StatCharrms:
An R Package for "Statistical Analysis of Chemistry, Histopathology, and Reproduction Endpoints Including Repeated Measures and Multi–Generation Studies"

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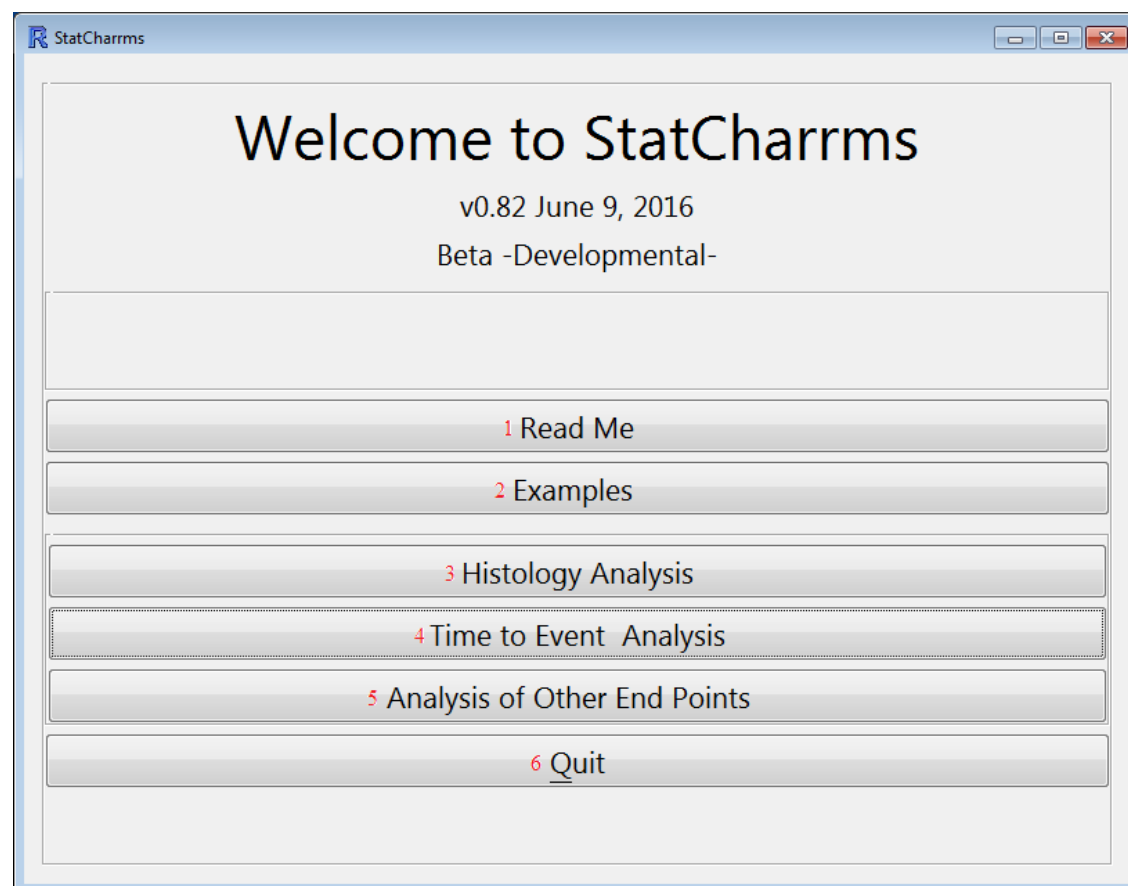June 19, 2017

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Introduction

StatCharrms (Statistical analysis of Chemistry, Histopathology, and Reproduction endpoints using Repeated measures and Multi–generation Studies) is a graphical user front–end for ease of use in analyzing data generated from the Medaka Extended One Generation Reproduction Test (MEOGRT)[10] and Larval Amphibian Growth and Development Assay (LAGDA) [11]. The analyses StatCharrms is capable of performing are: Rao–Scott adjusted Cochran–Armitage test for trend By Slices (RSCABS) [3], a Standard Cochran–Armitage test for trend By Slices (SCABS) [3], mixed effects Cox proportional model [5, 8,12,13, 14], Jonckheere–Terpstra step down trend test [6,9,13], Dunn test [1], one way ANOVA, weighted ANOVA, mixed effects ANOVA, repeated measures ANOVA, and Dunnett test [2].

Starting Screen

StatCharrms is a complete front–end to the analyses required for the MEOGRT and LAGDA containing graphical user interface (GUI) driven operations. To call the GUI for StatCharrms, type the following into the R console:

```
R Input
> install.packages('StatCharrms')        #Install StatCharrms from CRAN
> library(StatCharrms)                    #Load the StatCharrms library
> Run.StatCharrms()                       #Calls the GUI for StatCharrms
```

This should bring up the introduction window for StatCharrms.

At the top of the introduction window are the [**Read Me**] and [**Examples**] buttons . The [**Read Me**] button will display information about the authors, references, and complete log of changes. The [**Examples**] button will create a folder that is populated with the data and results for every example analysis used in this document. This includes an analysis for fecundity, length, weight, time–to–effect, and histopathological data.

Next is the [**Histology Analysis**] button. This button calls Histopath() from the RSCABS package allowing for the analysis of data sets from histopathological endpoints. Details on Histopath() can be found in the RSCABS vignette. If Run.StatCharrms() has been called the RSCABS vignette can be opened by typing the following into the R Console:

```
──────── R Input ────────
> vignette('RSCABS')
```

Continuing down the list is the [**Time to Event Analysis**] button, which calls the window used in performing Cox proportional mixed effect models [13, 14]. The [**Analysis of Other Endpoints**] button is used for analysis on quantitative endpoints (such as length, weight, and fecundity) using the following statistical tests: ANOVA, repeated measures ANOVA, mixed effects ANOVA, weighted ANOVA, Dunn's test [3], Dunnett's test [2], and a step down Jonckheere–Terpstra (JT) trend test [6,9,13]. Last and mostly definitely least is the [**Quit**] button which closes both StatCharrms and R.

Time to Event Analysis

Time to Event Analysis Main Window

Pressing the [**Time to Event Analysis**] button on the StatCharrms introductory window will generate the **Time to Event Analysis** window.
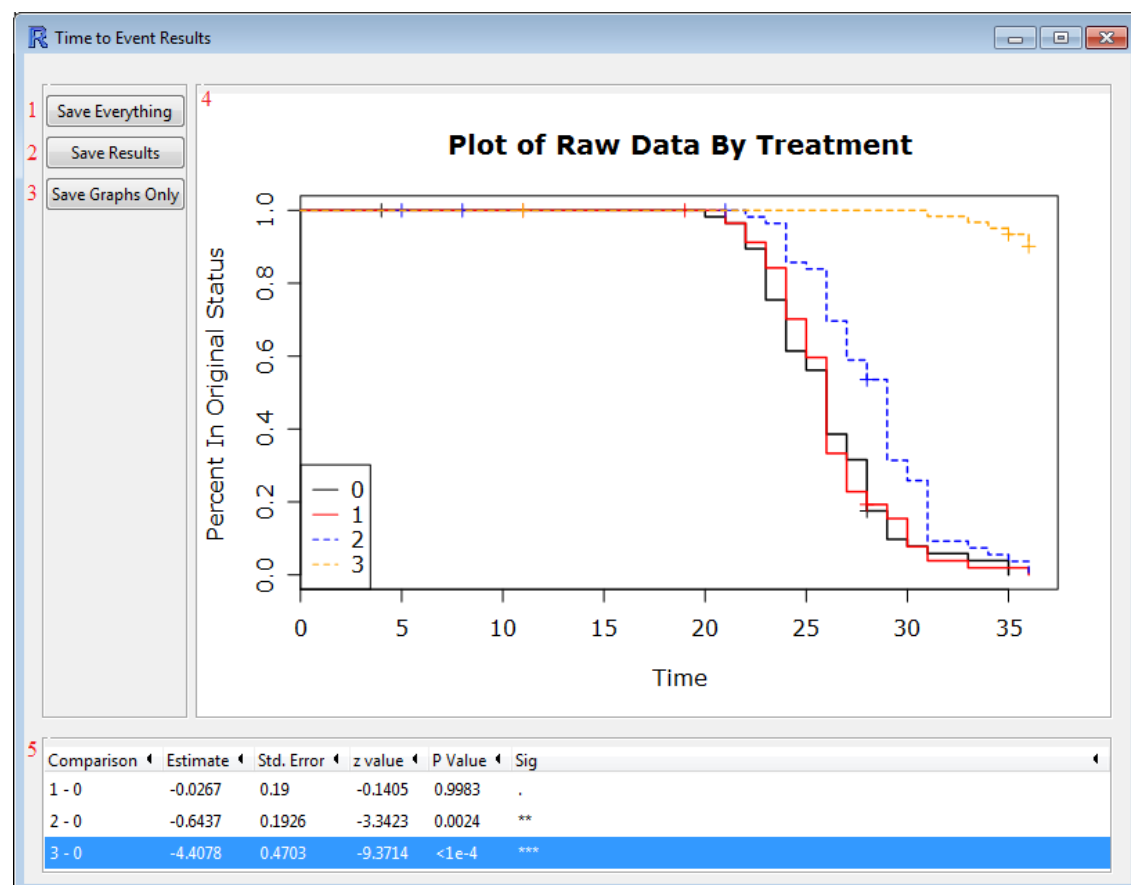
At first only the [**Load Data**] Button (1) will be available. Pressing this button will call a dialogue box where a data set can be navigated to and selected. Data files must be in csv format, have the first row contain the header information, and each row contain information about an observation. After the data is loaded items, 2–13 will appear while (14) will contain the load data. Information about each item in the window is listed below:

1: The [**Load Data**] button is used to load a data set into StatCharrms.

2: The name of the **Treatment** variable, where the lowest number indicates controls and every greater number is a more severe treatment. This must be selected for any analysis to run.

3: The name of the **Replicate** variable. The **Replicate** variable may either contain character strings or numeric values.

4: Used to specify the name of the **Gender Variable**, if applicable.

5: **Gender Value**: If a variable for gender is specified, the value of the gender used in the analysis is specified here.

6: The **Generation Variable** is used if the experiment spanned multiple generations. This is where the name of the variable that contains information about each generation can be specified.

7: **Generation Value** is the generation the analysis is performed on and is defined here.

8: The name of the **Time Variable** is the name of the variable that indicates when events happen. Values in the column can either be dates or integers representing a period of time since the start of the experiment. This must be selected for any analysis to run.

9: **Time Format**: The format the **Time Variable** is in. It can be any standard format for date or a number, which is indicated by selecting **Numeric**. Note, by convention StatCharrms will set the earliest date in the analysis to 0.

10: The **Status Variable** is the variable that indicates the final status of the subject, whether it is the event being tested for or a censoring event (see 11 and 12).

11: The **Event Value** is the value representing the event being testing for.

12: The **Censored Value** is used to indicate the value used to represent a censored event .

13: The [**Run Analysis**] Button can be used after each of the entries for **Treatment**, **Status Variable**, and **Event Value** have been specified.

14: This is were the data set is displayed. Always double check the data set to make sure it was loaded correctly.

Time to Event Results

After all the values have been specified pressing the [**Run Analysis**] button (13) will run a mixed effects Cox proportional hazards analysis on the data then generate the **Time to Event Results** window.
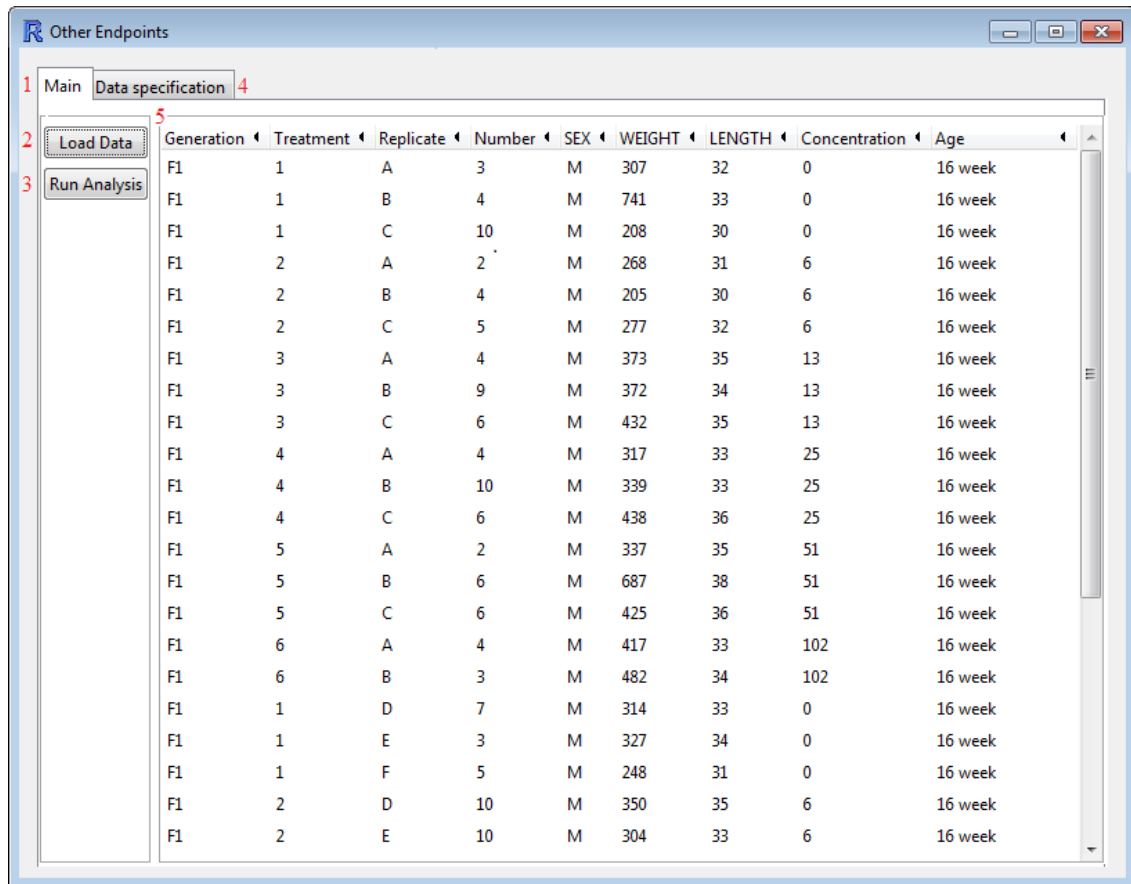

1: The [**Save Everything**] button will save the graph being displayed (4) as a pdf. Furthermore, this button will also save the main effect table (5) and the data table as an HTML file.

2: The [**Save Results**] button will save the data table and the main effect table (5) as an HTML file.

3: The [**Save Graphs Only**] button will save the graph being displayed (4) as a pdf.

4: A Kaplan–Meier plot [7]. This plot does not take into account the replicate structure of the data.

5: The main effects table. This table displays the results of a Dunnett's test from the Cox mixed effects models. The columns are:

- **Comparison**: The treatment levels being compared.
- **Std.Error**: The estimated difference between treatment levels.
- **z value**: The value of a z statistic.
- **P Value**: The corresponding p–value of the Dunnett's test.
- **Sig**: A significance flag where "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001.

Other Endpoints

Other Endpoints Main Window

This module of StatCharrms is used for endpoints with standard numerical values. Length, weight, and fecundity are examples of data with standard numerical values. When the [**Analysis of Other Endpoints**] button is pressed on the StatCharrms introductory window, the main analysis window for other endpoints will appear.

1: The **Main** tab for the **Analsis of Other Endpoints** module. Items on this tab are used to load, view, and analyze standard numerical data.

2: The [**Load Data**] button is used to load data into StatCharrms. After data is loaded a **Data specification** tab (4) will be created and (5) will be updated with the data set.

3: The [**Run Analysis**] button will only appear after the [**Confirm Selected Values and Variable**] button is pressed on the **Data specification** tab. Pressing this button will start running the analyses selected on the **Data specification** tab. After every analysis is ran a new window will appear with the results of each analysis.

4: The **Data specification** tab only appears after a data set has been loaded into StatCharrms. This tab is where information about the data is specified. If a new specification on the same data set is desired the **Data specification** tab can be navigated back to and changes can be made. After changes are made just press the [**Confirm Selected Values and Variable**] button on the **Data specification** tab to lock in the changes.

5: This is where the current data set is displayed. If any of the **generation**, **gender**, and/or **age** values (all of which are optional) are selected on the **Data specification** tab, the selection will be reflected here by subsetting the data.
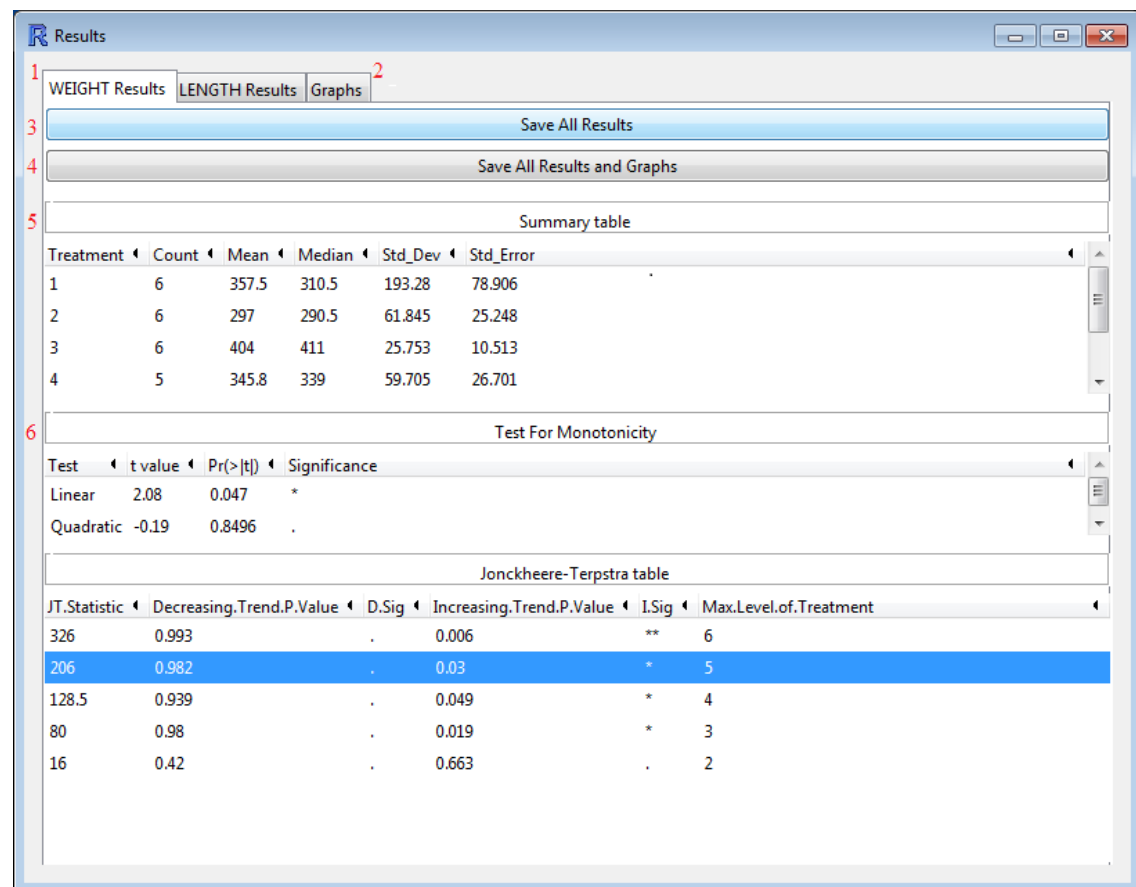
Other Endpoints Specification Tab

1: This is where the name of the **Treatment** variable is specified. The **Treatment** variable must contain only numbers where the lowest number indicates controls and every greater number indicates a more severe treatment. A **Treatment** variable must be specified before analysis can start.

2: The name of the **Replicate** variable. Values for the **Replicate** variable can be any combination of number and letters.

3: The name of the **Generation Variable**.

4: If different generations are used the **Generation Value** is the generation the analysis is performed on.

5: The name of the **Age Variable**.

6: If there are multiple ages in within the data set, the **Age Value** age the analysis is performed on is specified here.

7: The name of the **Gender Variable**.

8: **Gender Value**: The gender the analysis is performed on.

9: If a weighted ANOVA is to be used, the **ANOVA Weight** is the name of the variable containing the weights for the ANOVA.

10: The **Test Direction** can be set to **Both** for a two–tailed test or either of; **Decreasing** or **Increasing** for a one–tailed test. Since MEOGRT [[10] calls for one–tailed test, the default setting is **Decreasing**.

11: The **Alpha Level**. This is only used in the Jonckheere-Terpstra trend test. The The p–value of a step of the Jonckheere-Terpstra trend test need to be less then the textbfAlpha Level for the test to proceed to the next step.

12: After a **Treatment** variable is specified the [**Select Endpoints To Test**] button can be pressed to specify every endpoint an analysis is performed on. Each selected endpoint will appear in (14) contained within a frame named after the endpoint.

13: After endpoints are selected the [**Confirm Selected Values and Variables**] button is used to update StatCharrms with all the values specified in this form. At any time the **Data specification** tab can be navigated back to and the values of the entries can be changed. After the changes are made pressing the **Confirm Selected Values and Variables** button will update the information in StatCharrms.

14: At first this will only contain A–F, however after endpoints are specified it will also contain a frame for each endpoint selected.

A–F: All of these are only used if repeated observations on the same subject are recorded during different times over the course of the study.

    A: The name of the **Time Variable**, which indicates when the observation occurred.

    B: If the time is listed as dates, the **Time Format** is used to specify the format the date is in. The **Time Variable** can also be a list of numbers, in which case (B) can be set to **Numeric**.

    C: The **Analysis Interval** is the number of units in time (e.g. number of days) each endpoint is averaged over for the analysis.

    D: The **Graph Interval** is the number of units in time (e.g. number of days) each endpoint is averaged over for the purpose of graphing.

    E: The [**Select Excluded Times**] button allows for the selection of each time to be excluded from the analysis.

    F: Every excluded time will be displayed within this frame.

15: **Test Type** will appear after an endpoint is selected. This controls for the specific type of statistical test used for that endpoint. This can be set to **Auto**, which follows MEOGRT guidelines, or any specific test type the StatCharrms is capable of performing.

16: The **Transformation** used on the endpoint. As with (15) this only appears after an endpoint is selected. This can be **None** for no transformation, **Square_Root** commonly used for count data, **Log** (base 10) for data that spans multiple orders of magnitude, **Log + 1** if the data both spans multiple orders of magnitude and includes a value of 0, and finally **Arcsine** (of the square root of the response) for analysis of percents.

After the [**Confirm Selected Values and Variables**] button is pressed, the analysis can be ran from the **Main** tab using the [**Run Analysis button**]. This will start analyzing every endpoint. After every analysis is finished (which may take a while) the results window will appear.

Other Endpoints Results

1: The endpoint tabs. There is a tab for every endpoint used in the analysis.

2: Clicking on the **Graphs** tab will navigate you to a tab were the data can be plotted.

3: The [**Save All Results**] button will save all the results (and data tables) for all the endpoints as HTML files.

4: The [**Save All Results and Graphs**] button will perform the same operations as the [**Save All Results**] button with the addition of saving every possible graph for every endpoint as a pdf.

5: The **Summary table** is included in every analysis, and includes entries for:

- **Treatment**: The level of the treatment variable.
- **Count**: The number of observations for the level of the treatment variable.
- **Mean**: The mean of the response for the level of the treatment variable.
- **Median**: The median of the response for the level of the treatment variable.
- **Std_Dev**: The standard deviation of the response for the level of the treatment variable.
- **Std_Error**: The standard error of the response for the level of the treatment variable.

6: The rest of the tables depends on what analyses were ran. The two tables listed in the figure above are the **Test For Monotonicity** table and the **Johnckheere–Terpstra table**. Other tables could include: a **Wilks table**, a **Levene table**, an **Anova table**, a **Dunnett table**, and a **Dunns table**. A description for each table is listed below.

- **Test For Monotonicity**: This is the test for monotonicity as described in the OECD guidance for statistics [9]. According to the test, data is considered to be monotonic unless a **Quadratic** term is significant and the **Linear** term is not. The columns of the table are described below:

Test For Monotonicity				
Test	t value	Pr(>|t|)	Significance	
Linear	2.08	0.047	*	
Quadratic	-0.19	0.8496	.	

  - **Test**: The test for a linear or quadratic trend.
  - **t value**: The t–value for that test.
  - **Pr(>|t|)**: The associated p–value.
  - **Significance**: A significance flag where "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001.

- **Johnckheere–Terpstra table**: This is the table associated with the step down Johnck-heere–Terpstra test for trend. This test will keep stepping down until a non–significant result is found.

Jonckheere-Terpstra table					
JT.Statistic	Decreasing.Trend.P.Value	D.Sig	Increasing.Trend.P.Value	I.Sig	Max.Level.of.Treatment
326	0.993	.	0.006	**	6
206	0.982	.	0.03	*	5
128.5	0.939	.	0.049	*	4
80	0.98	.	0.019	*	3
16	0.42	.	0.663	.	2

  - **JT.Statistic**: The JT test statistic.
  - **Decreasing.Trend.P.Value**: The p–value for a decreasing trend.
  - **D.Sig**: The significance flag for the decreasing tend where "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001.
  - **Increasing.Trend.P.Value**: The p–value for a increasing trend.
  - **I.Sig**: The significance flag for the increasing tend where "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001.
  - **Max Level of Treatment**: The treatment level the test statistic corresponds to.

- **Wilks table**: A table for the Shapiro–Wilk test for normality, with additional statistics about the data's statistical distribution.

Wilks table						
OBS	STD	SKEW	KURT	SW_STAT	P_VALUE	Signif
34	112.8643	1.17283	3.61345	0.88327	0.0017	*

  - **OBS**: The number of observations in the data set.
  - **STD**: The standard deviation of the residuals in the data set.
  - **SKEW**: The skew statistic [4]. Positive skew could indicate the right skewed distribution while a negative skew could indicate the left skewed distribution.
  - **KURT**: The value of the kurtosis of the residuals. Kurtosis [15] is a measure of how heavy tailed a distribution is. A large kurtosis could be indicative of an outlier.

- **SW_STAT**: The value of the Shapiro–Wilk test statistic.
- **P_VALUE**: The p–value associated with the test statistic.
- **Signif**: A significance flag which uses "*" for p–value ≤ 0.01 and a "." otherwise.

- **Levene table**: The Levene's test is a test for non–homogeneity of variance.

Levene table			
Treatment ◄ Df ◄ F value ◄ Pr(>F)			◄
Treatment	5	0.6684019	0.6505773

- **Df**: The degrees of freedom.
- **F value**: The value of the test statistic.
- **Pr(>F)**: The p–value associated with the test statistic. If the p–value is low then the variance is non–homogeneous, meaning the Dunnett and ANOVA tests should not be use. In which case either use a different data transformation or use a non-parametric test such as a Johnckheere–Terpstra or a Dunns. If the **Test Type** is set to **Auto** in the **Data specification** tab, StatCharrms will treat p–values less than 0.01 as indicators of non–homogeneity.

- **Anova table**:

Anova table				
	Sum Sq ◄ Df ◄ F value ◄ Pr(>F) ◄ Signif			
Treatment	70594.67	5	0.9404432 0.4703198	.
Residuals	420365.8	28	NA NA	NA

- **Sum Sq**: The between treatment sum of square (top) and the within treatment sum of squares (bottom).
- **Df**: The degrees of freedom.
- **F value**: The value of the F statistic.
- **Pr(>F)**: The p–value associated with the F statistic.
- **Signif**: The significance flag where "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001.

- **Dunnett table**:

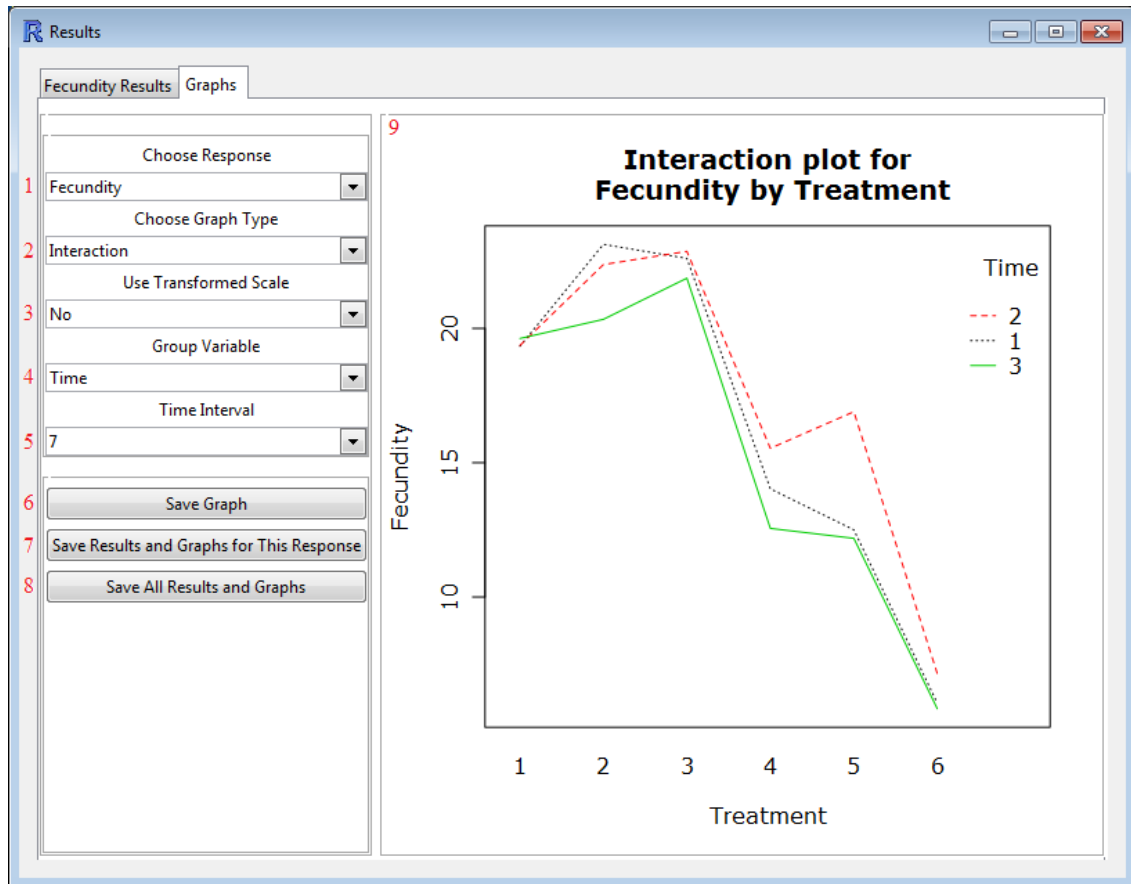| Dunnett table								
Treatment ◄	Levels ◄	Estimate ◄	Std. Error ◄	Df ◄	t.value ◄	p.value ◄	AdjP ◄	Signif
Treatment	2 - 1	-60.5	70.74	28	-0.8552	0.3997	0.8643	.
Treatment	3 - 1	46.5	70.74	28	0.6573	0.5163	0.9473	.
Treatment	4 - 1	-11.7	74.19	28	-0.1577	0.8758	0.9999	.
Treatment	5 - 1	71.5	74.19	28	0.9637	0.3435	0.8025	.
Treatment	6 - 1	55	70.74	28	0.7775	0.4434	0.9019	.

- **Treatment**: The name of the effect being test. This is often either the **Treatment** variable or the **Time Variable**.
- **Levels**: The two levels of effects being tested. If a **Time Variable** was not used then the comparison will always be with a treatment group to a control.
- **Estimate**: The estimated difference in transformed means between the levels.
- **StdErr**: The standard error.
- **Df**: The degrees of freedom.
- **T.Value**: The values of the student t statistic.

- **P.Value**: The p–value associated with the test statistic.
- **AdjP**: The p–value from the Dunnett's test.
- **Sig**: A significance flag for the p–value of the Dunnett's test. An "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001.

- **Dunns table**: A non–parametric Dunnett's test. This tests for differences in distributions (both mean and variance) in every treatment level compared to the controls.

				Dunns table	
Treatment	NTreatment	Rank	Difference	P Value	Signif
1	6	12	0	1	.
2	6	9.75	-2.25	1	.
3	6	23.41667	11.41667	0.1176	.
4	5	15.7	3.7	1	.
5	5	20.9	8.9	0.3497	.
6	6	23.5	11.5	0.1136	.

- **Treatment**: The level of the treatment variable being tested.
- **NTreatment**: The number of observations for the treatment level.
- **Rank**: The average rank of the data within that treatment.
- **Difference**: The difference in average rank compared to controls.
- **P Value**: The p–value associated with the level of treatment.
- **Signif**: The significance flag where "." is a p–value > 0.05, "*" is a $0.01 <$ p–value ≤ 0.05, "**" for $0.001 <$ p–value ≤ 0.01, and "***" for p–value ≤ 0.001. A "significant" p–value could indicate a difference in either the mean or variance of the data within the treatment compared to the controls.

Clicking on the **Graphs** tab will navigate to the graphing portion of the results window. Below is an example **Graphs** tab for the example fecundity data set included in StatCharrms.

1: **Choose Response** is where the graphed response is chosen. Any endpoint chosen on the **Data specification tab** can be selected here.

2: **Choose Graph Type**: This controls the type of graph plotted. Any of a: box and whisker plot, quantile–quantile, violin, or (if a **Time Variable** was specified) an interaction plot can be chosen.

3: **Use Transformed Scale**: This controls the scale the data is plotted in. Choosing "No" plots the data in the untransformed scale, while "Yes" will use the transformation selected for the analysis.

4: This is only available if a **Time Variable** was specified on the **Data specification tab**. It allows for selection of the variable for the x–axis. This can be either the **Time Variable** or the **Treatment** variable.

5: This is only available if a **Time Variable** was specified on the **Data specification tab**. It allows for changing the interval of time the response variable is averaged over. For example, select 1 for a daily response or 7 for a weekly one.

6: The [**Save Graph**] button saves the graph currently being displayed as a pdf.

7: The [**Save Results and Graph for this Response**] button saves the results for the current response as a HTML file and all possible graphs for the response as a pdf.

8: The [**Save All Results and Graphs**] Saves the results for all responses as a HTML files and all possible graphs for all responses as pdfs.

9: A display of the current graph.

Acknowledgments

References

1. Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**:241—252.

2. Dunnett, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. *Journal of the American Statistical Association*, **50**:1096–1121.

3. Green, J. W., Springer, T. A., Saulnier, A. N., and Swintek, J. A. (2014). Statistical analysis of histopathological endpoints. *Environmental Toxicology and Chemistry*, **33**(5): 1108—1116

4. Groeneveld, R. and Meeden, G. (1984). Measuring Skewness and Kurtosis. *Journal of the Royal Statistical Society. Series D (The Statistician)* **33**(4): 391—399.

5. Hosmer, D.W. Jr and Lemeshow, S. (1999). *Applied Survival Analysis: Regression Modeling of Time to Event Data.* Wiley—Interscience.

6. Jonckheere, A. R. (1954). A distribution–free k–sample test against ordered alternatives. *Biometrika* **41**:133—145.

7. Kaplan E. L. and Meier P. (1958). Non–parametric estimation from incomplete observations. *Journal of the American Statistical Association* **53**:457—481

8. Lee, E.T. and Wang, J.W., (2003). *Statistical Methods for Survival Data Analysis, 3th edition.* Wiley—Interscience.

9. OECD, (2014). Current Approaches in the Statistical Analysis of Ecotoxicity Data, OECD Publishing, Paris.
   DOI: http://dx.doi.org/10.1787/9789264085275—en

10. OECD, (2015). Test No. 240: Medaka Extended One Generation Reproduction Test (MEOGRT). OECD Publishing, Paris.
    DOI: http://dx.doi.org/10.1787/9789264242258—en

11. OECD, (2015). OECD Guidelines for the Testing of Chemicals, Section 2. Test No. 241: The Larval Amphibian Growth and Development Assay (LAGDA). OECD Publishing, Paris.
    DOI: http://dx.doi.org/10.1787/9789264242340—en

12. Ripatti and Palmgren, J. (2000). Estimation of multivariate frailty models using penalized partial likelihood, *Biometrics* **56**:1016—1022.

13. Terpstra, T. J. (1952). The asymptotic normality and consistency of Kendall's test against trend, when ties are present in one ranking. *Indagationes Mathematicae* **14**:327—333.

14. Therneau T., Grambsch P., and Pankratz VS. (2003). Penalized survival models and frailty, *Journal of Computational and Graphical Statistics* **12**:156—175.

15. Westfall P.H. (2014). Kurtosis as Peakedness, 1905–2014. R.I.P.. *The American Statistician* **68**(3):191—195, DOI: 10.1080/00031305.2014.917055

Appendix

Analysis flow chart (Abridged from [10]):