Stream Restoration Analysis

Investigators

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Introduction

Stream restoration is work conducted to improve the environmental health of a river or stream, in support of biodiversity, recreation, flood management and/or landscape development [1]. However, the return of biological conditions often lags substantially behind the return of physical habitat and water quality conditions following stream restoration. This project focuses on accelerating the recovery of biological conditions in previously restored streams. Our investigator proposes an approach for enhancing recruitment of adult stream insects that can be readily integrated into restoration practices to accelerate the return of biological conditions in restored streams. The researchers added suitable emergent rocks (substrates) into the restored stream, which is considered as the treatment, so that adult stream insects are able to adapt to them quickly and attach their eggs to the underside of the rocks. Our main goal is to determine whether increasing the number of suitable egg-laying substrates in a restored stream increases egg recruitment into the stream. The client would also like to understand if the insect abundance in the treatment streams comes close to the insect abundance in the reference streams. Finally, we want to determine if individual insect types are affected differently by the treatment.

Data

Study Design

The design of blocks and split plots is shown in Table 1. In 2019, the researchers blocked streams primarily by proximity to one another and secondarily by stream characteristics, such as channel size and presence or absence of a culvert [3] above the restored reach. They randomly assigned one stream from each of five blocks to receive the treatment (addition of suitable rocks for ovipositor [4]) using a coin toss, resulting in five restored-untreated and five restored-treated streams. In order to check whether the treatment results in an increase in biological condition that approaches the reference condition, three reference streams were selected based on their

Table 1: Visualization of Study Design

Block 1	Treatment	2019
		2020
	Restored	2019
		2020
Block 2	Treatment	2019
		2020
	Restored	2019
		2020
Block 3	Treatment	2019
		2020
	Restored	2019
		2020
Block 4	Treatment	2019
		2020
	Restored	2019
		2020
	Reference	2019
		2020
Block 5	Treatment	2019
		2020
	Restored	2019
		2020
	Reference	2019
		2020
Block 6	Reference	2019
		2020

proximity to restored streams, accessibility, similar catchment size, and for having predominantly forested intact watersheds. These three reference streams are regarded as streams with optimal biological conditions. The State of NC Division of Environmental Quality reference streams are relatively undisturbed and have not been restored. Each stream is 50 meters long. In 2020, in five of the ten restored streams, the researchers increased the number of suitable oviposition sites by approximately 100% by positioning new rocks or repositioning existing rocks during the flight and egg laying period, thus creating the preferred characteristics for adult aquatic insects to oviposit their eggs; these will be referred to as simply "treatment streams". In five other restored streams, they handle similar numbers and sizes of substrates but will not change their positioning relative to water velocity or the water surface, thereby maintaining the number and quality of oviposition substrates from standard restoration techniques; these will be referred to as "restored streams". Oviposition sites in the reference streams were not altered.

Data collection

Data were collected from 5 restored-untreated streams, 5 restored-treated streams and 3 reference streams in 2019 and 2020. The response variables include: number of emergent rocks, number of egg masses per stream area or per rock area, and the proportion of rocks that rolled. Refer to Figure 1 for illustration of egg masses. To determine whether the manipulation of oviposition sites impacts egg recruitment, our investigators census the egg masses on the underside of each rock protruding above the water surface in the delineated study reaches of the three stream types bi-weekly during the summer egg laying season. They estimate the cumulative number of egg masses of each taxa [5] in each stream. To test whether increasing the number of suitable oviposition sites also increases the abundance and richness of stream insects, they collect ten 0.09 m^2 benthic samples from each of the 13 streams. They count, weigh, and identify individuals to genus or subfamily and compare metrics of diversity (e.g., richness, evenness, EPT, Biotic Index per NCDEQ) that are standardized for differences in abundance among streams by rarefaction.
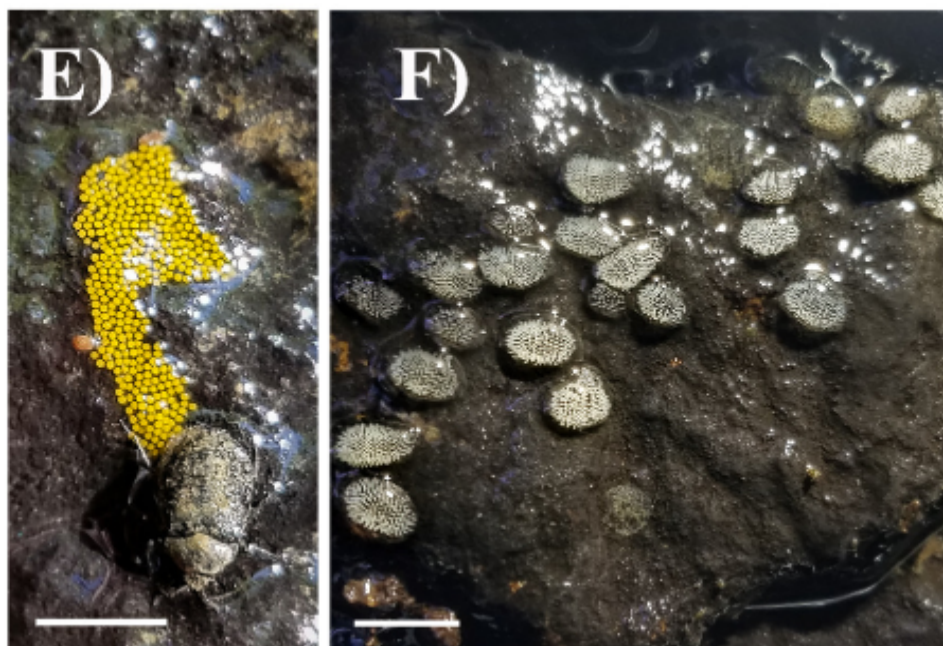

Figure 1: Illustration of Egg Masses [2]

Data Processing and Visualization

With the dataset given, the enhanced egg laying habitat treatment treatment effect will be analyzed. There will be two comparisons. First is the treatment streams versus restored streams. Second is the treatment streams versus reference streams. These two comparisons are performed in order to assess the value-added benefits of restoring the egg-laying habitats and to determine whether the treatment streams are able to come near to the biological conditions of reference streams.

Prior to performing statistical analysis, examination of the quality of the data and some exploratory data analysis were conducted. The dataset was sorted by block for the future analysis and a box plot was created to visualize the mean egg masses of streams through categorizing by type of streams and grouping by time (Figure 2). There is an outlier in the restored streams. This outlier causes unequal variance for the response variable. We clarified with the investigators that this data point was indeed collected correctly and it was caused by a storm.
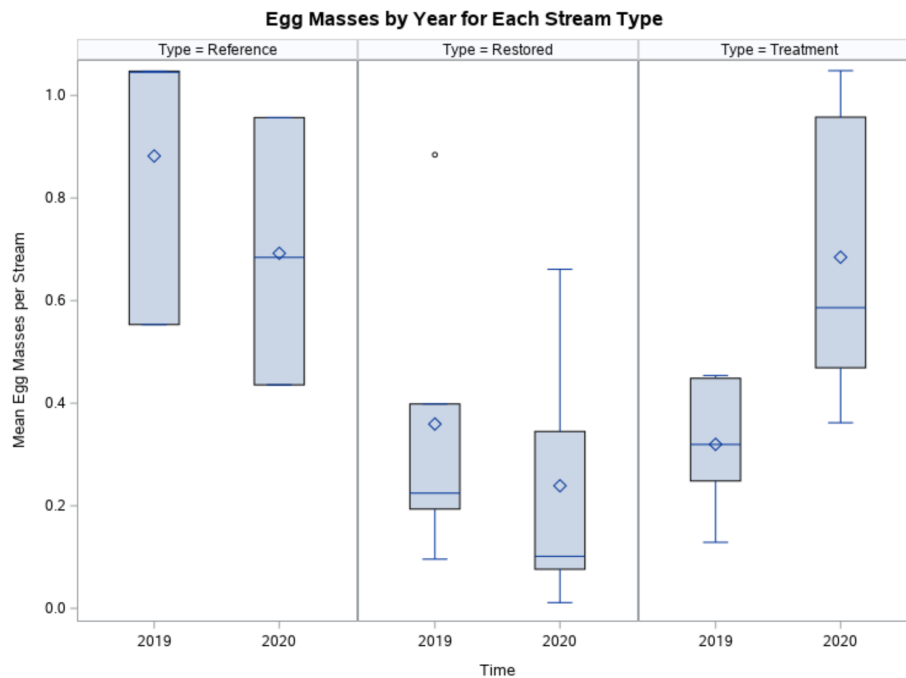

Figure 2: Visual of Egg Masses by Type and Time

After the outlier issue, the residuals against treatments from the model fitted were plotted. The response variable was mean egg mass and the explanatory variables were Time, Type, Block, Type*Time. The residual plot on the left side of Figure 3 shows increasing variance. A square root transformation stabilized the variance, and these diagnostics are shown on the right. After the square root transformation for the response variable, it shows better R^2 and smaller mean squared error (MSE), suggesting that the transformation improves the model.
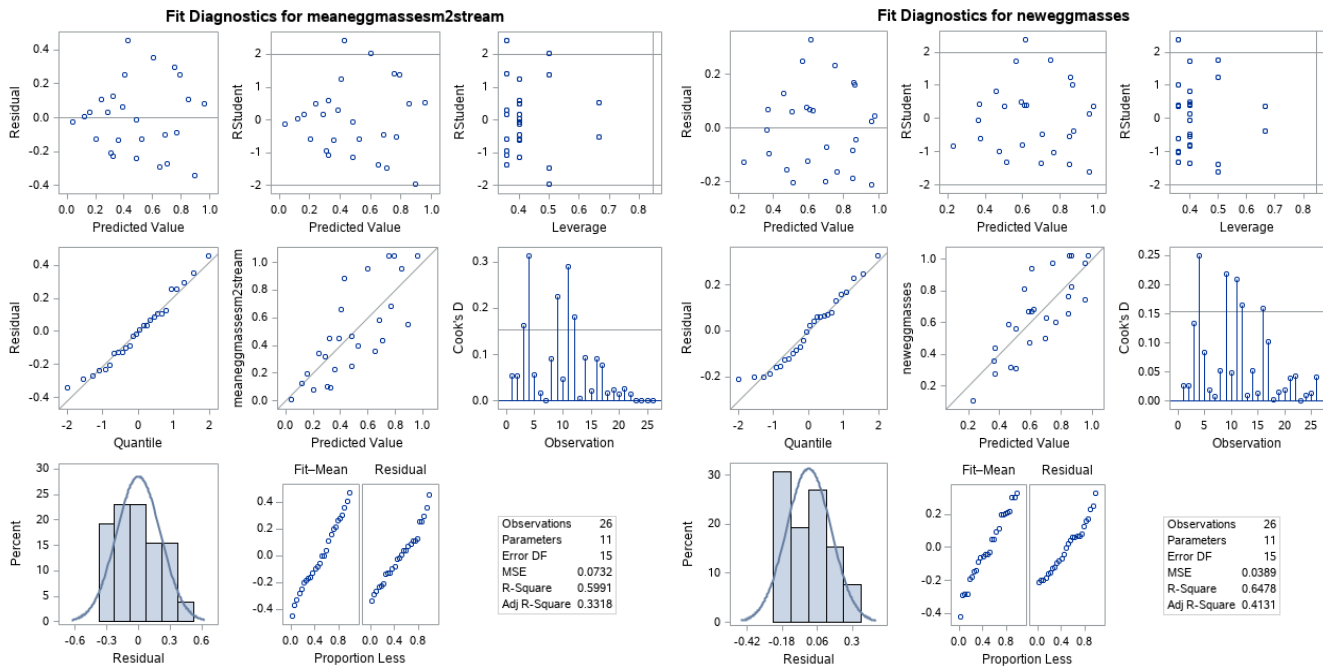
Figure 3: Fit Diagnostics for Original Response (Left) and Transformed Response (Right)

The original dataset has columns for egg masses for the total number of insect types, as well as split into each type of insect for each column. For reference, Table 2 below shows the alias which will be used to refer to specific insect types going forward.

Table 2: Names of insect types

Alias	Insect
ephem	Mayflies (Ephemeroptera)
coleo	Beetle (Coleoptera)
trich	Caddis flies (Trichoptera)
dipgg	True flies (Diptera)

Statistical Methods

Exploratory data analysis was performed to get an understanding of the relationship between the variables. The split plot design was chosen with subplot factor time to control the factors that cause variability between subjects. The levels of factor Block were assigned to main plots, and then levels of factor Treatment were assigned to plots within each block. Levels of factor Time were assigned to subplots within each plot. There were two reasons for using the split plot design for this project. First, some factors were difficult to change in the experiment, so a completely randomized design was not possible for this study design. In this situation, split plots were nested within the block. Having a split plot with two time points not only controls for factors that cause variability between subjects, but also can reduce the number of subjects used in the experiment. This is essential, as there are only 13 streams.

The factors include time of measurement, type of stream, and the block. The statistical model used is:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + B_k + (\alpha\beta)_{ij} + (\alpha B)_{ik} + \varepsilon_{ijkl}$$

where i = 1, 2, 3 and represents the stream type, j = 1, 2, and represents year, and k = 1 to 6 and represents the block. The interaction term of α (Type) and beta β (Time) was added to test whether the effect of Type or Time differs depending on the level of the other variable. The interaction term of α (Type) and B (Block) was added to the model and set to be random to account for variability of streams within the block. The block effect was regarded as random since blocks are used for replication to control variation.

To show if the improvement in insect abundance from 2019 to 2020 is significantly different for the restored and treated streams, the interaction effect between the year of study and stream types, excluding reference streams, was tested. Since the time effect across treatment types (treatment streams versus restored streams) is tested, our null hypothesis is H$_0$: $\mu_{32} - \mu_{31} - (\mu_{22} - \mu_{21}) = 0$ vs alternative test H$_A$: $\mu_{32} - \mu_{31} - (\mu_{22} - \mu_{21}) \neq 0$.

To explore whether the treatment stream approaches the reference stream in insect abundance, the means for the reference and treatment stream in 2020 were compared to determine if there is a significant difference. The null hypothesis is H$_0$: $\mu_{32} - \mu_{12} = 0$ vs alternative test H$_A$: $\mu_{32} - \mu_{12} \neq 0$). For a second check, an equivalence test, which checks if the ratio between two groups within a chosen range close to 1 was performed. The null hypothesis for this test is H$_0$: $\mu_{32}/\mu_{12} < H_L$ or $\mu_{32}/\mu_{12} > H_H$, where H$_L$ and H$_H$ are the lower and upper bounds to the ratio we are testing. The alternative hypothesis is H$_A$: $H_L < \mu_{32} / \mu_{12} < H_H$.

To investigate the effect of the treatment on individual insect species, the univariate analysis and the multivariate analysis were carried out. Multivariate ANOVA (MANOVA) determines whether the difference of egg masses of insect species depends on the treatment, since the treatment in the model can affect the relationship between those dependent variables (egg masses of different insect species). It tests the treatment effects on all types of insects simultaneously. The null hypothesis for MANOVA is that the stream type has no effect on the four different insect species and the alternative is that there is an effect.

To balance the design, the reference streams were excluded from the analysis because they were not present in all blocks. We used GLM with MANOVA statements to do the multivariate analysis (See Appendix). The new variable insect type is added into our model:

$$Y_{ijkhl} = \mu + \alpha_i + \beta_j + B_k + \gamma_h + (\alpha\beta)_{ij} + (\alpha B)_{ik} + \varepsilon_{ijkhl}$$

The response variable is the egg masses for each type of insect. α represents the Treatment (Type), i = 1, 2, and β represents Time, j = 1, 2. B represents the Block k = 1, 2, 3, 4, 5. γ represents the insect type , h = 1, 2, 3, 4. Block, interaction between block and type, and insect type are randomized in this model to control the variation. The MANOVA statements in Proc Glm were used to test Time effect, insect type effect, and time across type effect. The Proc Glm procedure also provided a univariate output we used to test individual differences for each insect type.

Results

Improvement of Treatment versus Restored Streams

Figure 4 shows the least squares means for each stream type over the two years. The red and green lines represent the restored and treatment streams, respectively. We see that the treatment line increases while the

restored line decreases, and the lines cross, which may indicate a strong interaction. The results of the tests confirm this.
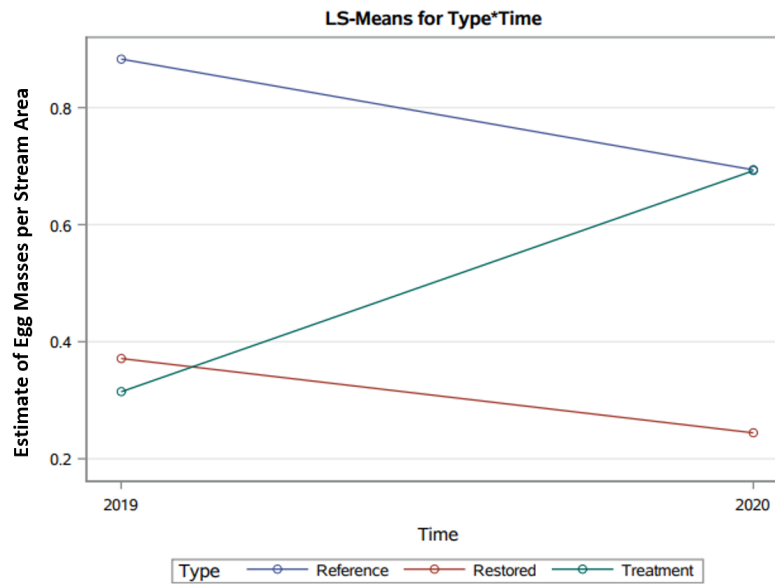

Figure 4: LS Means for the Streams Across Years

We were able to reject the null hypothesis for the first research question testing whether there was a difference between restored streams and treatment streams from 2019 to 2020 (p-value = 0.038). The difference in differences is 0.149. This contrast takes into account the large egg mass variation between streams. The 95% confidence interval (0.000713, 0.5563), sits just above 0. This difference represents how much more the treatment stream improved compared to the reference stream. The data suggests that the treated stream has improved over the restored stream. Table 3 shows the estimates for each level of Type and Time.

Table 3: Egg Mass Estimates for Each Stream Type

Type/Time	Egg Mass Mean	Mean Standard Error
Reference 2019	0.883	0.175
Reference 2020	0.694	0.166
Restored 2019	0.371	0.114
Restored 2020	0.244	0.108
Treatment 2019	0.314	0.112
Treatment 2020	0.692	0.128

Comparison of the Treatment Stream to the Reference Stream

In Figure 4, we can see that the estimated mean for the treatment (green line) and reference streams (blue line) in 2020 are very similar. Additionally, in the previous figure, it does not appear that the treatment stream in 2020 differs much from either of the reference stream numbers. This visual similarity indicates there is not much difference between the two streams in 2020. To test if the treatment streams approach the reference streams,

we tested if the averages of the egg masses for both years in the reference stream is different from the treatment stream in 2020. After contrasting the two means, we did not find evidence of a difference between reference streams and treatment streams in 2020 (p-value = 0.115). The difference is estimated to be 0.03.

Additionally, we conducted an equivalence test to determine whether the ratio of the difference between years for reference and treatment streams was approximately 1. The 95% confidence interval for this ratio is estimated to be 0.76 to 1.37 with a point estimate of 1.02. This means that the reference streams are estimated to be very near equal with the treated streams. It appears that the treated streams have no difference from the reference streams in the number of egg masses.

Investigation of Differences between Insect Types

Figure 5 shows two graphs for the visualizations of the different egg masses of the insect type for different treatments and years. The means are connected with lines. It appears that for the treatment streams, the mean egg masses of insect type Coleoptera, Diptera, and Trichoptera have increased from year 2019 to 2020.
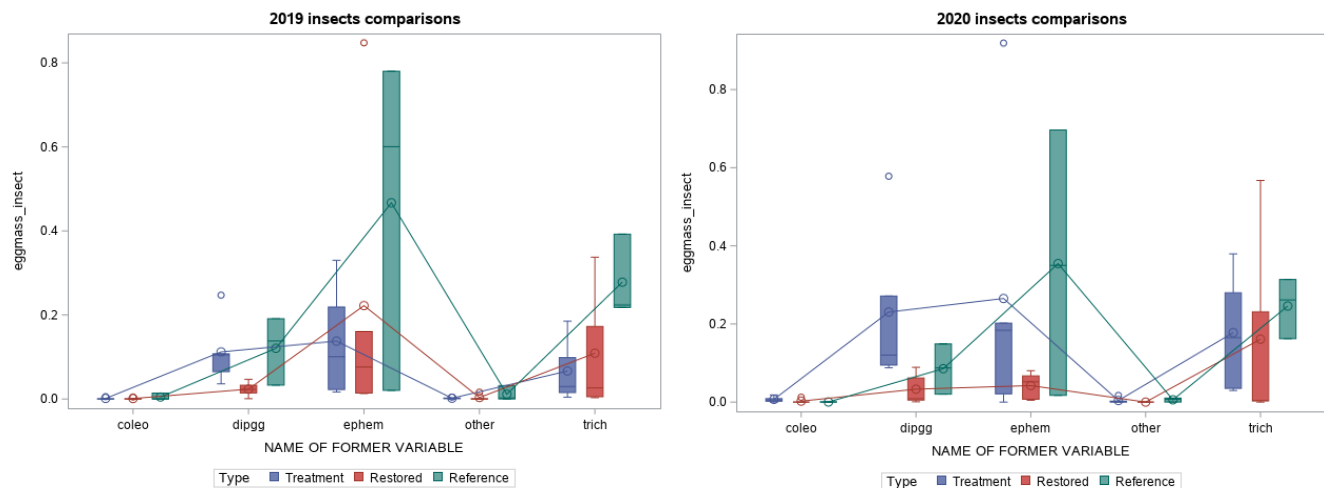

Figure 5: The different egg masses of the insect type for different treatments and years

To determine if there are differences in the behavior of insect types, a Proc Glm with a MANOVA statement was run. The p-value of the Wilk's Lambda (see Table 3) for the overall MANOVA test was 0.23, which indicates that there were no significant differences in the behavior of insect types across streams over time.

Table 3: MANOVA test for Differences Between Insect Types

MANOVA Test Criteria and Exact F Statistics for the Hypothesis of No Overall Time*Type Effect H = Type III SSCP Matrix for Time*Type E = Error SSCP Matrix S=1 M=1 N=1.5					
Statistic	**Value**	**F Value**	**Num DF**	**Den DF**	**Pr > F**
Wilks' Lambda	0.38446767	2.00	4	5	0.2327
Pillai's Trace	0.61553233	2.00	4	5	0.2327
Hotelling-Lawley Trace	1.60099892	2.00	4	5	0.2327
Roy's Greatest Root	1.60099892	2.00	4	5	0.2327

Because we did not see a difference in the behavior of insect types and because of the unbalanced design, a univariate test for each insect type was more feasible and informative for this experiment. Figure 6 shows the LSMeans from the univariate test for each individual insect type. The red line represents the treatment stream and the blue line represents the restored stream.
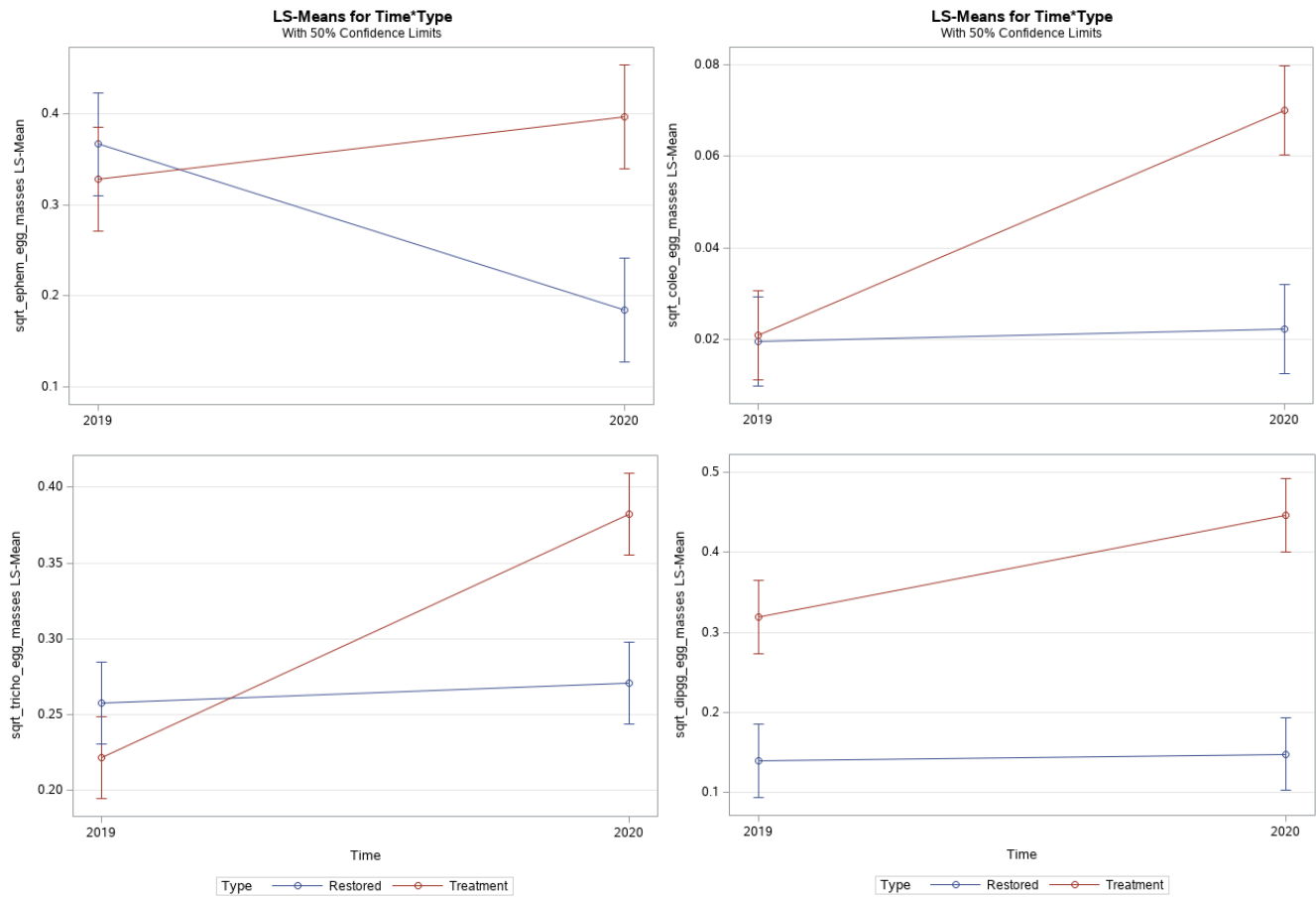

Figure 6: LSMeans for Insect Types (Top Left: Ephemeroptera, Top Right: Coleoptera, Bottom Left: Trichoptera, Bottom Right: Diptera)

It appears that every insect increases more for the treatment stream than the restored stream over the two years. Table 4 shows the estimate of the change in egg masses from 2019 to 2020 for the restored versus the treatment stream on the square root scale along with the p-values for each insect type.

Table 4: Univariate Analysis for Each Insect Type

Insect	Contrast Estimate (Square Root Scale)	p-value
Mayflies (Ephemeroptera)	0.25	0.16
Beetle (Coleoptera)	0.05	0.13
Caddis flies (Trichoptera)	0.15	0.09
True flies (Diptera)	0.12	0.39

Though we saw a significant difference between the treatment and restored streams from 2019 to 2020 for the overall insect count, we were unable to see any significant differences when comparing the insect types individually probably because of the variation, which is too extreme when looking at the insect types individually. When looking at the combined egg masses, the variation balanced out and we were able to see a significant difference.

Summary

Improving the stream restoration process is essential because there are many resources and dollars spent on this annually. This project provides both a proof-of-concept and a set of guidelines and informational tools that can be applied to previous and/or future stream restoration projects to enhance biological conditions that are fundamental to stream ecosystem structure and function and assessment [6]. In this project, we made use of the data collected from reference streams, restored streams without treatment, and restored streams with treatment. Our goal was to study whether enhancing the egg-laying habitat for adult stream insects can help accelerate the recovery of biological conditions in restored streams. Our analysis showed the following results:

- There is a significant increase in the abundance of insect egg masses between the treated and untreated restored streams.
- The abundance of insect egg masses in the treated restored streams has no significant difference from the reference streams.
- The differences for each species insect in the treated and untreated restored streams are not significant.

While the results established in this project are interesting, we would like to note the following areas for future research:

- Data heterogeneity. Our investigators had tried to design the experiment so that the reference streams and the restored streams have similar conditions. However, the data collected may still have data heterogeneity. It shall be great to further improve the experiment to deal with this issue.
- Methodology. We thought of trying the ratio of 2020 to 2019(egg mass) before fitting the model. This can make the analysis procedure easier. But there was a problem including the block effect for the overall MANOVA test. The univariate analysis for the ratio one gives the same result as what we mentioned above in the result section. If more time were given, we would like to delve further into different methods.
- Small sample size. In this study, we only have 26 sample points. To draw more conclusive results, more data points need to be monitored and the data may need to span over multiple years.
- Missing or rolled rocks. It may be interesting in further analysis to analyze how the number of missing or rolled rocks in a stream affects the number of egg masses. This was not addressed in this analysis but it could help the researchers understand the results more.
- Unbalanced study design. Proc GLM and MANOVA will work perfectly with this design if the data is balanced, but there is only one type of stream in Block six, and due to Covid-19 and some restrictions, data was only gathered from 3 reference streams rather than the planned 5.

Overall, the treatment evaluated in this analysis shows promise and was shown to be effective. Though further research is recommended, it seems likely that the treatment will shorten the length of time it takes naturally for biological conditions to return following the stream restoration process.

References

[1] https://en.wikipedia.org/wiki/Stream_restoration

[2] S. Jordt and B. W. Taylor. A rolling stone gathers no eggs: the importance of stream insect egg laying natural history for stream restoration.

[3] https://en.wikipedia.org/wiki/Culvert

[4] https://en.wikipedia.org/wiki/Ovipositor

[5] https://en.wikipedia.org/wiki/Taxon

[6] B. Taylor and S. Jordt. Accelerating the recovery of biological conditions in restored streams by enhancing the egg-laying habitat for adult stream insects. NC State Project Description.

Appendix: SAS Program Code

(for performing all data analyses described in Report)

```
data work.stream;
        infile streams firstobs = 2 dsd;
        length Block Subsite $ 15 Type $ 10;
        input Block SubSite    $  Type  $  Time  meansuitablerocksm2stream  meaneggmassesm2stream
meanmassxindirockarea          meanephemeggmassesxm2stream          meancoleopeggmassesxm2stream
meantrichopeggmassesxm2stream meandipggmassesxm2stream nsitevisits;
run;


*main ANOVA to test the interaction between time and type (question 1);
*sqrt transformed anova;
title "Sqrt transformed ANOVA";
proc glimmix data=stream plots=(RESIDUALPANEL); *use glimmix to transform response variable;
        class Type Time Block;
        model meaneggmassesm2stream = Type|Time / link=power(0.5);         *transform  the  response  to
the square root;
        random Block / subject = Type(Block); *random effects for block and stream;
        estimate 'D-I-D' Type*Time 0 0 1 -1 -1 1 / ilink cl; *estimate treatment vs restored from 2019 to 2020,
note that ilink transforms back to original scale;
        estimate 'Ref Avg vs Trt 2020' Type -2 0 2 Type*Time -1 -1 0 0 1 1 / ilink divisor=2; *estimate treatment
2020 vs average of reference;
        lsmeans Type|Time / ilink diff plot=meanplot(ilink sliceby=Type join);
run;


*run equivalence test to test is treatment and reference streams similar;
proc sort data = stream;
```

```
        by Subsite Type;
run;
proc transpose data = stream out = wide(drop=_NAME_);
        by Subsite Block Type;
        var meaneggmassesm2stream;
run;
proc sort data = wide;
        by Type;
run;
data difference;
        set wide;
        by Type;
        diff = col2 - col1;
        if first.Type then index = 1;
        index+1;
run;
proc sort data=difference;
        by index;
run;

proc transpose data = difference out = diff_sort;
        by index;
        var col2;
        id type;
Run;

*equivalence test;
proc ttest data=diff_sort dist=lognormal tost(0.75, 1.25);
  paired Reference*Treatment;
run;


*dataset for MANOVA use;
data stream_manova;
        set stream;
        sqrt_egg_masses = sqrt(meaneggmassesm2stream);
        sqrt_ephem_egg_masses = sqrt(meanephemeggmassesxm2stream);
        sqrt_coleo_egg_masses = sqrt(meancoleopeggmassesxm2stream);
        sqrt_tricho_egg_masses = sqrt(meantrichopeggmassesxm2stream);
        sqrt_dipgg_egg_masses = sqrt(meandipggmassesxm2stream);
        *where Block ne '6';
        where Type ne 'Reference';
run;

*run overall MANOVA test;
proc glm data = stream_manova plots=(diagnostics residuals);
        *where Type ne 'Reference';
        class Time Type Block;
```

```
        model          sqrt_ephem_egg_masses          sqrt_coleo_egg_masses          sqrt_tricho_egg_masses
sqrt_dipgg_egg_masses = Time|Type Block Block*Type;
        random Block Block*Type;
        *manova h = _all_ e = Block*Type;
        manova h=type e=block*type;
        manova h = time time*type;
        test h=type e=time*type;
        test h = type*time e = block*type;
        estimate 'D-I-D' Type*Time 1 -1 -1 1;
        lsmeans Type*Time / diff cl alpha=0.5 slice=Type plot=meanplot(join cl); *compare to when I take out
reference streams entirely;
run;


title "";
```