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Regression Analysis

Final Project

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Abstract

Macroinvertebrate biomass is considered an ecological indicator of stream health and is often used to estimate the energy available in a system for use by organisms throughout many different trophic levels. The traditional way of estimating macroinvertebrate biomass is converting specimen length measurements to ash-free dry mass and then to caloric content. Though a well-accepted and used tool for measuring the energetics of aquatic systems, length to dry mass relationships may suffer from inaccuracy due to complex morphological differences between taxonomic levels and the lack of a standard procedure for acquiring length measurements. This study investigates whether other size measurements of macroinvertebrates, such as surface area, may more-accurately predict dry mass and provide less-subjective measurement through computer-based measurements from images rather than measurements done by hand. The study also considers whether the relationship between size and mass of macroinvertebrates is more linear or following a natural log pattern. Trichoptera specimens were collected Sweden and the Midwest United States, measured for size using a camera-based software program, preserved in various mediums, re-measured, dried and weighed. Linear and natural log-based regressions were created for both surface area and length to dry mass relationships, and the two were compared. Overall surface area to dry mass relationships were stronger than length to dry mass relationships, and the natural log models were more accurate than the linear models. The results of this study suggest that traditional methods for measuring macroinvertebrates may not be the most accurate, and that

current studies that use length to dry mass relationships established from literature may benefit from conducting their own measurements using surface area instead of length.

Introduction

Macroinvertebrates, the larval stage of an insect's life cycle, contribute immensely to the ecosystem energetics of marine and aquatic systems. Because of this, measuring and interpreting the energetic content of these water-based insect larvae can greatly assist scientists in studying energy-associated ecosystem processes such as predator-prey selectivity and trophic-level energetic transfer (Gilinsky 1984, Oertli 1993, Turesson et al. 2002, Zhao et al. 2005, Helmus et al. 2013). The standard length-to-mass relationship has been used since the 1980's to relate macroinvertebrate size to dry mass, which is then converted to caloric content via relationships measured and recorded since the 1960's (Cummins and Wuycheck 1971, Smock 1980, Brodmann and Reyer 1999, Wilt et al. 2014).

What is not standard, however, is the protocol for measuring the length of the macroinvertebrate specimen; measurements may include, but are not limited to, the length from the anterior labium to posterior of the last abdominal segment of the insect larvae, the width of the mesothorax, labium and head capsule, and any other measurements the researcher deems it fit to add (Sample et. al 1993, Johnston and Culjak 1999, Eklöf et al. 2017). Lengths can be determined by hand-measuring, using simple digital imaging and analysis software to compute the measurements or by taking measurements from previous literature (Sample et. al 1993, Paavo et al. 2008, Velghe and Gregory-Eaves 2013, Pond et al. 2016).Because of the wide variety of unique body parts and length-mass relationships between macroinvertebrate taxa, it is generally accepted that length-dry mass relationships must be taxon specific at the very least to the family level (Smock 1980, Mährlein et al. 2016, Eklöf et al. 2017). Because of the wide variety of measurement techniques for macroinvertebrate length-dry mass relationships that are oftentimes researcher-

specific, getting accurate, reusable relationships has proven incredibly difficult. Additionally, the wide variety of morphological characteristics between macroinvertebrate families makes standardizing length measurement protocols nearly impossible (Image 1).

Image 1. Image illustrating the unique morphologies of different macroinvertebrate families. Images taken at 1x power with a Motic 3.0 Camera. Size bars for each macroinvertebrate are included. Macroinvertebrate familes from left to right: Ephemeroptera, Plecoptera, Trichoptera. Images taken by Rachel Prokopius using a Motic 3.0 camera.

With advancements in technology and increased access to inexpensive computer programs for analyzing images (for example, ImageJ), it is possible to obtain potentially more-accurate measurements for aquatic organisms, namely the surface-area of the organism. Because of the variety of morphological characteristics unique to certain macroinvertebrate families and the relative subjectivity of length measurements, measurement of surface area from macroinvertebrate images may be a better measurement when compared to the standard length measurements. Previous analysis of the dataset obtained for this study have suggested this hypothesis to be true. However, visual analysis of the relationship between both surface area and length to

macroinvertebrate dry mass appear to be more curvilinear than linear. Therefore, this project aims to determine whether a transformed model is statistically a better predictor of dry mass from surface area and length of macroinvertebrates. It is hypothesized that the comparison of measurement methods for caddisfly larvae (Trichoptera) will show that surface area is a stronger predictor (i.e. has a higher R^2 value) of macroinvertebrate dry mass than length when the surface area and dry mass measurements are transformed using a natural log function.

Methods

Samples were taken once a month from November 2017 through May 2018 from Ranån, a stream about forty minutes north of Karlstad, Sweden that connects to Klarälven, the large river present in the area (Figure 1). Similar data was collected and analyzed in the Northern Kentucky Area, in a tributary of the Ohio River known as Four Mile Creek. Data was collected in October of 2018 (Figure 2).

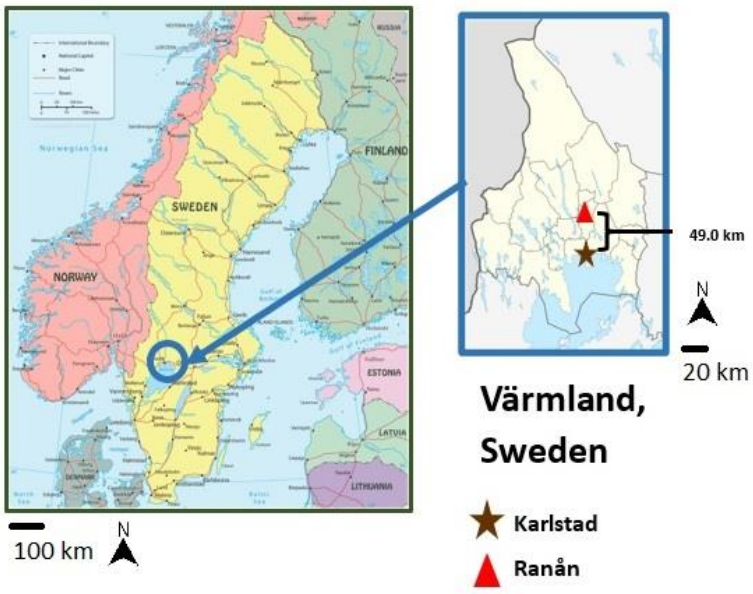

Figure 1. Maps of study area in Värmland, Sweden. Scale bars and north arrows are supplied for each map portion in the figure. Maps supplied by http://www.geographicguide.com/europe-maps/sweden.htm and https://en.wikipedia.org/wiki/V%C3%A4rmland_County.

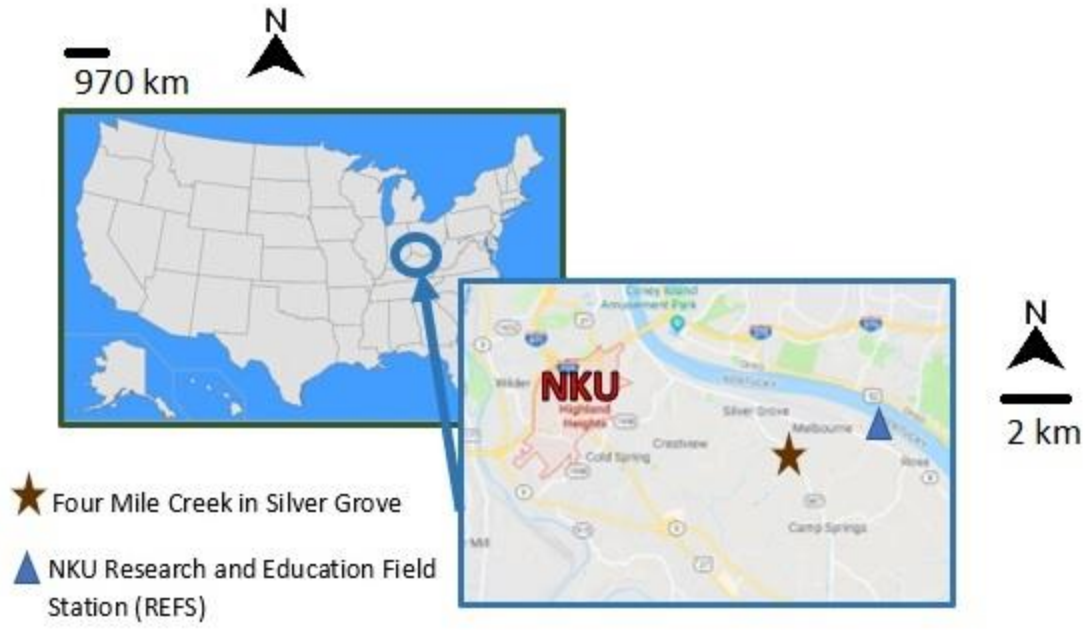

Figure 2. Maps of study area in Northern Kentucky. Red "NKU" symbolizes the university. Scale bars and north arrows are supplied for each map portion in the figure. Maps supplied by https://www.sporcle.com/games/Matt/find_the_states and Google Maps

Each sampling excursion included analysis of stream conditions such as water height, temperature, dissolved oxygen, etc. Macroinvertebrate sampling comprised of one individual positioning a fine-mesh seine towards the flow of water and another individual using his/her feet and macroinvertebrate extraction device to dislodge debris from up to one meter in front of the seine. The individual would work his/her way towards the seine, continuing the kicking and dislodging of rocks and debris. Once at the seine, both individuals would lift the seine so it stayed taut and parallel to the surface of the water. The seine would then be carefully exposed to the water to wash the collected specimens towards the center of the seine, which made collection of specimens in a bucket much easier and quicker (Image 2). This process was repeated four more times for a total of five samples from the sample area. Additional collection methods included using D-nets to dislodge macroinvertebrates from the rocks and debris at the edges of the stream,

and placing drift-foraging collection apparatuses in the water column for a couple of hours to catch macroinvertebrates traveling freely through the water.

Image 2. Research at the Ranån study site in Sweden. Upper left: holding kicknet for catching macroinvertebrates. Upper right: research students collecting macroinvertebrates. Lower left: setting up the drift net system. Lower right: learning to use field equipment. Photos taken by Dr. Richard Durtsche and Rachel Prokopius.

The bucket containing the specimens and water from the sample area were then taken to Karlstad Universitet and Northern Kentucky respectively, placed in a refrigerator in the lab analysis area with a bubbler running constantly to provide aeration. This method kept specimens alive for days or even weeks, depending on the time available to analyze the sample each day. During sample analysis, the monthly sample was removed from the bucket and picked through with little water in order to better see specimen movement. Specimens were removed from the debris and detritus in the bucket and sorted by Order, the majority of which were further identified to Family.

Imaging of specimens was performed using a Motic Images Plus 3.0 multi output digital microscope camera and accompanying software. The camera attached to an imaging cone with 1x power, which combined with the 1x power of the camera itself to have an overall power of 1x. On rare occasions an individual specimen would be analyzed under the dissecting microscope; the camera would attach to a 10x lens that fit into one of the eyepiece holes of the microscope, and the microscope would be set at 0.63x, giving an overall power of 6.3x. The cone and microscope were each calibrated in the imaging software, and each calibration was used accordingly. When using the cone, it would be placed with lights over a petri dish of specimens. Anywhere from ten to twenty specimens could be analyzed in a single image, depending on the size of the specimens. Lighting, color, contrast, exposure, etc. were manipulated on the program in order to isolate each specimen from the dish and from one another (Image 1). Two images were taken of each dish, one for measuring and one for reference. The specimens in the measuring image were labeled with numbers and surface area, perimeter and length were either recorded on the spot or done at a later time with the imaging software. The measurements could take place at a later time because the program could upload the saved image and still recognize the individual specimens, which allowed for many specimens to be imaged in one day, preserved and measured later. The specimens would then be placed into individual capped vials and suspended either in water and frozen or suspended in 70% ethanol for at least 24 hours.

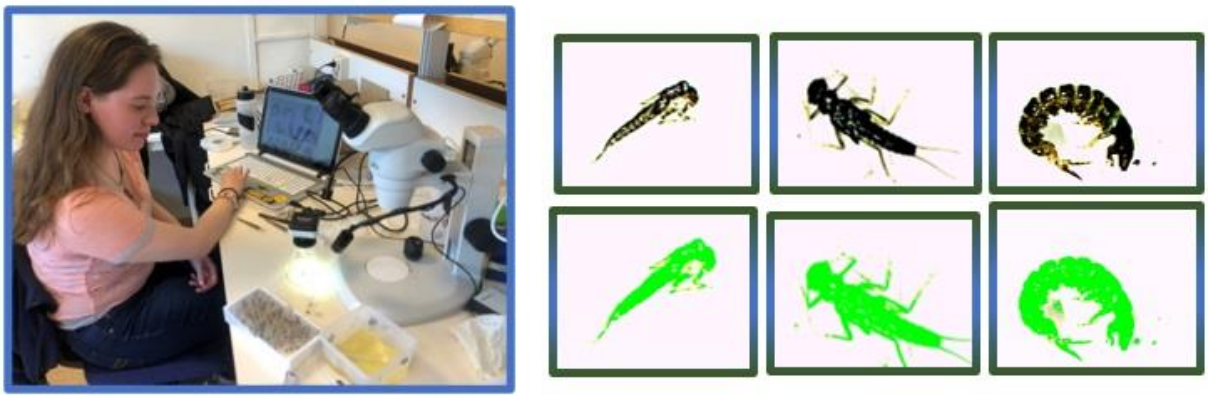

Image 3. Analysis of macroinvertebrates in the lab. Left: setup of imaging and data collection from macroinvertebrates. Photo taken by Dr. Richard Durtsche. Right: Surface area of macroinvertebrates highlighted in green and calculated in square mm by the Motic 3.0 Imaging software. Photos taken by Rachel Prokopius.

After specimens were preserved for at least 24 hours in their respective mediums, they were reimaged using the process illustrated above and their new measurements recorded for comparison to fresh specimen measurements. Each specimen was returned to its respective vial without a preservation medium and dried uncapped in a drying oven at 60°C for at least 24 hours. Once dried, the specimens were removed from the drying oven and quickly capped in order to minimize condensation on the specimens as they were moved from a warmer to a cooler environment. Each specimen was massed in milligrams on a microgram scale that measured to two places passed the decimal.

Statistical analyses were performed with the data collected from the macroinvertebrate specimens. Normality plots and residual plots were created for the Order Trichoptera to identify any patterns in the data pre and post-transformation. Regressions were created for surface area-mass relationships and length-mass relationships for specimens in different preservative mediums (fresh, frozen or alcohol), and an ANOVA run on each regression to test for significance. All statistical analyses were performed with R 3.6.2.

Results

Out of all of the data collected on the Order Trichoptera in Sweden and Northern Kentucky, the Family Hydropsychidae was most-often found. Therefore, analysis of results will include the entire Order Trichoptera and the individual Family Hydropsychidae in order to apply the technique to different taxonomic levels (Image 4).

Image 4. <u>Left</u>: Trichoptera families found in Sweden and Northern Kentucky. From top left to top right: *Hydropsychidae, Rhyacophilidae, Polycentropodidae. From bottom left to bottom right: Phryganeidae, Bracgycentridae. <u>Right:</u> Hydropsychidae specimen enlarged to better see details. Size bars for each macroinvertebrate are included. Photos taken by Rachel Prokopius.*

Plots of normality were constructed for the linear and natural log-transformed models for each of the preservation techniques (fresh, alcohol and frozen) for both the Order Trichoptera and the Family Hydropsychidae (Figures 3-8).

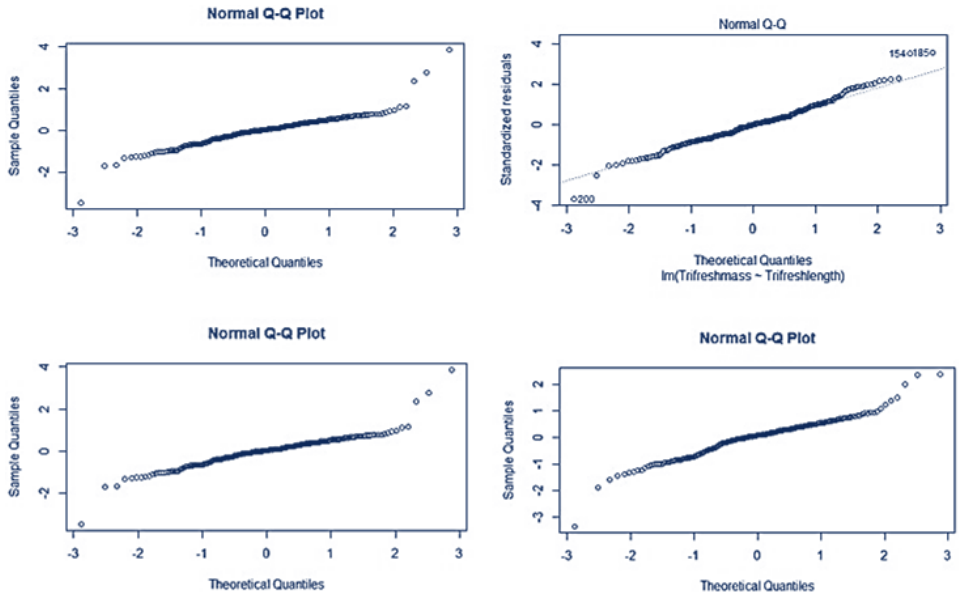

Figure 3: Plots of normality for the residuals of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of fresh specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

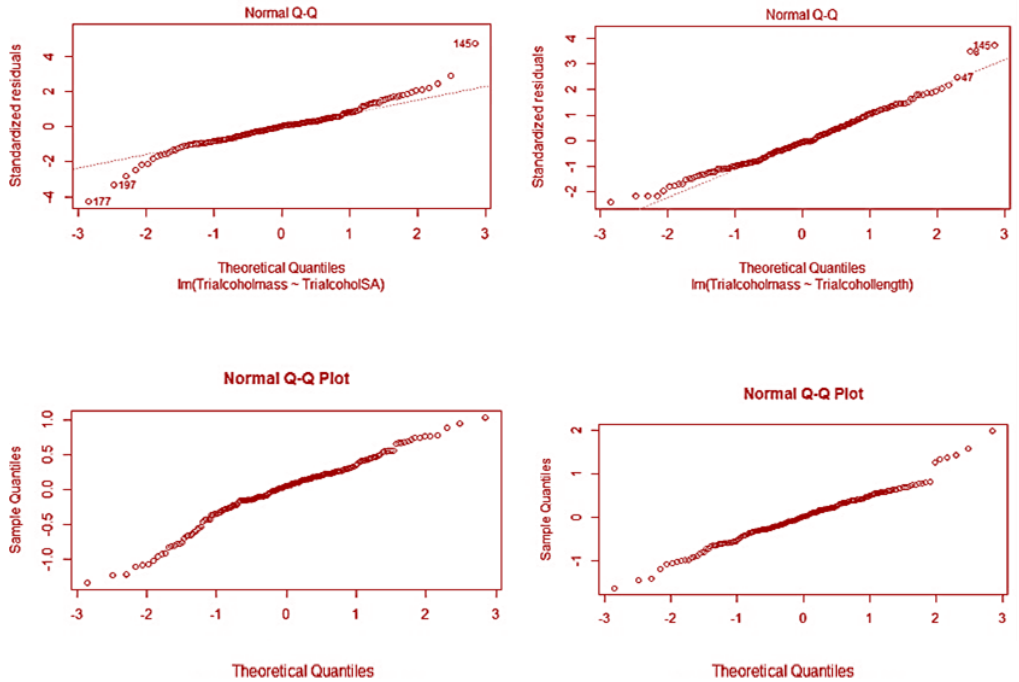

Figure 4: Plots of normality for the residuals of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of alcohol-preserved specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

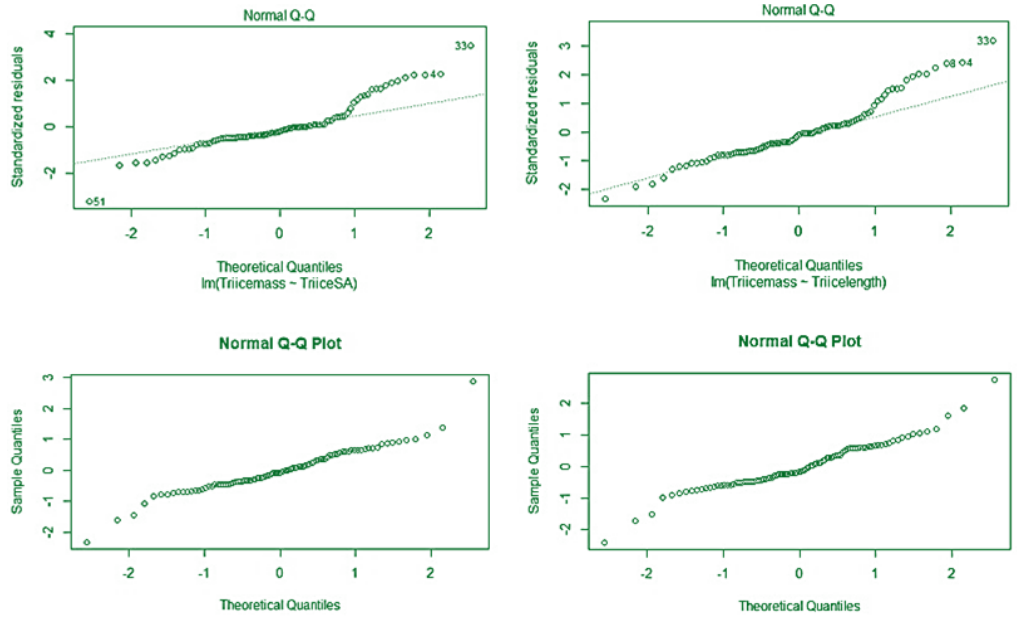

Figure 5: Plots of normality for the residuals of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of frozen-preserved specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

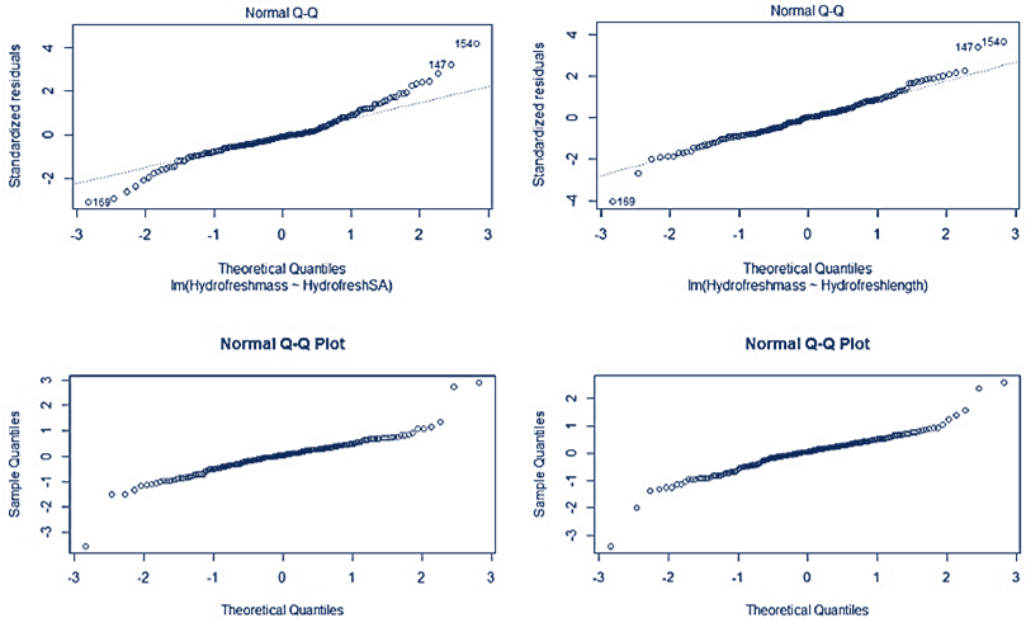

Figure 6: Plots of normality for the residuals of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of fresh specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

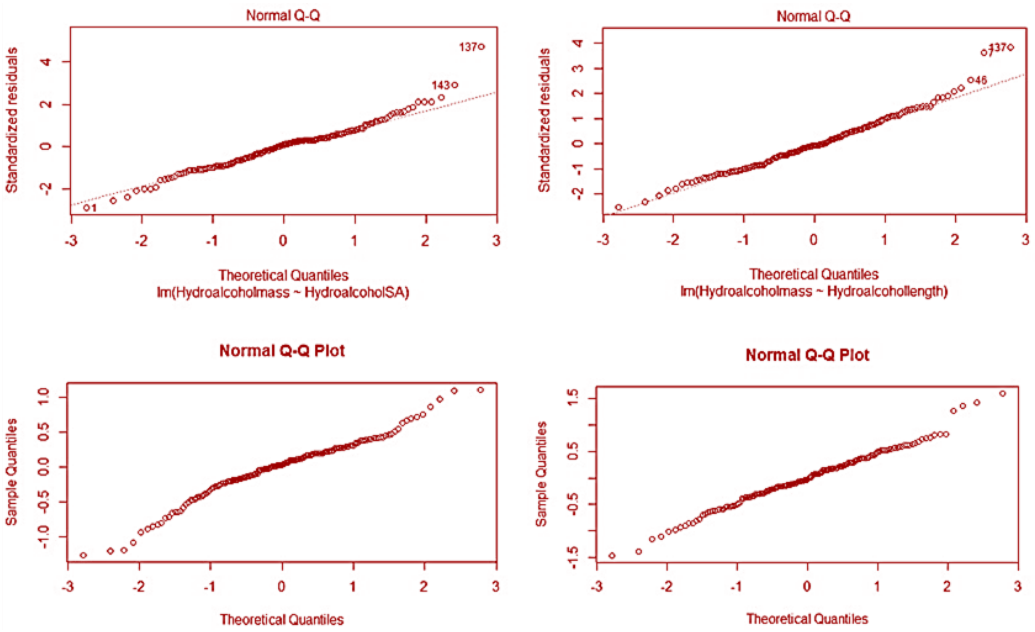

Figure 7: Plots of normality for the residuals of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of alcohol-preserved specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

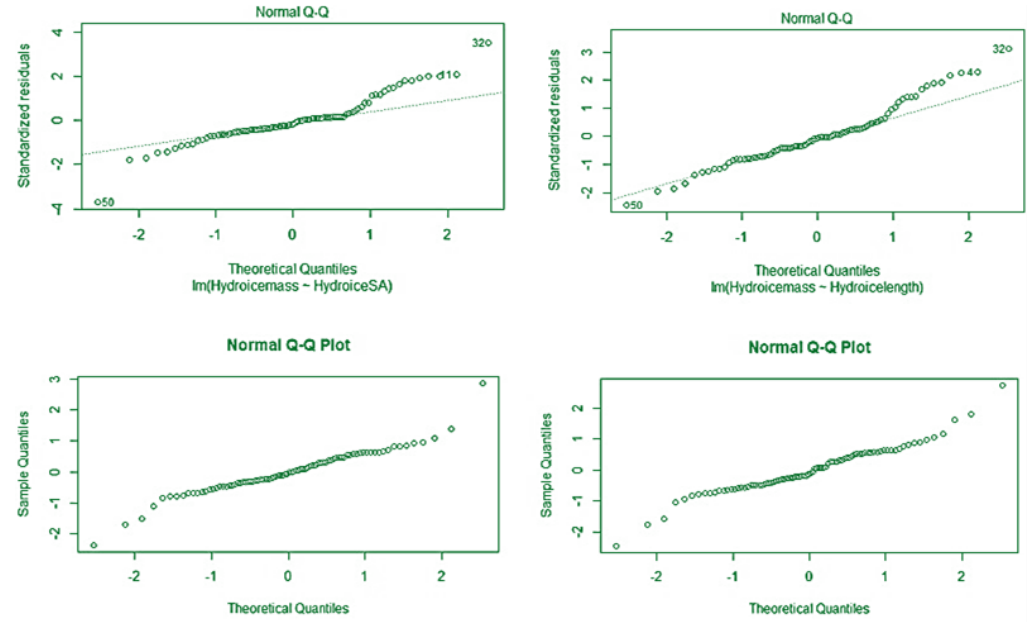

Figure 8: Plots of normality for the residuals of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of frozen-preserved specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

Plots of residuals versus predicted response values were constructed for the linear and natural log-transformed models for each of the preservation techniques (fresh, alcohol and frozen) for both the Order Trichoptera and the Family Hydropsychidae (Figures 9-14). A pattern is seen for smaller predicted responses in all of the linear models, with less of a pattern for higher predicted responses. For the natural log-transformed models, there is less of a pattern seen for the plots overall regardless of the size of the predicted response.

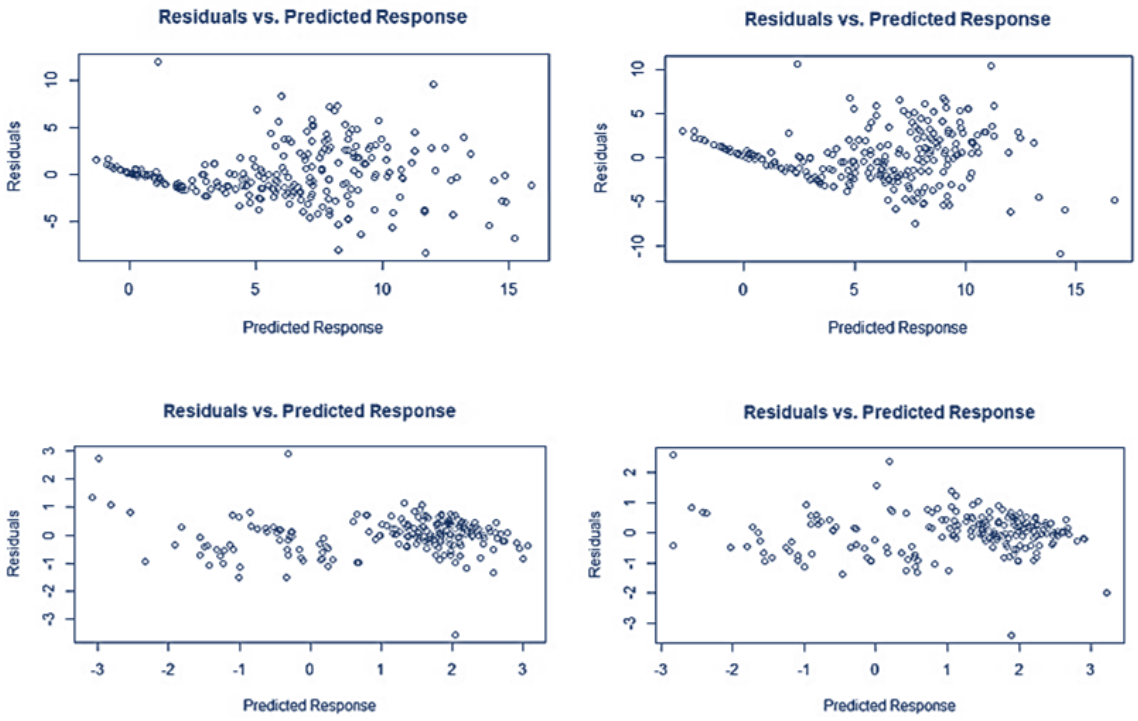

Figure 9: Plots of residuals versus predicted response values of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of fresh specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

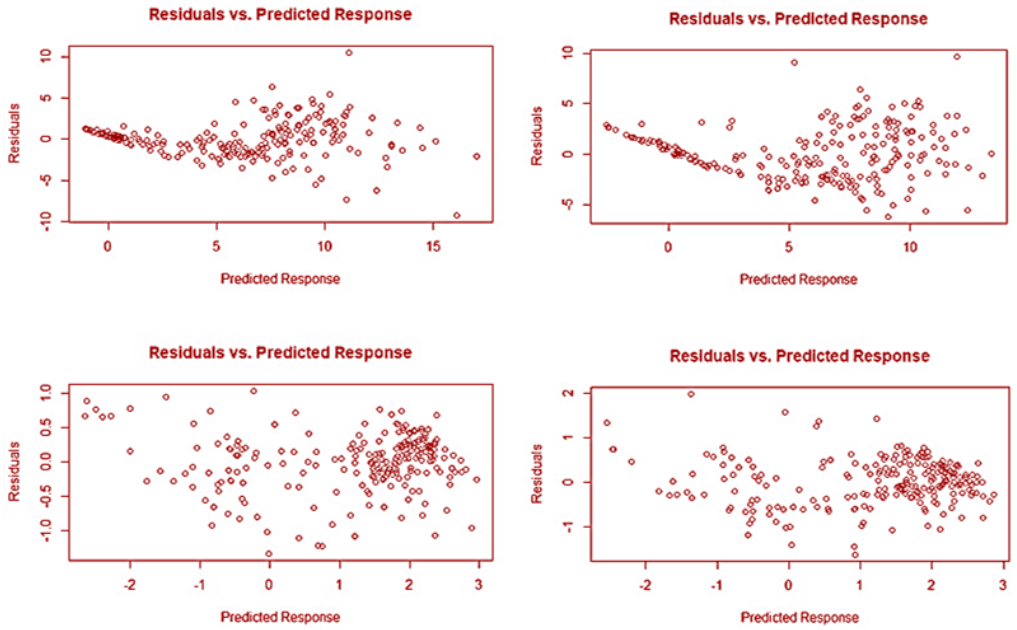

Figure 10: Plots of residuals versus predicted response values of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of alcohol-preserved specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

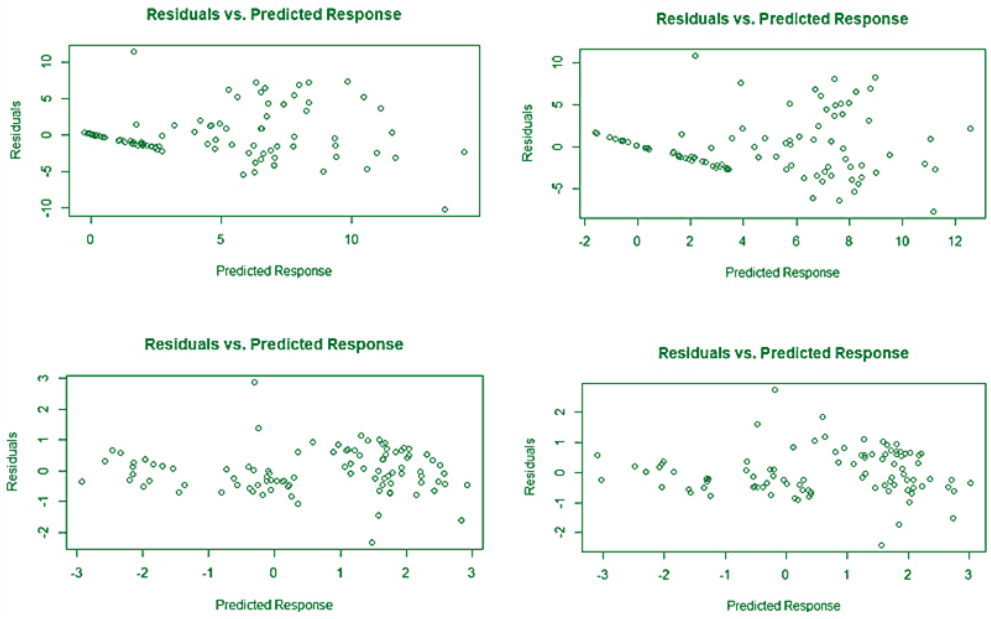

Figure 11: Plots of residuals versus predicted response values of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of frozen-preserved specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

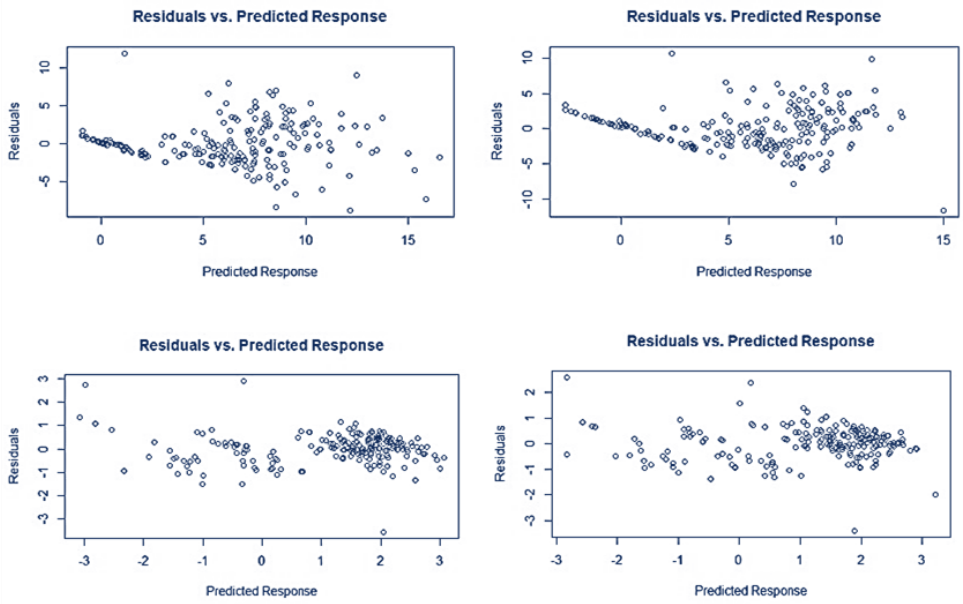

Figure 12: Plots of residuals versus predicted response values of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of fresh specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

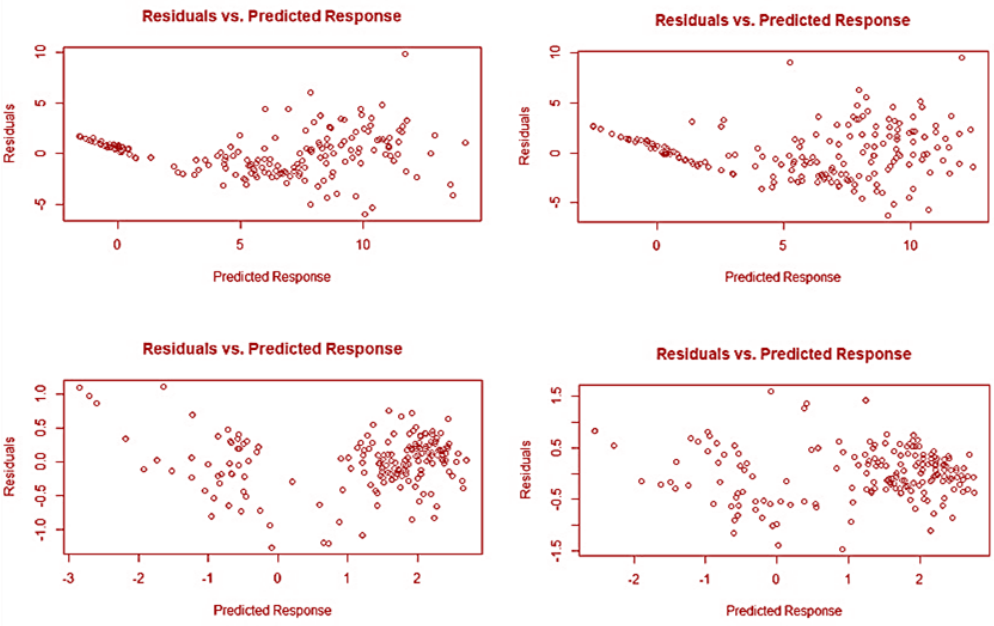

Figure 13: Plots of residuals versus predicted response values of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of alcohol-preserved specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

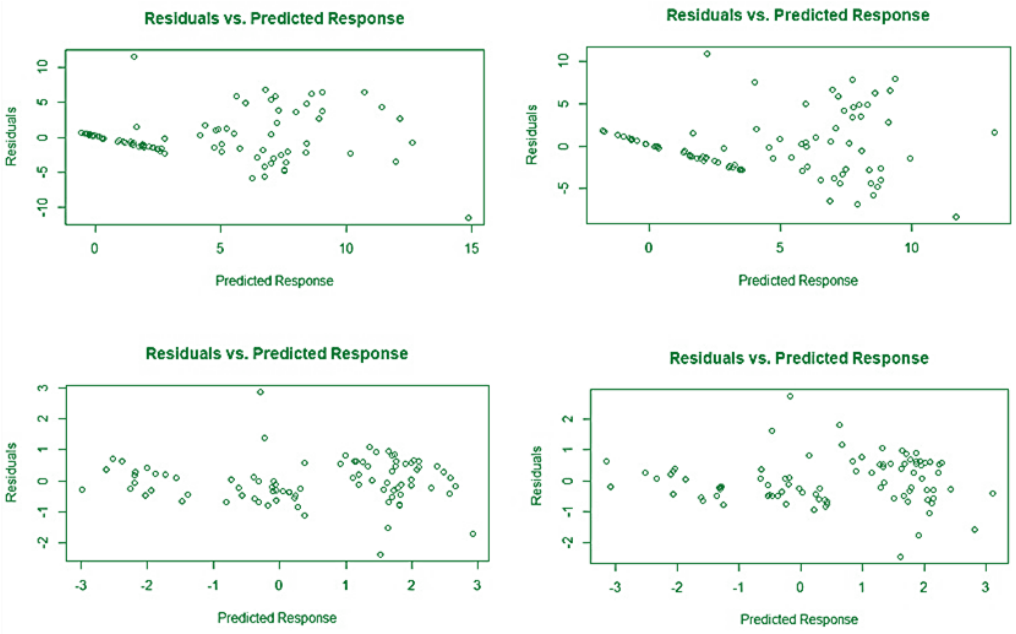

Figure 14: Plots of residuals versus predicted response values of the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of frozen-preserved specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

All regression analyses for the Order Trichoptera and the Family Hydropsychidae were significant to an alpha level of 0.05, regardless of whether the model was transformed. Natural log transformation increased the R^2 value for all of the regressions compared to their respective linear models. All surface area to dry mass models, except for the fresh surface area to dry mass linear model for the Order Trichoptera, had a larger R^2 value than the corresponding length to dry mass relationships (Table 1, Figures 15-19).

Taxonomic level	Model Type	Measurement	F-statistic	df	R^2 value
Order-Trichoptera	Linear	Surface Area	478.8	1, 250	0.6569
Order-Trichoptera	Natural log transformed	Surface Area	878	1, 250	**0.7784**
Order-Trichoptera	Linear	Length	388	1, 250	0.6081
Order-Trichoptera	Natural log transformed	Length	888.8	1, 250	**0.7805**
Family-Hydropsychidae	Linear	Surface Area	392.1	1, 212	0.6491
Family-Hydropsychidae	Natural log transformed	Surface Area	904.9	1, 212	**0.8102**
Family-Hydropsychidae	Linear	Length	367.1	1, 212	0.6339
Family-Hydropsychidae	Natural log transformed	Length	870.7	1, 212	**0.8042**
Order-Trichoptera	Linear	Surface Area	808.7	1, 228	0.7801
Order-Trichoptera	Natural log transformed	Surface Area	2073	1, 228	**0.9009**
Order-Trichoptera	Linear	Length	520.7	1, 228	0.6955
Order-Trichoptera	Natural log transformed	Length	1236	1, 228	**0.8443**
Family-Hydropsychidae	Linear	Surface Area	713.4	1, 183	0.7958
Family-Hydropsychidae	Natural log transformed	Surface Area	1911	1, 183	**0.9126**
Family-Hydropsychidae	Linear	Length	450.8	1, 183	0.7113
Family-Hydropsychidae	Natural log transformed	Length	1180	1, 183	**0.8657**
Order-Trichoptera	Linear	Surface Area	113.7	1, 94	0.5474
Order-Trichoptera	Natural log transformed	Surface Area	457.1	1, 94	**0.8294**
Order-Trichoptera	Linear	Length	98.74	1, 94	0.5123
Order-Trichoptera	Natural log transformed	Length	379.9	1, 94	**0.8016**
Family-Hydropsychidae	Linear	Surface Area	112.6	1, 86	0.5671
Family-Hydropsychidae	Natural log transformed	Surface Area	421	1, 86	**0.8304**
Family-Hydropsychidae	Linear	Length	89.06	1, 86	0.5087
Family-Hydropsychidae	Natural log transformed	Length	347.1	1, 86	**0.8014**

Table 1: Results of ANOVAs run on the linear and natural log-transformed models relating surface area (sq mm) and length (mm) to dry mass (mg) of specimens. Color corresponds to preservation technique (blue: fresh, red: alcohol-preserved, green: frozen-preserved). Models within preservation techniques are separated by taxonomic level (Order-Trichoptera and Family-Hydropsychidae) by darker lines. The higher R^2 value for each model comparison is bolded, and the highest R^2 value per taxonomic level per preservation technique is underlined. Every ANOVA result is significant to an alpha level of 0.05 and with a p-value less than 0.0001, and are not included with each ANOVA result for simplicity of table design.

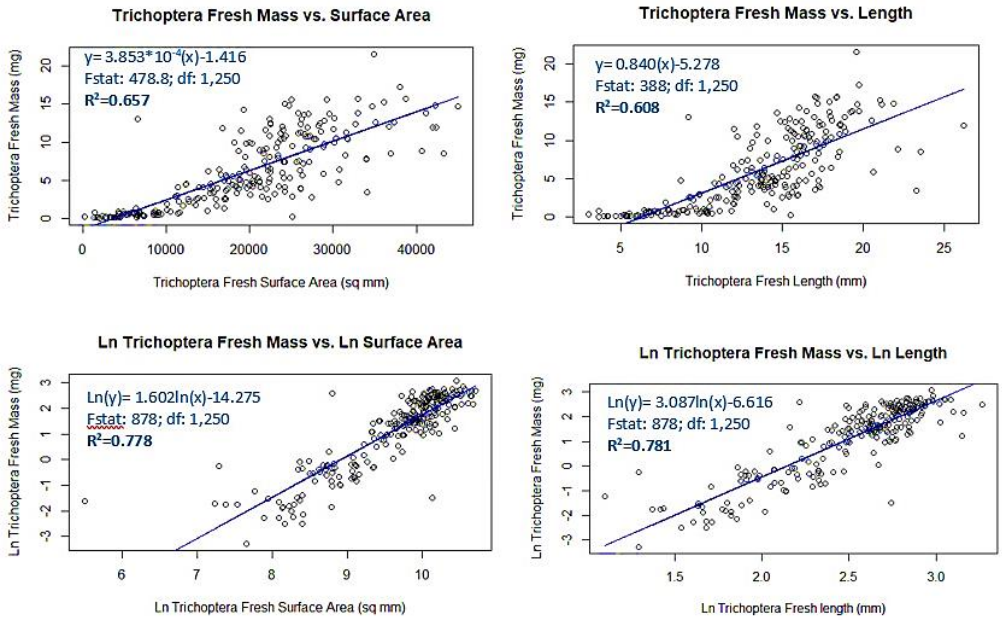

Figure 15: Plot of dry mass (mg) versus surface area (sq mm) and length of linear and natural log-transformed models of fresh specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

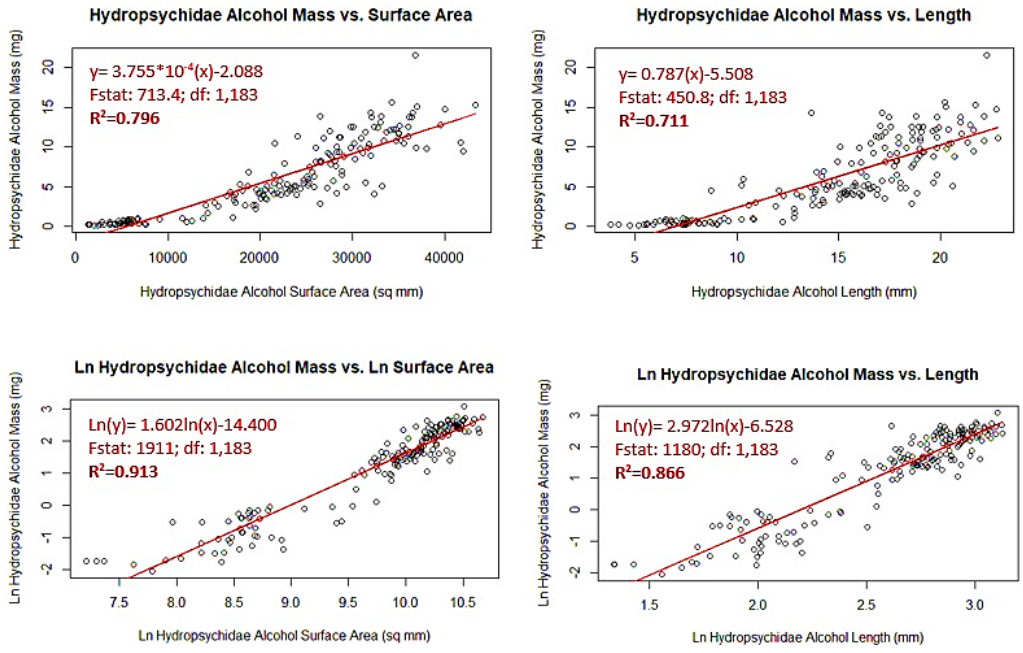

Figure 16: Plot of dry mass (mg) versus surface area (sq mm) and length of linear and natural log-transformed models of alcohol-preserved specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

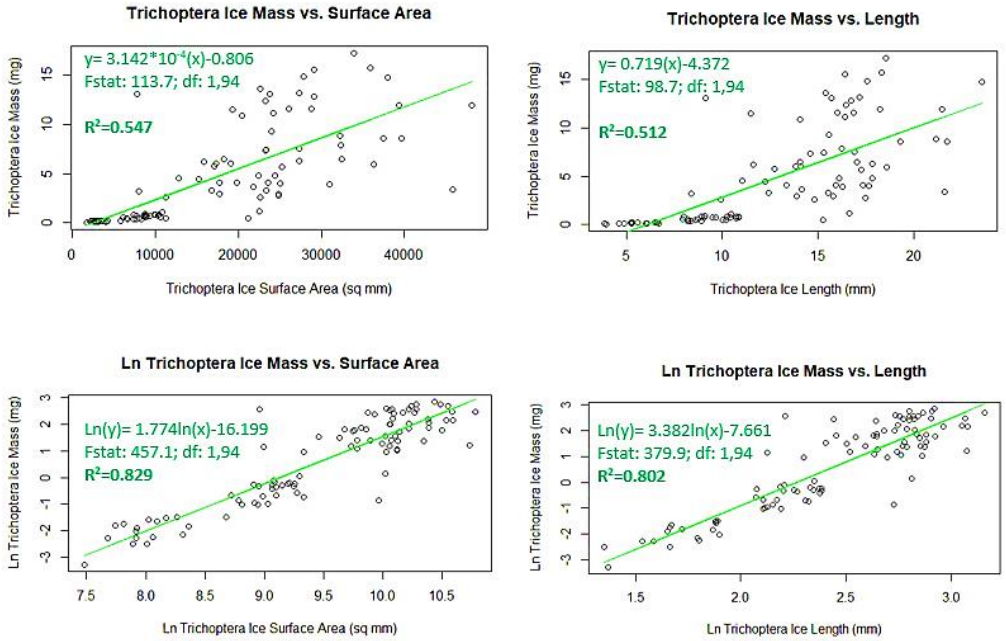

Figure 17: Plot of dry mass (mg) versus surface area (sq mm) and length of linear and natural log-transformed models of frozen-preserved specimens in the Order Trichoptera. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

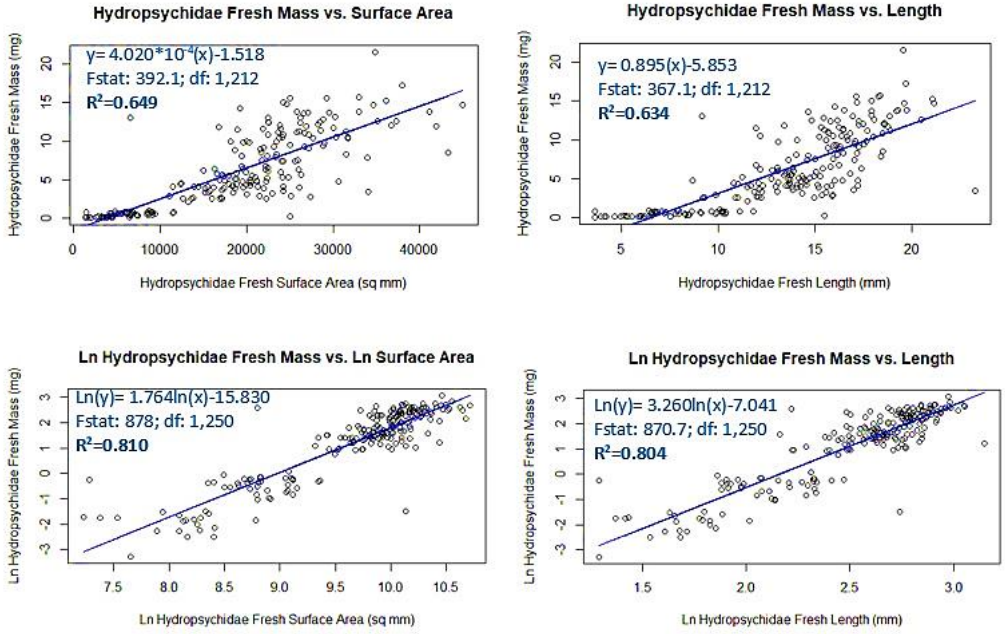

Figure 17: Plot of dry mass (mg) versus surface area (sq mm) and length of linear and natural log-transformed models of fresh specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

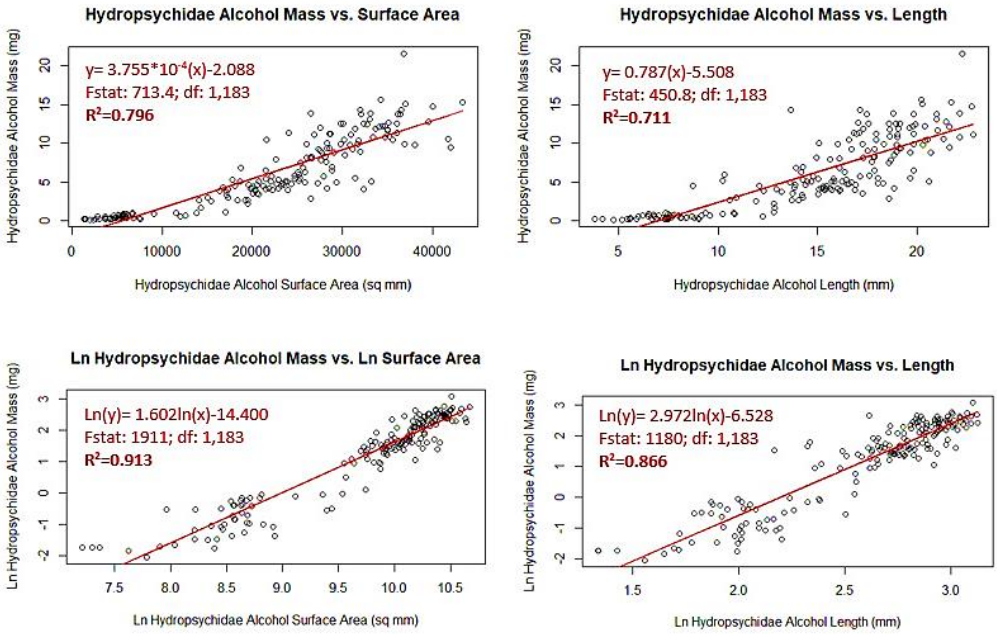

Figure 18: Plot of dry mass (mg) versus surface area (sq mm) and length of linear and natural log-transformed models of alcohol-preserved specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

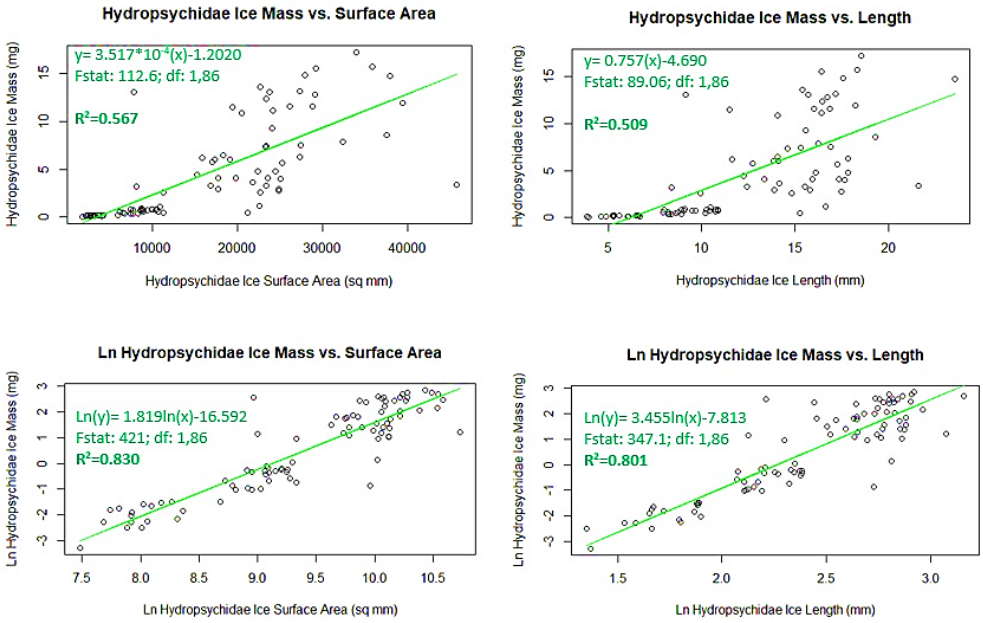

Figure 19: Plot of dry mass (mg) versus surface area (sq mm) and length of linear and natural log-transformed models of frozen-preserved specimens in the Family Hydropsychidae. Top left: linear surface area model. Top right: linear length model. Bottom left: natural log-transformed surface area model. Bottom right: natural log-transformed length model.

Discussion:

The original normality plots for the fresh and alcohol-preserved specimens at both the Order and Family levels do not appear to change drastically with transformation, with a few outliers on the edges of the normality plots but overall the residuals falling on a straight line. For the frozen-preserved specimens, the data points with higher standardized residuals do not fall on a straight line for the linear model. However, once the data is log-transformed these residuals fall on the straight line of the normality plot (Figures 3-8). All natural log transformations yielded more randomly-distributed residuals on the residual vs. predicted response plots, especially at lower levels of predicted response (Figures 9-14). Ideal normality plots show residuals falling on a straight line to indicate a normal distribution of data, one of the key elements needed in a data set for accurate statistical analysis. Additionally, ideal residual plots are randomly distributed above and below zero to indicate constant variance and independence of data, which are also requirements for a data set for accurate statistical results. Even before looking at the difference in R^2 values between the linear and natural log-transformed plots, it is clear that natural log transformation has reconfigured the data set in such a way that it is better able to be analyzed statistically.

The data represented here collected from both Sweden and Northern Kentucky aquatic systems suggest that, overall, surface area measurements of macroinvertebrate taxa are more accurate than the traditional length-mass relationship previously established and used in literature (Smock 1980, Benke et al. 1999, Eklöf et al. 2017). R^2 values in the 0.65-1.0 range are ideal for using regressions to make comparisons between variables, obviously with values closer to 1.0 being overall preferred. R^2 values were higher for all of the surface area to dry mass models compared to the corresponding length to dry mass models except for the fresh Trichoptera

transformed model, where the length to dry mass model had an R^2 value very slightly higher than the surface area to dry mass transformed model (R^2= 0.781 and 0.778 respectively) (Table 1, Figure 15). As can be seen in the figure comparing the models for fresh Trichoptera specimens and in the corresponding normality plots, there may be outliers in the data set that are altering the fit of the models. Further analysis of these points may be helpful in teasing out the true difference between the models and whether the current form of the model is the most accurate (Figures 3 and 15).

The data presented here also yield some new avenues of study to continue finding the best way to gather data on macroinvertebrates for subsequent use in analyzing energetics and health of aquatic systems. The comparison of natural log-transformed models that had the closest R^2 values between the surface area to dry mass and length to dry mass relationships was also the comparison that had the most specimens analyzed (i.e. fresh Trichoptera, 251 specimens) (Table 1, Figure 15). This suggests there may be similar levels of accuracy of models with a high enough sample size of macroinvertebrates, which would need further collection and analysis to determine.

Natural log-transformation may also be more effective in macroinvertebrate size models with a smaller sample size; only 87 Hydropsychidae specimens were analyzed after being preserved by freezing, and the R^2 values between linear and natural-log transformed models for both surface area and length increased by almost 0.3 (Table 1, Figure 19). Once again, because of the nature of the data analysis and sampling method in this study this concept can’t be seen definitively. Perhaps preservation by freezing does not allow for accurate measurements and data transformation is needed because of this and not because of the smaller sample size. Further collection and data analysis would need to be done in order to tease out these concepts.

A final direction to consider furthering this research is determining whether size to dry mass relationships are needed for more-specific taxonomic levels (i.e. Family) or are accurate at less-specific taxonomic levels (i.e. Order). Looking at the data presented, the R^2 values for the Hydropsychidae Family are not very much higher than the R^2 values for the Trichoptera Order as a whole in the same preservation medium. For example, the natural log-transformed surface area to dry mass relationship R^2 value is 0.9009 for the Trichoptera Order and 0.9126 for the Hydropsychidae Family (Table 1, Figures 16 and 18). However, the analysis of the Trichoptera Order includes all of the specimens from the Hydropsychidae Family. With a total of 229 Trichoptera specimens analyzed after alcohol preservation and 184 of these specimens being in the Hydropsychidae Family, this leaves only 45 specimens from the other Trichoptera Families represented in this data set (Table 1, Image 4). More collection of the underrepresented Trichoptera Families would need to be done in order to determine whether individual regressions by Trichoptera Family are needed or if one for the Trichoptera Order is sufficient to get accurate dry mass measurements from size values.

The innerworkings and possible ecological processes of an ecosystem are greatly determined by its energetic content (Zhao et al. 2005, Helmus et al. 2013, Bernhardt et al. 2018). The use of macroinvertebrates to determine the energetics of aquatic systems is widely-accepted and used, and therefore must be as accurate as possible in order to yield usable results in scientific studies. This study has shown that the traditional length to dry mass relationship that is currently used by scientists measuring macroinvertebrates may not be the most accurate or effective way of determining energetic content, and that other size measurements such as surface area may develop more-accurate size to dry mass relationships for scientists to use in order to predict the energetic content of aquatic systems.

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