to assess changes in functional diversity across different treatments.

By employing cluster analysis and Principal Coordinate Analysis (PCoA), it becomes possible to identify groups of species sharing similar traits and subsequently compare them with abundance values across all macroinvertebrate groups within each treatment. This methodology can provide valuable insights for drawing informed conclusions regarding the impact of crayfish, ALAN and their combined treatments on functional

Figure 31: Weighted average of locomotion trait in different treatment groups

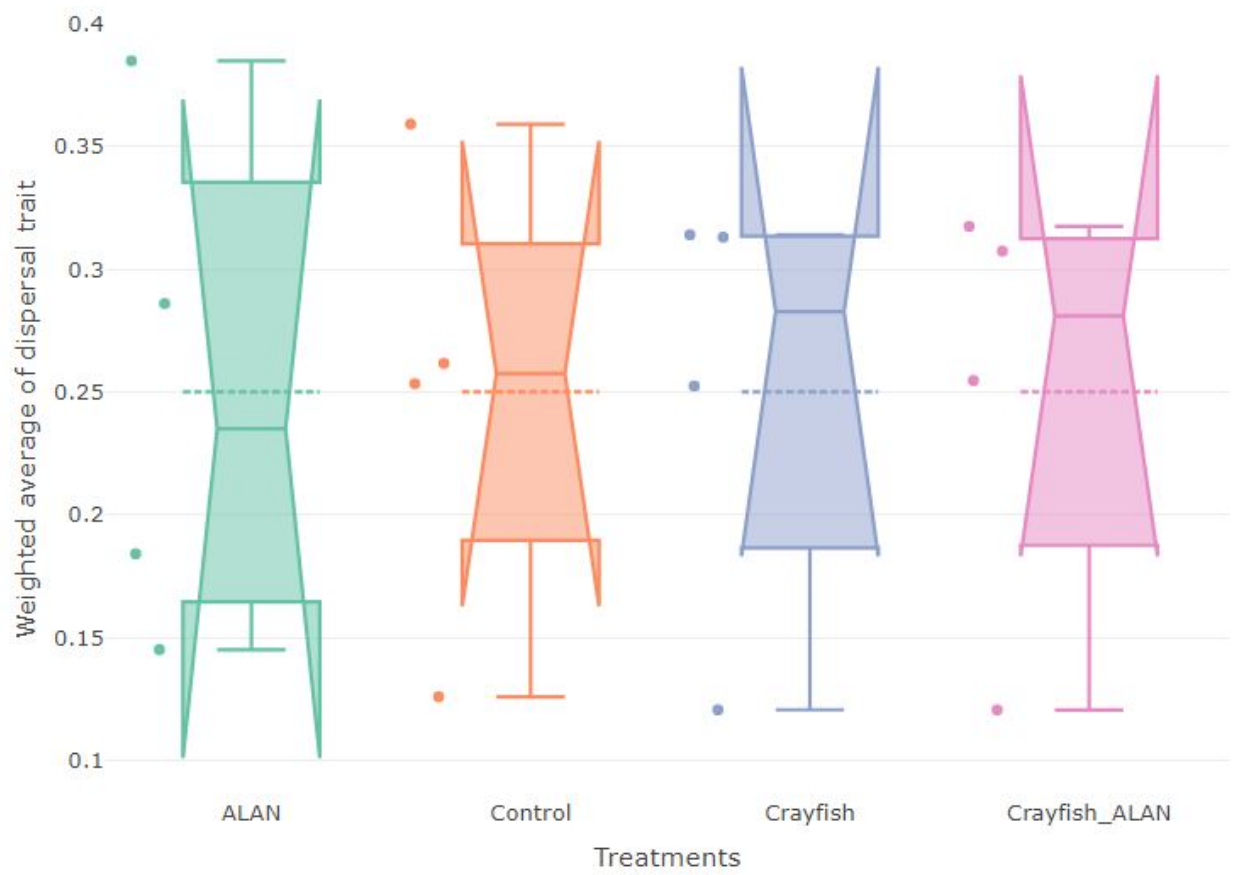Figure 32: Weighted average of locomotion size in different treatment groups

Figure 33: Weighted average of dispersal trait in different treatment groups

Figure 34: Weighted average of voltinism trait in different treatment groups

Figure 35: Weighted average of reproduction in different treatment groups

traits. These comparisons enable us to ascertain whether these stressors lead to a reduction or enhancement of functional traits within the macroinvertebrate community.

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