

BIOLOGY 708 - Final Project

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Scientific Background:

Our dataset consists of information on several morphological measurements of Lake Whitefish hatchlings that were reared in different water temperatures. The Age represents the number of days after hatching at which morphological measurements were taken. Treatment values represent the temperature (in degrees Celsius) of the water in which eggs were incubated. After the incubation treatment (post-hatch) larvae were all kept at the same temperature (8 degC). Spawning adults were caught in Lake Huron and stripped of eggs/milt, and fertilized embryos brought back to the lab. Sampling was lethal, so each row represents a different fish (different fish were sampled at different ages). This experiment was repeated several years in a row. This is only 1 year of the dataset, so we are working on acquiring several more years of data. All the response variables are correlated, as they all come from the same individual. As the fish grows, so do all the other body features that we measure (except the yolk, which diminishes). A simple linear model won't account for the fact that these variables are correlated, so I want to be able to fit a model that accounts for the correlations.

Scientific Questions:

1. What is the influence of temperature on length and biomass? How does temperature influence the relationship between length and biomass?
2. What is the relationship between length and other morphological characteristics, and how does temperature affect these relationships?
3. What is the effect of temperature on 'yolk efficiency' (i.e. relationship between growth rate (increase in length or biomass) and reduction in yolk volume/mass over time)?

General Statistical Approach:

We first created correlation plots to better understand the correlations between the different morphological traits in our dataset. Then, since the morphological traits were highly correlated with one another and we wanted to test the effect of temperature on all of them, we decided to create multivariate models. We created different models for the body morphology traits ('Log_Script_No_Yolk.R') and the yolk size traits ('Log_Script_Just_Yolk.R'), because the yolk was depleted after 7-14 days so it didn't make sense to include yolk data beyond this age. However, we wanted to be able to look at the effects on the body morphology traits up to 28 days. Though our data didn't allow us to directly test yolk use efficiency, we created a

multivariate model that just included yolk weight and body weight ('Yolk_Plus_Body.R') so that we could look at how temperature treatment affected these two traits differently in an indirect way. To look at how temperature affected allometry, or specifically the relationship between body length and other morphological characters, we created another multivariate model that included the length as a predictor rather than as a response variable ('Allometry.R'). Finally, as an additional exploration, since the 8 degree temperature treatment is considered extreme and not as biologically relevant, we re-ran the body morphology multivariate model using just the 2 and 5 degree temperature treatments to see if we could see clear differences between the two biologically relevant temperatures ('No_Yolk_No_8C.R').

General Description of Scripts:

We divided our analysis into several scripts that were made to answer different scientific questions. While looking at the structure of our 2016-2017 dataset we realized that dry yolk weights were only collected before the 14 day age point, making all yolk weights beyond 7 days 0.0g. Because of this, we were unable to analyze yolk measurements with the rest of the response variables separated them out and created a second script. The first script (Log_Script_No_Yolk.R) looks at all other response variables, whereas the second script (Log_Script_Just_Yolk.R) only looks at yolk. In an effort to analyze the yolk measurements we created a 3rd script (Yolk_Plus_Body.R) that only used yolk weight and body weight as response variables to simplify the relationship. In this script we were trying to answer whether there is an interaction between temperature and age on the allocation of yolk weight to body weight. One of our important questions was to investigate the relationship between length and the other morphological traits, and in order to do this we created a 4th script (Allometry.R) that incorporated total length as a predictor variable. This allowed us to look at allometry in a relatively simple way. Previously we had discussed the fact that the difference between the 2C and 5C temperature groups is what we are really interested in and is the most biologically relevant, so we created a 5th script (No_Yolk_No_8C.R) that only considered the 2C and 5C treatment groups to compare model results.

Data Cleaning:

Before running our models, we omitted certain variables from the dataset to eliminate perfectly correlated and irrelevant variables. For the body morphology and allometry models ('Log_Script_No_Yolk.R', lines 10-23), we omitted the 'ID' variable, since it was not relevant, the 'Measure Body Length' variable since it was equivalent to the Length variable, the 'Eye Size' variable since it was equivalent to the Eye Diameter variable, the 'Fin indentation ratio' since it was a function of the other fin measurements, the 'total weight' variable, since it was a function of body weight and yolk weight, and the yolk variables, since we were using them in a separate model. We omitted any observations with 'NA' values in any of the morphology

measurements, since we believed that the NA values were distributed fairly randomly throughout the dataset and were likely due to imaging errors during data collection. We also converted all of the response variables to numeric variables (some of them were originally coded as character variables) (lines 25-27). We converted age and treatment to factors (lines 28-29), since although age and temperature are continuous, we only had a few discrete number values for each, so it made sense to treat them as categories.

For the yolk morphology model ('Log_Script_Just_Yolk.R', lines 10-25), we omitted the body morphology variables since we weren't using them in this model. We also omitted 'yolk volume', since it was a function of yolk height and width, and omitted the '% yolk weight' variable since it was a function of the yolk weight variable. We divided the width and height values by 1000 to get their values in mm, to make later interpretation easier. Similar to the body morphology models, we removed any observations containing NA values and converted treatment and age to factors. We then removed all observations for ages other than 0 and 7, since the yolk weight was reduced to zero after these ages.

We verified that the covariance matrices for body morphology and yolk morphology were not of full rank (no perfectly correlated variables) by calculating the product of the eigenvalues for the body morphology covariance matrix ('Log_Script_No_Yolk.R' lines 65-67) and for the yolk morphology covariance matrix ('Log_Script_Just_Yolk.R', lines 50-52). Neither of the products were vanishingly small, confirming that there were no perfectly correlated variables.

In the model using body weight and yolk weight as response variables, we eliminated all variables except the yolk weight, body weight, treatment and age ('Yolk_Plus_Body.R', lines 12-23). We eliminated any observations with NA values, used only the observations from ages 0 and 7 days (since yolk weight was zero beyond 7 days) and converted treatment and age to factors, similar to the other models.

Transformations:

Body morphology ('Log_Script_No_Yolk.R'):

Since some of the relationships between morphological traits appeared to be non-linear and there was heteroscedasticity in the models created using the un-transformed data (see diagnostics below), we decided to log-transform all of the response variables (lines 44-53). This increased the linearity of the relationships between morphological traits and, in some cases, reduced the heteroscedasticity in the data (discussed further below). Furthermore, the log scale is usually more appropriate for biological phenomena, including allometric relationships. Thus, we created our multivariate models using the log of the morphological response variables.

Yolk Size ('Log_Script_Just_Yolk.R'):

For all models that included response traits related to yolk size (including the model using body weight and yolk weight as the response variables), we chose to scale the response variables by their standard deviations rather than using the log transformation (lines 40-45). This is because even at age 7 days, some fish had a yolk weight of zero (it was below the detectable level) and so a log transformation was not possible. Scaling allowed us to compare effects of treatments on all the response variables on a common scale.

Box-Cox Transformations:

Since there was still some heteroscedasticity in the model after the log-transformation, we explored the use of a box-cox transformation instead of a log-transformation for the body morphology data ('Log_Script_No_Yolk.R'). We used the 'boxcox' function on separate linear models constructed for each response variable to determine the optimal lambda value for each (e.g. Figure 5), and then created a new dataset where we transformed each response variable using its respective optimal lambda value and the equation $Y = (X^{\lambda} - 1)/\lambda$ (lines 87-130). However, many of the optimal lambda values provided by the boxcox function were negative, suggesting an inverse power law relationship between the predictors and the morphological trait, which did not make much biological sense. Furthermore, when we created the diagnostic plots for the models using the box-cox transformed data (lines 133-147), the transformations did not help meet the model assumptions. In fact, in some cases they caused the residuals to be less normal and the data to be more heteroscedastic. Therefore, we did not use the box-cox transformed data in our multivariate linear models and did not attempt this transformation in later models (e.g. the model not including the 8 degree temperature treatment).

Correlation Plots, etc:

We knew in advance our response variables would be highly correlated, which is why we decided to use multivariate statistics in the first place. We created a few different plots to analyze the correlations between response variables in order to better understand the nature of the relationship.

Log_Script_No_Yolk.R:

Lines 40 + 57 (pairs plot)

We started with a pairs plot, as this is a simple way to visualize correlations between variables. These plots (unlike the corplot) also communicate how linear the relationship is, or if it exponential. We created two pairs plots, one before and one after the data transformation to show how it influenced correlations. The ideal pairs plot would show randomly distributed points about the center value, however most of our plots showed an increasing linear trend, suggesting correlations between variables. Some of our plots hinted at an exponential relationship, which was accounted for by log transforming the data.

Lines 36-41 (corrplot)

The results of the pair plots suggested correlations, so this plot displays the extent of correlation between each pair of variables. The pairs plot is showing the "shape" of the relationship, whereas the corrplot shows the "magnitude" of correlation. This plot showed that most of our variables are highly correlated, with body length and weight being the most highly correlated. Most relationships are positive, however Fin_Min tends to have a negative relationship since it decreases as the fish grows.

Lines 61-63 (scatterplot)

This plot helps us compare the different correlation "shapes" between our treatment groups. Using this plot we can determine if the relationship between variables is affected by our temperature treatment. If the shape/size of the ellipses are different than this suggests an effect of treatment. Most of the plots have altered ellipse shapes, which infers a strong effect of temperature on the correlations between response variables. We chose not to display the regression line with the ellipse, as it makes it more confusing to interpret and doesn't add much info.

Multivariate Models:

We used the `lm()` function to create all of the multivariate models since we didn't have any random effects to add to the model. Age and temperature treatment (as factors) were used as fixed effects, and we included the interaction between age and treatment in the models, since we expected that the effect of temperature may differ between ages.

Body Morphology ('Log_Script_No_Yolk.R'):

We incorporated a matrix of our seven log-transformed body morphology response traits into the model, using temperature treatment and age as predictors (line 150). We produced a summary of the model, using the Wilks test statistic, as this is one of the most commonly used test statistics for this scenario in biology (line 151). We then printed the coefficients for the model (line 152). To understand the effects on a biologically relevant scale, we back-transformed the coefficients by exponentiating them (line 153). The model of body morphology in which we removed the data for the 8 degree treatment was identical, but just used only the 2 and 5 degree treatment data ('No_Yolk_No_8C.R', lines 95-98).

Yolk Morphology ('Log_Script_Just_Yolk.R'):

We incorporated a matrix of our three scaled yolk size traits (width, height and weight) into the model, using temperature, age and their interactions as predictors (line 64). Similar to the body morphology model, we produced a summary using the Wilks test statistic, and then printed the model coefficients (lines 65-66). To understand the effects on a biologically relevant scale, we

back-transformed the coefficients by multiplying the coefficient for each response variable by its standard deviation (lines 69-71).

Allometry ('Allometry.R'):

This model was similar to the body morphology model described above, except that the logged 'length' variable was incorporated as a predictor rather than a response variable (lines 48-51). We included all interactions in the model, as the interaction between temperature and length would tell us how temperature influenced the relationship between length and the other morphological traits.

Body Weight and Yolk Weight ('Yolk_Plus_Body.R'):

In this model, the scaled body weight and yolk weight variables were included as response variables, with treatment and age as categorical predictors (line 44). We included the interaction between treatment and age, as above. Similar to the other models, we printed the summary using the Wilks test statistic and then printed the coefficients (lines 45-46). We also back-transformed the coefficients by multiplying the coefficients for each response variable by its respective standard deviation (lines 49-50).

Diagnostics:

Log_Script_No_Yolk.R --> lines 69 - 84

Most packages that produce diagnostic plots are not able to accommodate a 'mlm object' so our options for diagnostic plots were somewhat limited. We chose (with the advice of Dr. Dushoff) to look at individual lm diagnostic plots instead of trying to produce a single mlm diagnostic plot on our own. There are 4 plots that are produced from the default plot() function including: 1) Residuals vs Fitted - linearity, 2) Q-Q plot - normality, 3) Scale-Location - Heteroscedasticity, 4) Residuals vs Leverage - Cook's Distance. The most important plot is (1), and any evidence of non-linear patterns would suggest a problem with the model. After looking at (1) we would then look at (3) and (4), then finally (2). While we considered normality to be the least important model assumption, it was the only diagnostic plot to be consistently off. Some of the plots also showed a degree of heteroscedasticity, but it was not consistent. That being said we did not interpret any of the diagnostic plots as "alarming" and continued fitting our mlm.

Yolk_Plus_Body.R --> lines 54 - 63

Near the end of the project we were experimenting with sjplot to produce different styles of effect plots, and discovered an additional diagnostic plot that is not included with the default function. This plot analyzes Variance Inflation Factors (multicollinearity), and some of our response variables are in the "tolerable" range for multicollinearity. The wikipedia page says "a multivariate regression model with collinear predictors can indicate how well the entire bundle of predictors predicts the outcome variable, but it may not give valid results about any individual

predictor, or about which predictors are redundant with respect to others." This seems like a strange relationship for Age and Temperature to have (our only predictors) given that one could never be used to predict the other... but maybe we are interpreting it wrong?

Dr. Dworkin suggested that multivariate models very rarely will meet the assumptions of a linear model, so other approaches like permutations and bootstrapping are employed to validity of results.

Permutation Approach Using Geomorph package:

Since there was still some heteroscedasticity in our data that we weren't able to eliminate using transformations and we were worried that our inability to meet model assumptions may affect the results, we decided to use permutation to calculate p values for the effects of age and temperature treatment. The first approach we used was using the `procD.lm` function from the `geomorph` package. We used the same response variables and predictor variables as above, and 5000 iterations. In all cases, printing the summary revealed that the p values were still very low, and the coefficients were essentially identical to those obtained using the regular `lm` function ('Log_Script_No_Yolk.R' lines 168-171; 'Log_Script_Just_Yolk.R' lines 86-89; 'Yolk_Plus_Body.R' lines 80-83; 'Allometry.R' lines 76-79; 'No_Yolk_No_8C.R' lines 117-120).

Manual Permutation Approach:

We randomly sampled (without replacement) our values 1999 times and ran the same model to determine the likelihood that our model results were due to chance alone. We are essentially creating a null distribution with which we can compare our actual results. A histogram was produced showing the null distribution of the test statistic, and our actual test statistic was included as a red line for reference. For all of our permutation tests the p-value result was 1/1999, meaning it was incredibly unlikely that the extremely low p-value we got from our model was due to chance (basically, none of the randomly generated test statistics were as high as the one produced by the original model). The permutation tests confirmed that our model results were reliable.

Results:

Body Morphology ('Log_Script_No_Yolk.R'):

Based on the summary for the multivariate model incorporating the body morphology traits (line 151), both temperature treatment and age influenced the body morphology variables, as did the interaction between temperature and age. The p values for the manova using the Wilks test statistic was $< 2.2e^{-16}$ for both of the main effects and $2.256e^{-16}$ for the interaction (Figure 7).

We exponentiated the model coefficients so that we could more easily interpret them (line 153). A first glance at the coefficients shows that, in general, increasing the incubation temperature decreased the size of all body morphology measurements, while these measurements all increased with age. The exception to this was 'Fin_Min', which is a measure of the minimum fin width between the posterior and anterior fins and which decreases with age as the two fins become more separate. However, based on the coefficients and the effect plots (line 165, Figure 8), temperature appears to affect the different morphological traits in different ways (and thus should influence the relationships between the different morphological traits). For example, at the average age, the 5 degree temperature treatment decreases length by 5% over the 2 degree treatment, and the 8 degree temperature decreases length by 8% over the 2 degree treatment. In contrast, temperature has a much greater effect on the anterior fin width ('Fin_Anterior'), reducing its size by 11% in the 5 degree treatment and 24% in the 8 degree treatment. The effect plots also allow us to view the interaction between temperature and age. The interaction effect is perhaps seen most clearly in the plots of Fin_Min and Fin_Posterior. In the Fin_Min plots, we can see that while the minimum fin width decreases gradually from 1 to 14 days in the 2 degree treatment, it remains the same during this time period in the 5 degree treatment and even shows some sign of increasing during this time period in the 8 degree treatment. In the Fin_Posterior plot, the posterior fin width does not increase in size in the 2 degree treatment until the 7 to 14 day period, while in the 5 and 8 degree treatments, it increases in size mostly during the 0 to 7 day period. These effects can also be visualized using the boxplots we created using the untransformed data ('ggplots.R', lines 8-41; Figure 9).

We calculated the lengths of the contrast vectors for treatment, age, and their interaction (lines 156-158) so that we could get a sense of the relative effects of each. The length of the interaction contrast vector was largest, suggesting it had the greatest effect on the response variables, while the length of the Treatment vector was smallest, suggesting it had the least effect (less than age and the interaction between treatment and age). We also calculated the coefficient of determination (line 161), which is roughly equivalent to the R squared in a regular anova model. The value was ~0.70, so 70% of the variation in the response variables was explained by the model.

The permutation tests executed using both the `procD.lm` function from `geomorph` (lines 168-171) and the manual approach both (lines 176-200) resulted in very small p values for all predictor terms – in fact, none of the iterations resulted in a test statistic equal to or greater than the true statistic from the original model. The coefficients from the `procD.lm` function matched those from the original model created using the `lm` function (line 171).

There were no major differences in our results when we re-ran the model using only the 2 and 5 degree treatments ('No_Yolk_No_8C.R'). The p values for all terms were very small (line 96),

the estimated coefficients for the 5 degree treatment were identical to the original model, as expected (lines 97-98), and the model explained a similar amount of variance, ~68% (line 106).

Yolk Size Traits ('Log_Script_Just_Yolk.R'):

Similar to the body morphology model, the main effects of treatment and age and the interaction between them were highly significant based on the results of the manova on the model (lines 62-63). The p values based on the Wilks test statistic for the main effects of temperature treatment and age were both $< 2.2e^{-16}$ and the p value for the interaction was $4.83e^{-6}$.

By examining the coefficients from the model using the scaled data (line 66), we can see that yolk size is larger in the higher temperature treatments, and it decreases with age. Since these coefficients have been produced with the scaled data, we can compare them to see how the effect of temperature varies among the response variables. Based on these coefficients and on the effect plots (line 83), we see that temperature treatment has the greatest effect on yolk weight (with the largest positive coefficients for both the 5 and 8 degree treatments), while it is yolk height that decreases the most with age (i.e. yolk height has the most negative coefficient for the 7 day age). We then back-transformed the model coefficients for each response variable by multiplying by each response variable's standard deviation (since scaling involves dividing by the standard deviation) to be able to better interpret the effects on a biologically relevant scale (lines 69-71). When the incubation temperature is increased from 2 to 5 degrees, yolk width increases by ~0.25mm, yolk height increases by ~0.20mm and yolk weight increases by ~0.22g. When the incubation temperature is increased from 2 to 8 degrees, yolk width increases by ~0.44mm, yolk height increases by ~0.33mm and yolk weight increases by ~0.33g. Based on the effect plots (line 83), the most obvious interaction between treatment and age is visible in the yolk width plot, where at 0 days post-hatch there are similar differences between the 2 and 5 degree treatments and the 5 and 8 degree treatments, while at 7 days post-hatch there is a greater difference between the 2 and 5 degree treatments than the 5 and 8 degree treatments. These effects can also be visualized using the boxplots we created using the unscaled data ('ggplots.R', lines 43-56; Figure 10).

Calculation of the length of the contrast vectors for treatment, age and their interaction (lines 74-76) revealed a similar pattern as in the body morphology model, with the interaction having the largest value (greatest effect) and temperature treatment having the smallest value/effect. Calculating the coefficient of determination (line 79), we can determine that the model accounted for 52% of the variance in the response variables.

As with the body morphology model, permutation tests gave similar results to the original model (lines 92-119).

Allometry Model ('Allometry.R'):

When we incorporated the logged length variable as a predictor instead of a response variable, we found significant effects (indicated by very low p values) of treatment, age, length and all of their interactions on the response variables after running a summary on the manova using the Wilks test statistic (line 49).

The purpose of this model was to look specifically at length, and the effect of treatment on the relationship between length and the other morphological traits, which can be investigated by looking at the treatment*length interaction. The coefficient values (lines 50-51) show that the relationship between length and the other morphological traits does indeed vary with treatment, as the coefficients for the interaction between treatment and length differ (i.e. 'Treatment5:Length' is different from 'Treatment8:Length'). This is made even more clear when viewing the effect plots (lines 60-63), which we separated by predictor term since they were too difficult to view when all three predictors were shown together. In particular, in the treatment*Length effect plots (line 63), we can see that for most variables, the relationship between log(length) and the morphological trait being examined is less positive in the 5 and 8 degree treatments compared to the two degree treatment. The 'Fin_Min' variable shows an even more interesting relationship to log(length): the relationship is positive in the 2 degree treatment, negative in the 5 degree treatment and positive again in the 8 degree treatment. Thus, incubation temperature clearly influences the allometric relationships between length and other morphological traits. We also visualized these relationships by creating ggplots showing the relationship between the untransformed length and other body morphology characters ('ggplots.R', lines 61-101; Figure 11).

Calculating the length of the contrast vectors shows that length and the three way interaction between length, treatment and age have the largest effects, while the interaction between age and length has the smallest effect. The coefficient of determination is ~ 0.77 , so the model accounts for 77% of the variation in the responses.

The permutation tests produced similar results when compared to the original model (lines 75-133).

Model Incorporating Body Weight and Yolk Weight ('Yolk_Plus_Body.R'):

Similar to the other models, in this model that incorporated body weight and yolk weight as the response variables, the main effects of treatment and age and their interaction were all significant, with very low p values ($< 2.2e^{-16}$) calculated using the Wilks test statistic (line 45).

To interpret the effects, we first back-transformed the coefficients. As we already knew from previous models, body weight is lower and yolk weight is higher in higher temperature treatments. Also, while body weight increases with age, yolk weight decreases. The main purpose of this model, however, was to compare the effects of temperature and age on body

weight and yolk weight. We can do this using the coefficients (lines 46-50) and the effect plots (line 73). While increasing the temperature treatment from 2 to 5 degrees reduces body size by $\sim 0.18\text{g}$, it increases the yolk size by a great amount, $\sim 0.22\text{g}$. In contrast, increasing the temperature treatment from 2 degrees to 8 degrees reduces body size by $\sim 0.38\text{g}$, while it increases yolk size by a smaller amount, $\sim 0.33\text{g}$. Furthermore, while body weight is, on average, 0.85g larger at 7 days post hatch compared to 0 days post hatch, yolk weight is only reduced by $\sim 0.46\text{g}$ at 7 days post hatch compared to 0 days post-hatch. Finally, examining the effect plots reveals that at 7 days post-hatch, there are strong differences in body size between temperature treatments, but weaker differences in yolk size between treatments. We also visualized the relationship between body weight and yolk weight by creating ggplots, which clearly show differences in the relationship between body weight and yolk weight across treatments and ages ('ggplots.R', lines 105-116; Figure 12). Overall, although we can't directly investigate how yolk use efficiency changes with temperature treatment using this data, we can say that treatment and age seem to affect body weight and yolk size differently, so it might be worthwhile to investigate this further in future studies.

Examining the lengths of the contrast vectors (lines 53055), we can see that, similar to the other models, the interaction between treatment and age has the greatest effect, while the main effect of treatment is the weakest. Based on the coefficient of determination (line 58), the model explains 71% of the observed variance.

P values calculated using permutation tests were also very low, and the coefficients estimated using the geomorph package were essentially identical to those produced by the original model (lines 85-112).

General Conclusions

We can use our statistical analysis to address our original scientific questions:

1. Increasing temperature decreases length and biomass at a given age, and makes the relationship between length and biomass less positive. Increasing temperature also decreases the size of many other body morphology traits, but increases yolk size at a given age.
2. Length is highly positively correlated with most other body morphology traits, but temperature affects this relationship. In general, the relationship between length and other morphological characters was less positive in higher temperature treatments (Figure 13).
3. We could not determine the effect of temperature on yolk efficiency directly using this dataset, as it did not follow individuals over time. However, based on our model incorporating yolk

weight and body weight, temperature has different effects on these different traits, so there is indirect evidence that it influences the ability for a fish to convert yolk mass into body mass.

Selected Examples of Plots and Output Tables:

We have selected examples of the different plots we created to show here and are generally showing one example per type of plot, rather than showing similar plots for all models. References to the code for the plots not shown here are provided when discussing them in the text.

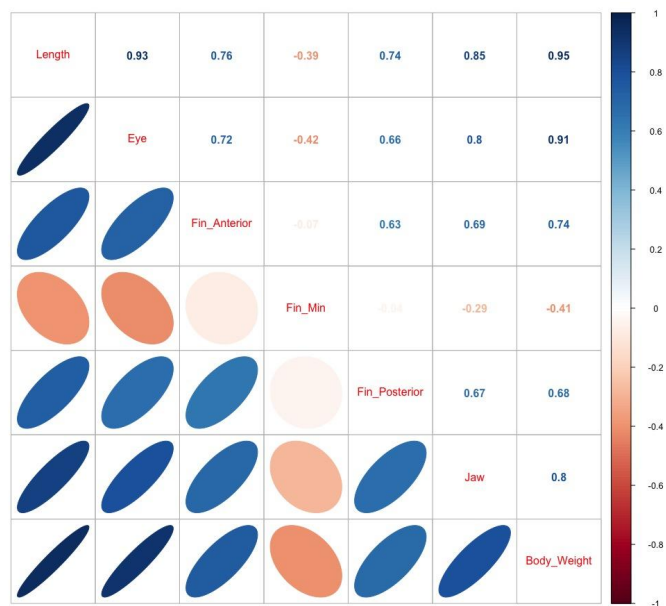


Figure 1. Correlation plot showing the correlation between various body morphology traits in lake whitefish (code = ‘Log_Script_No_Yolk.R’ lines 37-38).

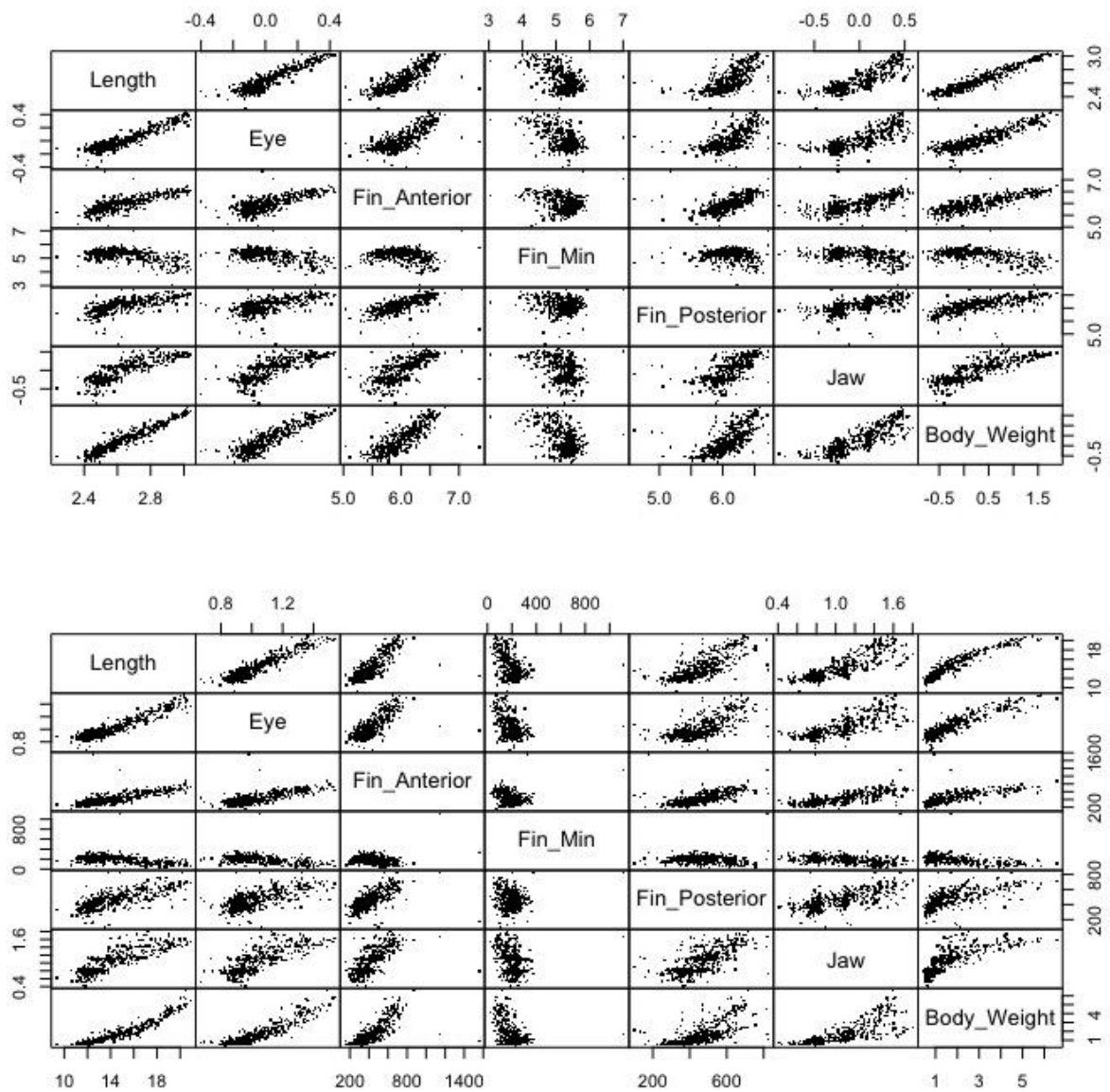


Figure 2. Untransformed (top) and log-transformed (bottom) measurements of body morphology traits in lake whitefish. Each point represents one fish. (code = 'Log_Script_No_Yolk.R' lines 40-41, 57-58).

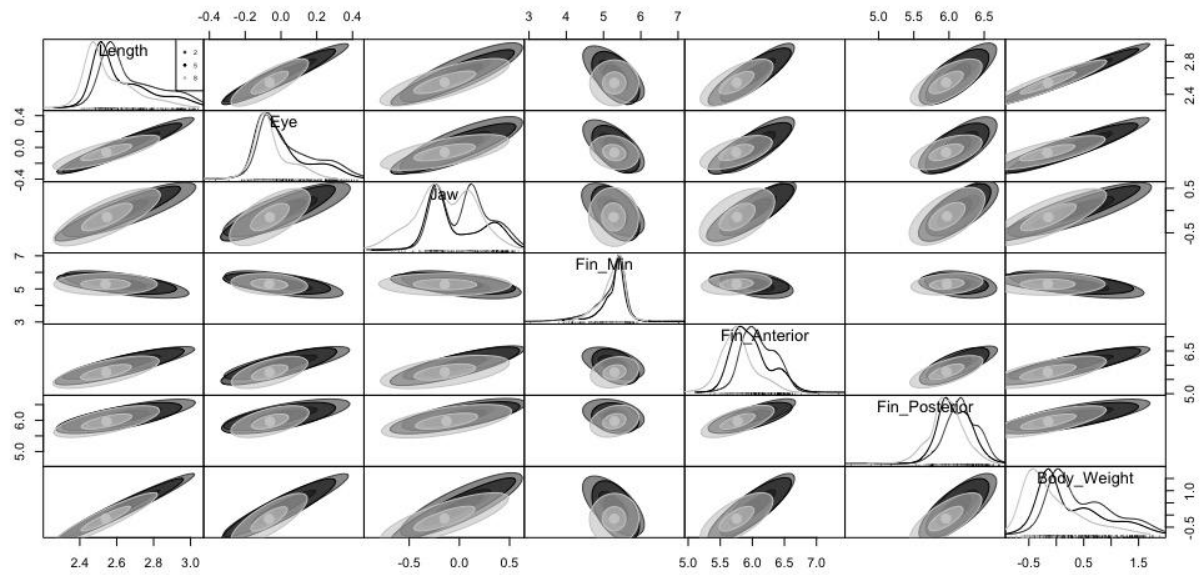


Figure 3. Scatterplot matrix showing the relationships between various body morphology measurements in lake whitefish, with different colours representing different incubation temperature treatments (2, 5 and 8 degrees Celsius). (code = 'Log_Script_No_Yolk.R' lines 61-63).

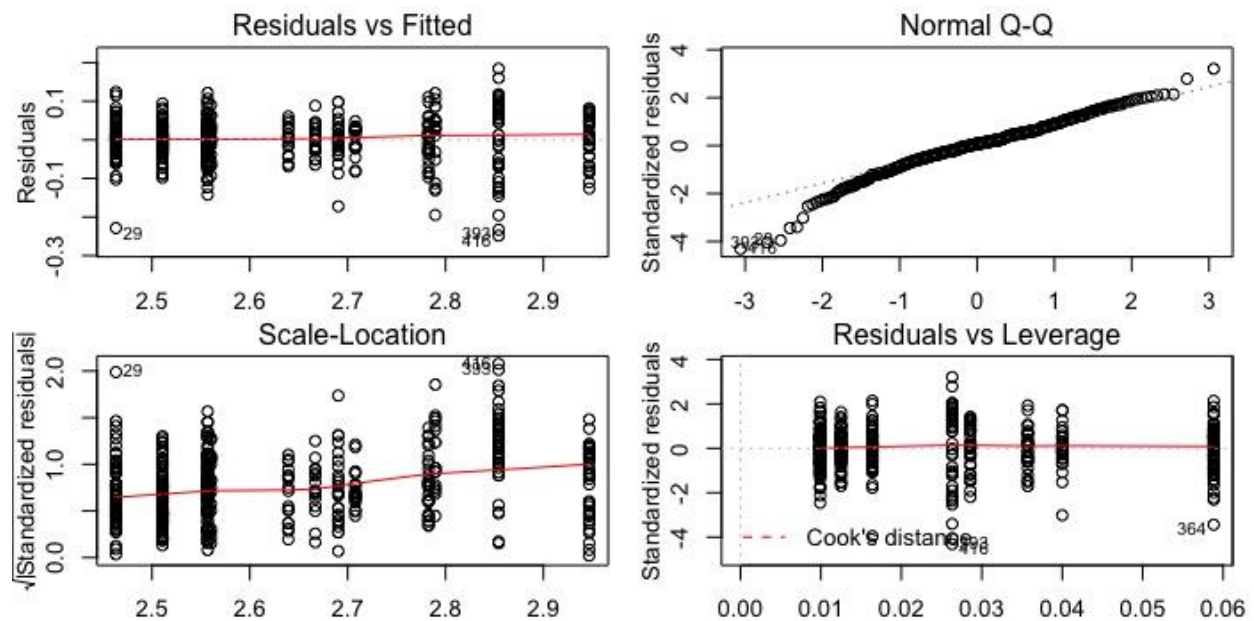


Figure 4. Diagnostic plots for the model Body length ~ Treatment*age. (code = 'Log_Script_No_Yolk.R' lines 70-72).

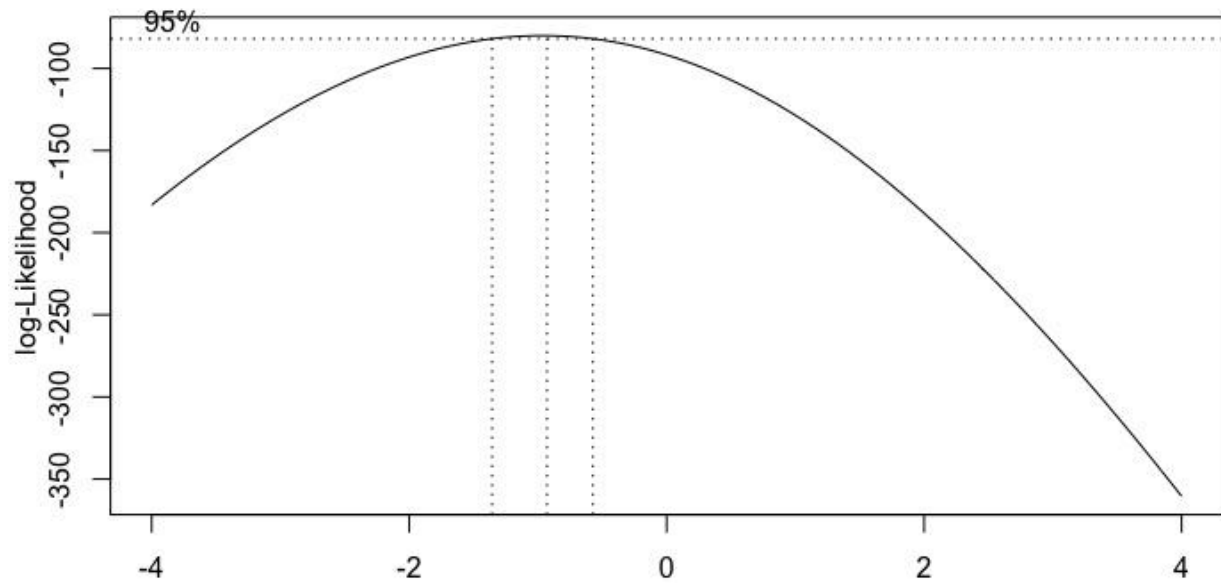


Figure 5. Box-cox plot showing the log-likelihood of the linear model 'Body Length ~ Treatment*age' using different lambda values to transform Body length using the equation $(\text{Length}^\lambda - 1)/\lambda$. (code = 'Log_Script_No_Yolk.R' line 87).

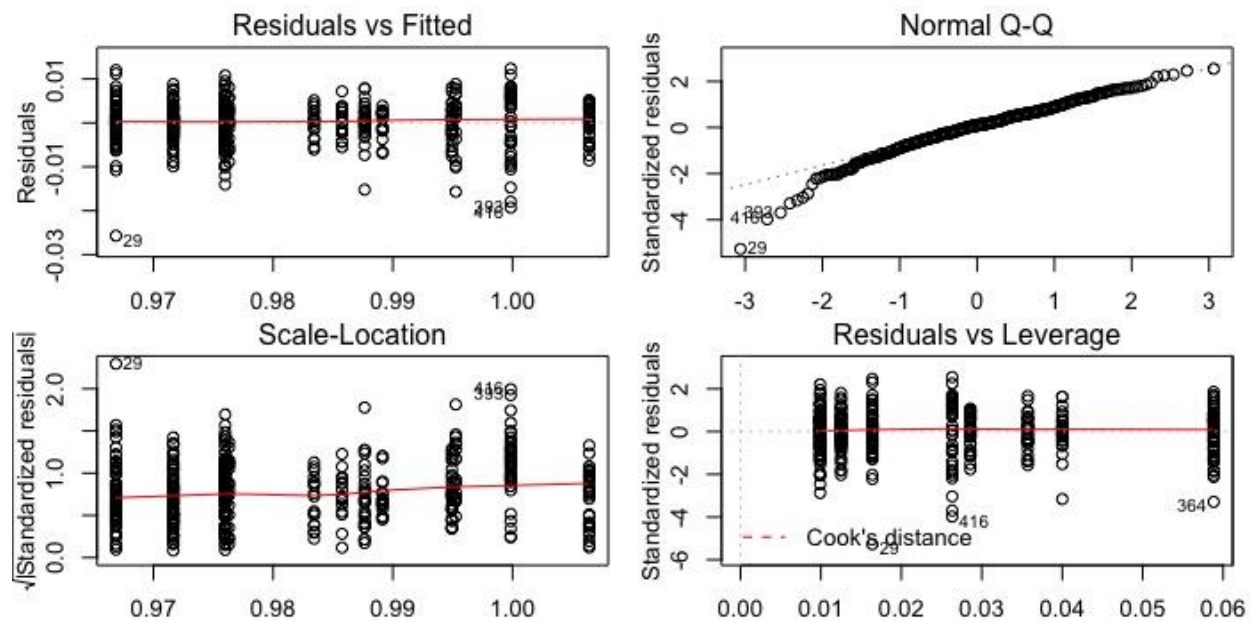


Figure 6. Diagnostic plots for the model Body length ~ Treatment*age after body length has been transformed using the box-cox equation. (code = 'Log_Script_No_Yolk.R' lines 87-90, 122-130, 133-135).

	Df	Wilks approx F	num Df	den Df	Pr(>F)
Treatment	2	0.46591	28.899	14	870.0 < 2.2e-16 ***
age	3	0.07476	87.322	21	1249.6 < 2.2e-16 ***
Treatment:age	6	0.68841	4.032	42	2043.8 2.256e-16 ***
Residuals	441				

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 7. Summary of the manova on the multivariate linear model using temperature treatment, age and their interaction as predictor variables and using 7 body morphology traits as response variables. (code = 'Log_Script_No_Yolk.R' lines 150-151).

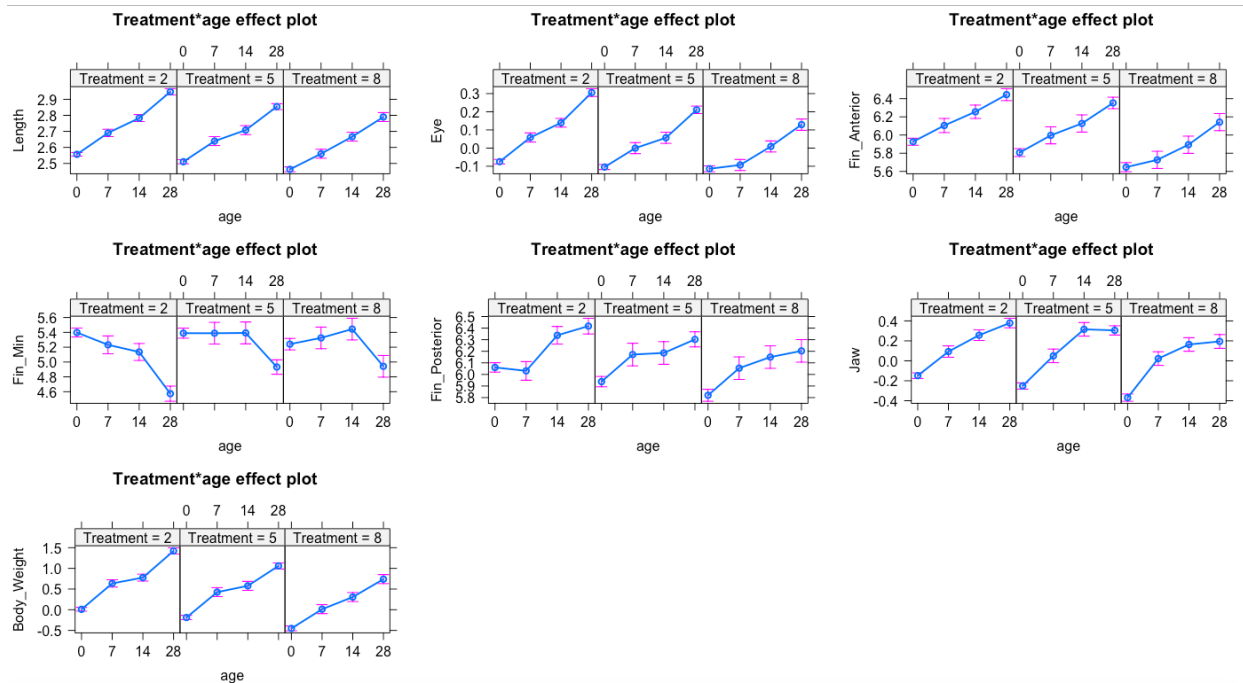


Figure 8. Effect plots based on the multivariate linear model incorporating age and temperature treatments as predictors and body morphology traits as responses. (code = 'Log_Script_No_Yolk.R' line 165)

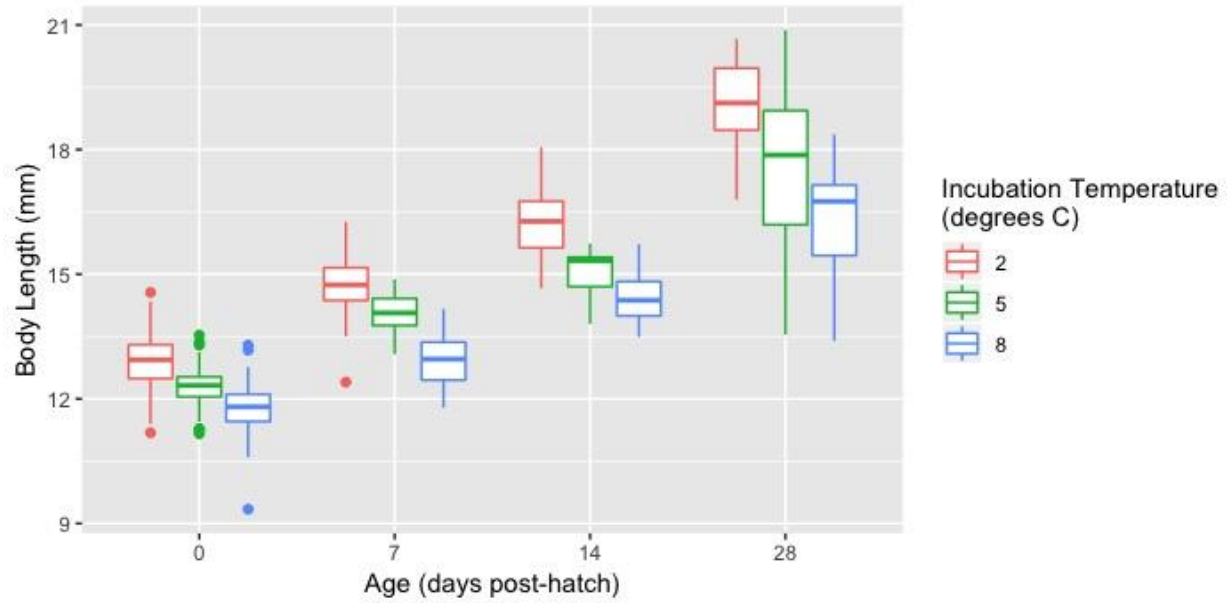


Figure 9. Boxplot of lake whitefish body lengths at different ages and from different incubation temperature treatments. (code = 'ggplots.R' lines 8-11)

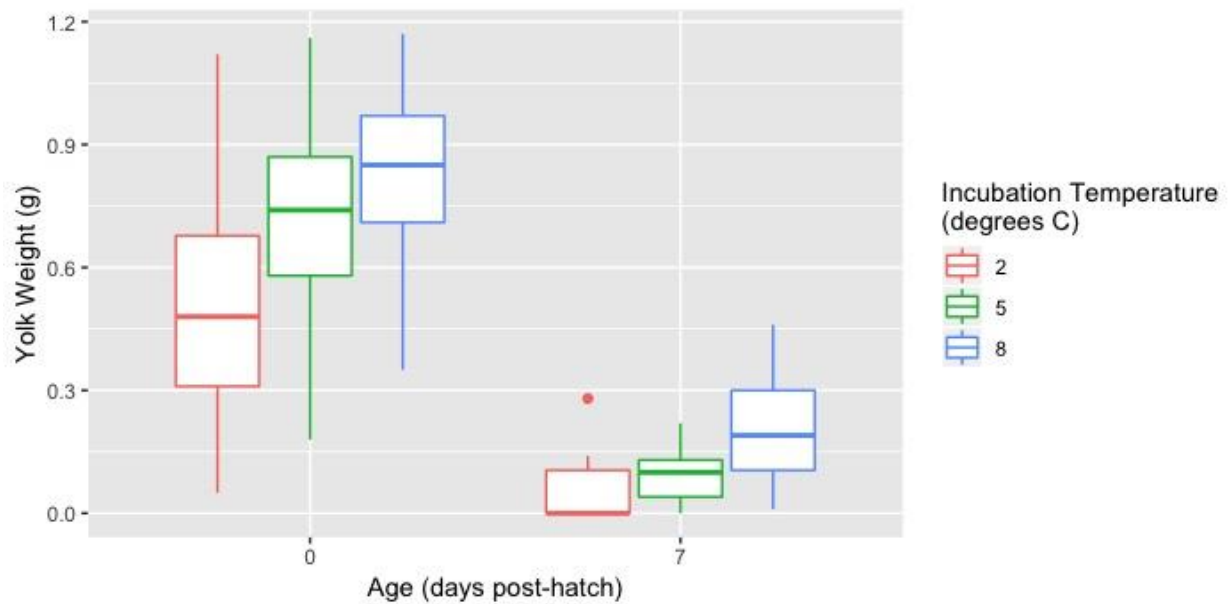


Figure 10. Boxplot of lake whitefish yolk weights at different ages and from different incubation temperature treatments. (code = 'ggplots.R' lines 43-46)

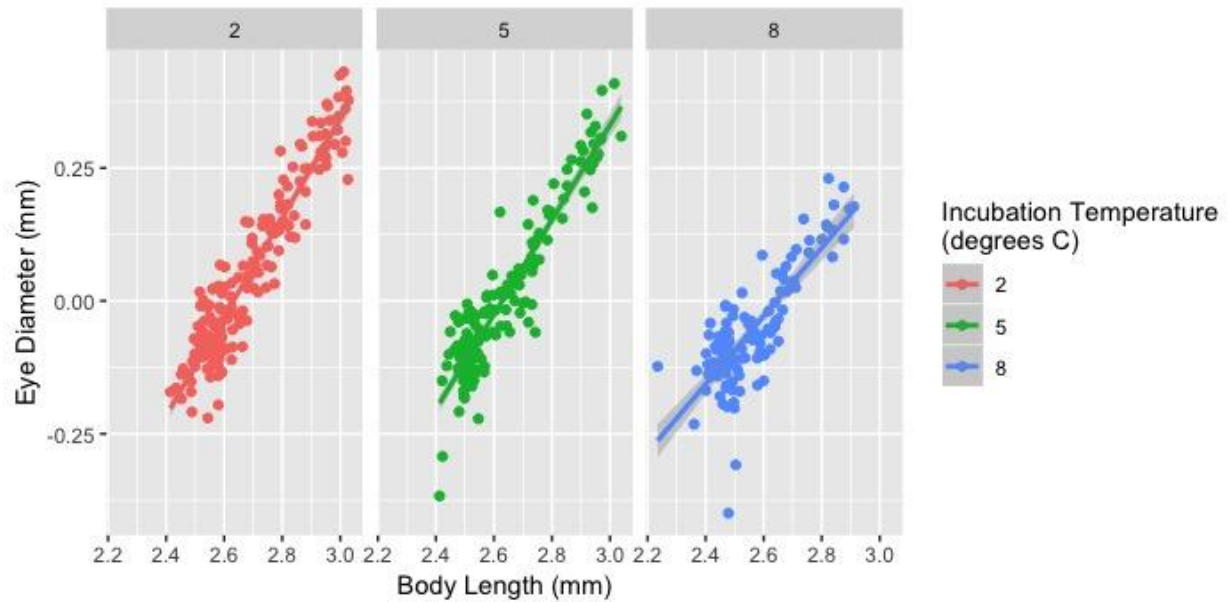


Figure 11. Plot of lake whitefish body length vs eye diameter in different incubation temperature treatments. (code = 'ggplots.R' lines 89-94)

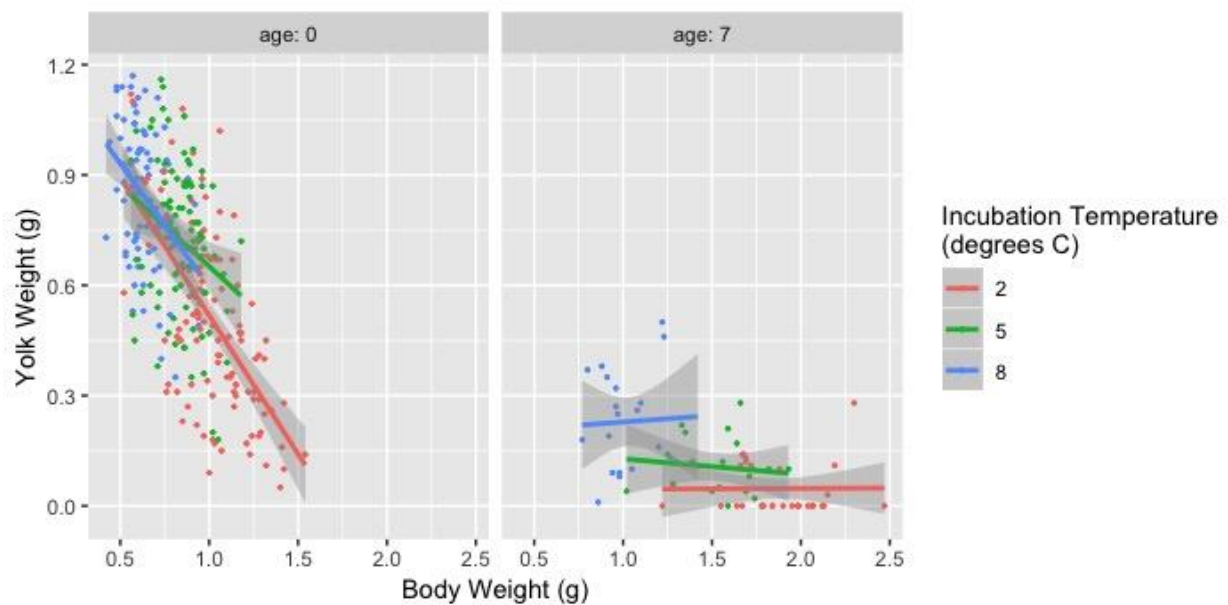


Figure 12. Yolk weight vs. body weight for lake whitefish at different ages and from different incubation temperature treatments. (code = 'ggplots.R' lines 111-116)

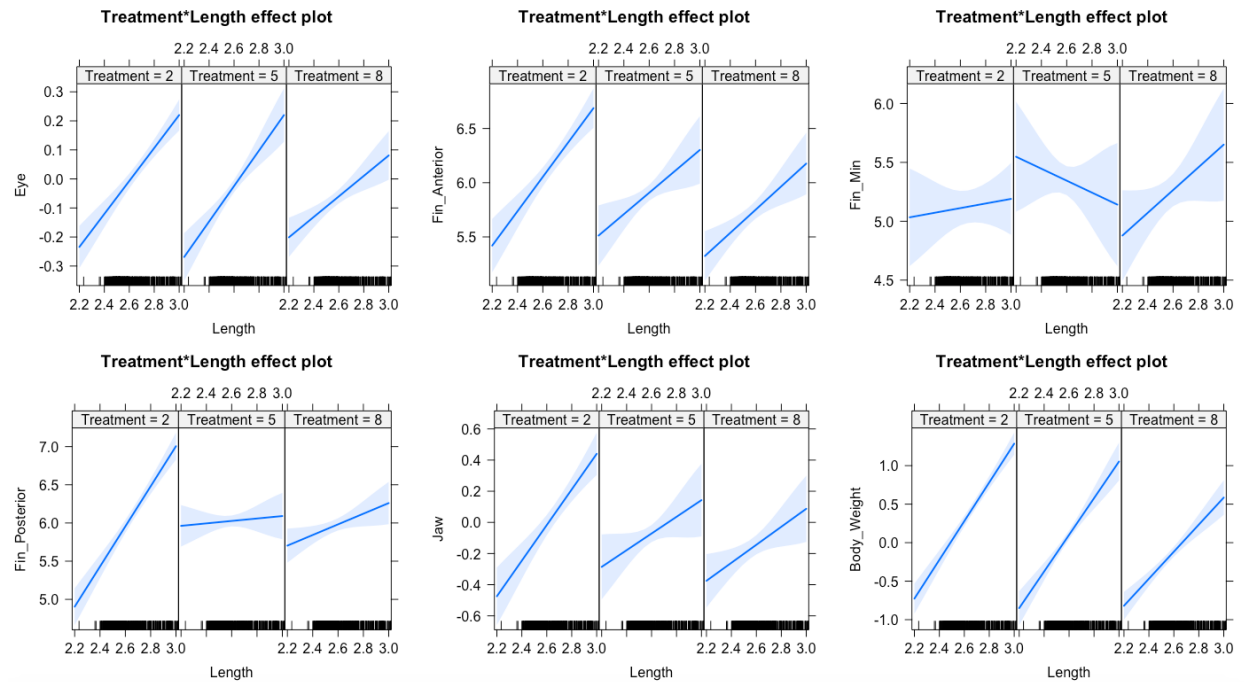


Figure 13. Effect plots based on the multivariate linear model incorporating age, temperature and log(length) treatments as predictors and body morphology traits as responses. (code = ‘Allometry.R’ line 73)