Final Project

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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).

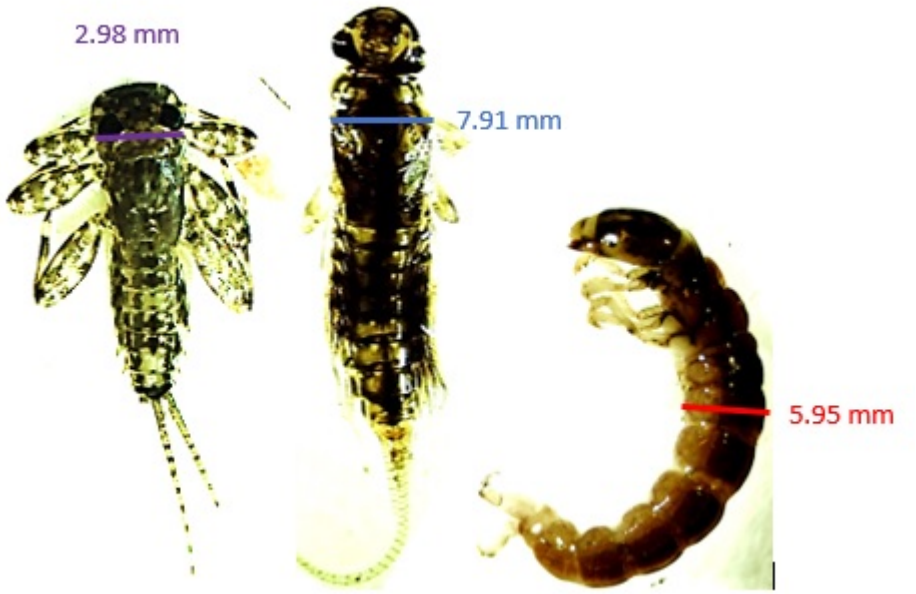

Image 1. Image illustrating the unique morphologies of different macroinvertebrate families. Images taken at 1x power with a Motic 3.0 Camera. Size bars for each macroinvertebrate are included. Macroinvertebrate familes from left to right: Ephemeroptera, Plecoptera, Trichoptera

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, analysis of the relationship between both surface area and length to macroinvertebrate dry mass appear to be more nonlinear than linear. Therefore, this project aims to determine whether a nonlinear 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 Rsquared value) of macroinvertebrate dry mass than length when the mathematical function relating the two variables is an exponential 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. Maps supplied by http://www.geographicguide.com/europe-maps/sweden.htm (http://www.geographicguide.com/europe-maps/sweden.htm) and https://en.wikipedia.org/wiki/V%C3%A4rmland_County (https://en.wikipedia.org/wiki/V%C3%A4rmland_County)

Figure 2. Maps of study area in Northern Kentucky.Red “NKU” symbolizes the university. Maps supplied by https://www.sporcle.com/games/Matt/find_the_states (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

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 and Genus, when possible.

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.

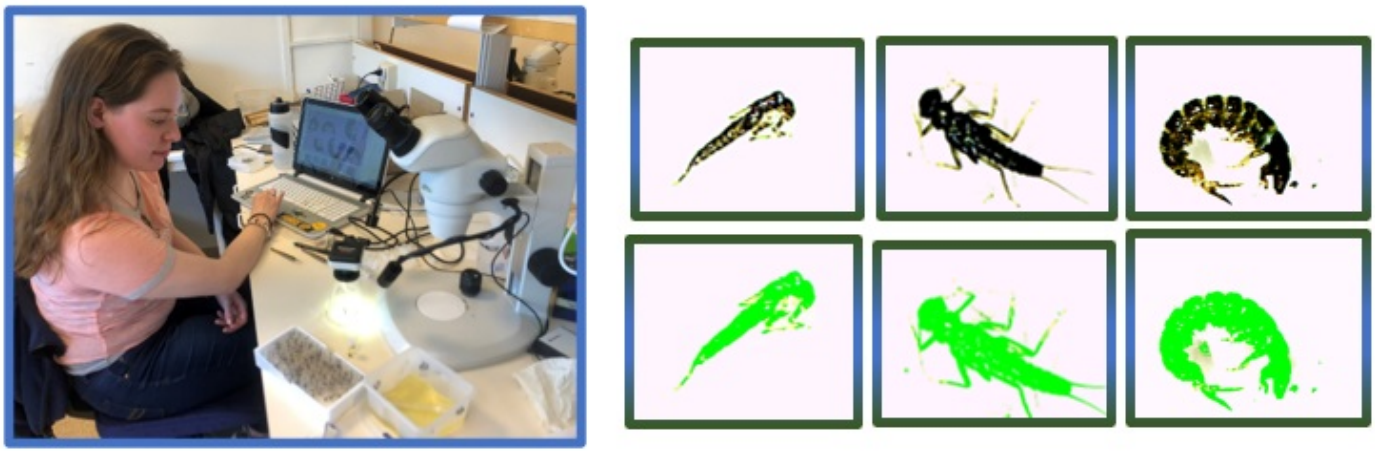

Image 3. Analysis of macroinvertebrates in the lab. Left: setup of imaging and data collection from macroinvertebrates. Right: Surface area of macroinvertebrates highlighted in green and calculated in square mm by the Motic 3.0 Imaging software.

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. Regressions and nonlinear models were generated to create surface area-mass relationships and length-mass relationships for different Orders of macroinvertebrates and in different preservative mediums (fresh, frozen or alcohol).All statistical analysis was performed with R 3.6.2, and the "nlstools" library was used to generate the nonlinear models.

Results

Linear models consistently show higher Rsquared values for surface area to mass relationships than length to mass relationships. All significant relationships to an alpha level of 0.05 for Trichoptera Order as a whole and for individual Trichoptera Families are included in Table 1.Relationships for Trichoptera Families are only included when both surface area and length to mass relationships are both significant to an alpha level of 0.05 (Table 1). Side-by-side graphs of surface area and length to mass relationships for the Trichoptera Order and the Hydropsychidae Family, the family of caddisfly larvae most prominent in the field collections at the Ranån field site in Sweden, are included (Figure 3).

Model Type	Measurement Type	Treatment	Standard Error of Regression	T-statistic	P-value
Trichoptera-Linear	Surface Area	Alcohol	1.21E-05	28.437	<0.0001
Trichoptera-Nonlinear	Surface Area	Alcohol	2.36E-06	18.68	<0.0001
Trichoptera-Linear	Length	Alcohol	0.03425	22.82	<0.0001
Trichoptera-Nonlinear	Length	Alcohol	0.007925	17.086	<0.0001
Hydropsychidae-Linear	Surface Area	Alcohol	1.41E-05	26.709	<0.0001
Hydropsychidae-Nonlinear	Surface Area	Alcohol	3.19E-06	19.134	<0.0001
Hydropsychidae-Linear	Length	Alcohol	0.03708	21.23	<0.0001
Hydropsychidae-Nonlinear	Length	Alcohol	0.009136	15.977	<0.0001
Polycentropodidae-Linear	Surface Area	Alcohol	5.92E-05	3.03	0.0105
Polycentropodidae-Nonlinear	Surface Area	Alcohol	7.01E-05	2.043	0.0636
Polycentropodidae-Linear	Length	Alcohol	0.06649	3.483	0.00452
Polycentropodidae-Nonlinear	Length	Alcohol	0.09128	2.307	0.0397
Rhyacophilidae-Linear	Surface Area	Alcohol	2.66E-05	13.02	<0.0001
Rhyacophilidae-Nonlinear	Surface Area	Alcohol	5.34E-06	7.182	<0.0001
Rhyacophilidae-Linear	Length	Alcohol	0.1843	5.406	0.00012
Rhyacophilidae-Nonlinear	Length	Alcohol	0.03091	3.788	0.00226
Bracgycentridae-Linear	Surface Area	Alcohol	6.30E-05	3.916	0.00241
Bracgycentridae-Nonlinear	Surface Area	Alcohol	1.17E-05	2.881	0.0149
Bracgycentridae-Linear	Length	Alcohol	0.2245	2.91	0.0142
Bracgycentridae-Nonlinear	Length	Alcohol	0.03942	2.209	0.0493

Table 1. Summary table of statistics for linear and nonlinear models for surface area and length to mass relationships for individual Trichoptera families

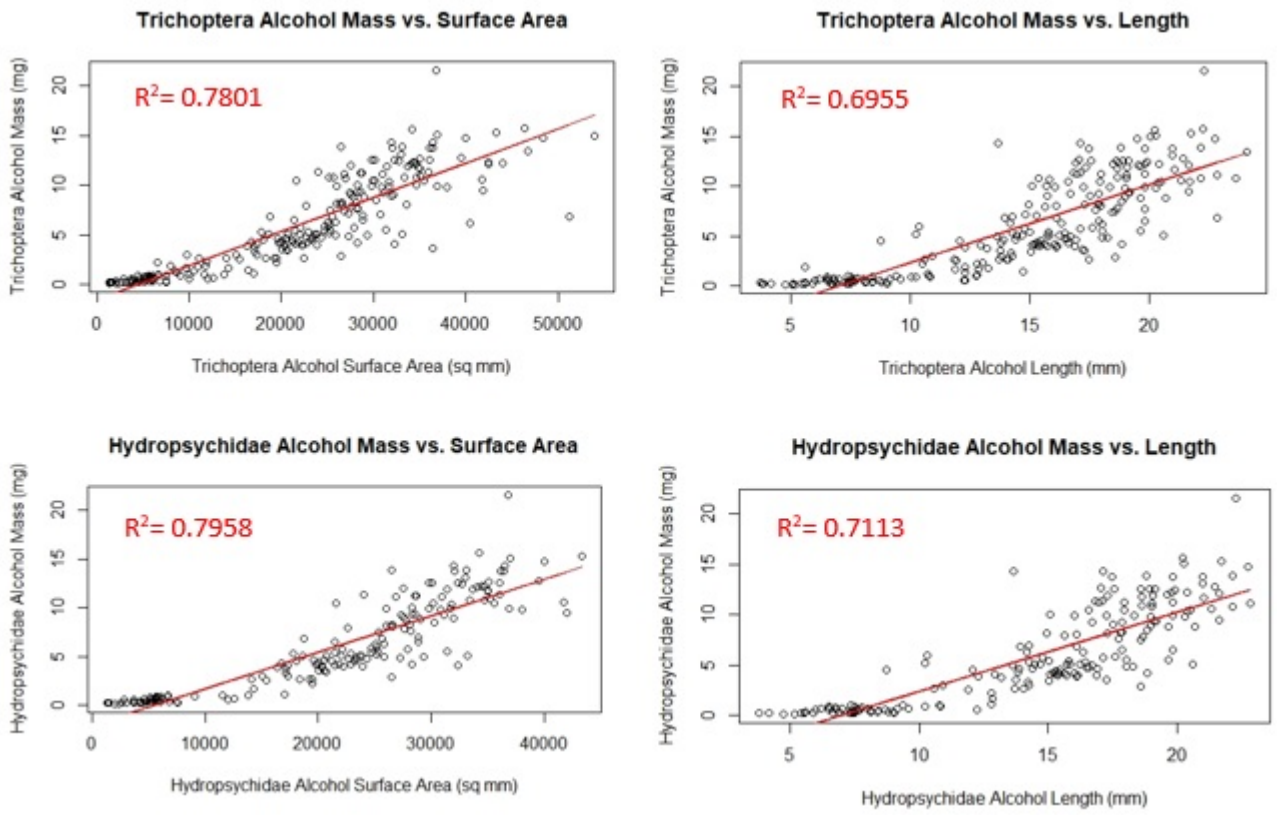

Figure 3. Linear surface area and length to mass relationships for the Trichoptera Order and the Hydropsychidae Family

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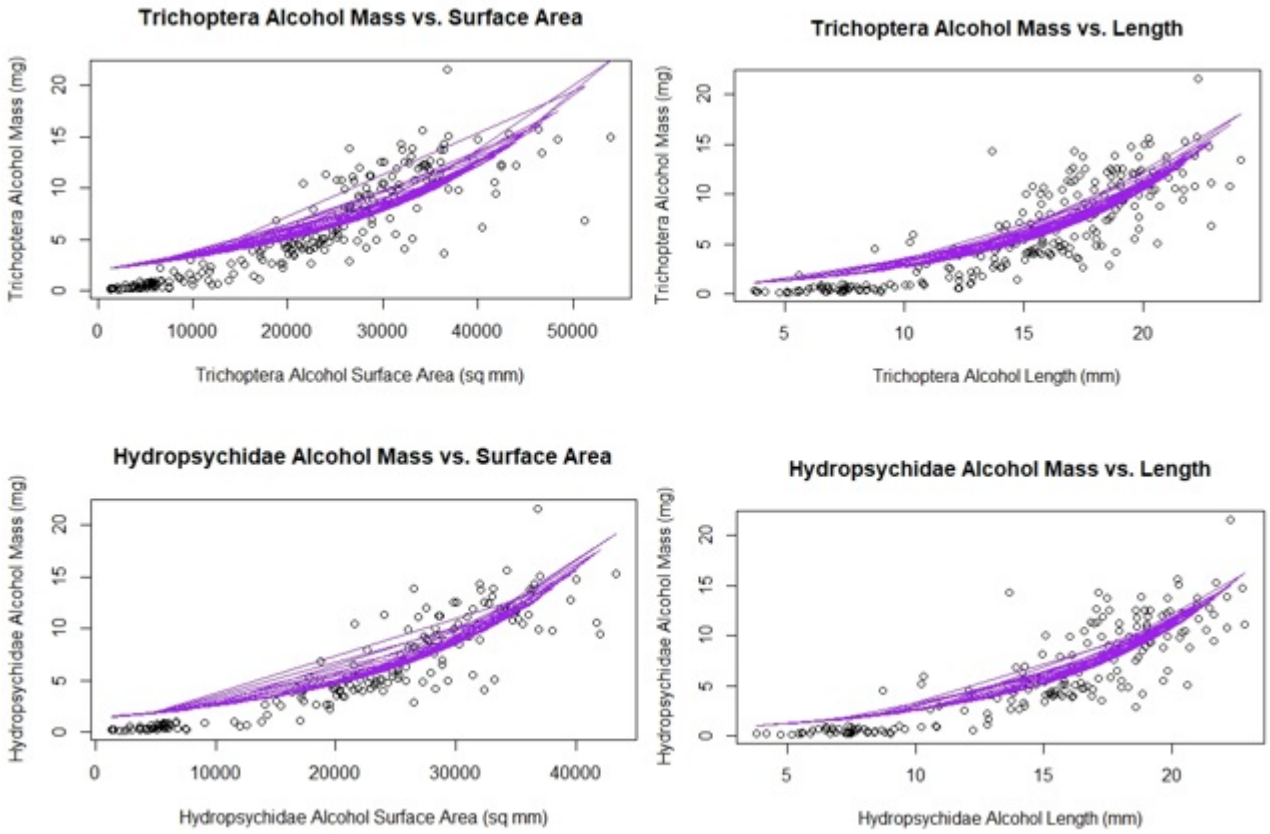

Figure 4. Nonlinear surface area and length to mass relationships for the Trichoptera Order and the Hydropsychidae Family

Discussion

Data collected from both Sweden and Northern Kentucky aquatic systems suggested that 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). Rsquared 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. Rsquared values tended to be higher for Trichoptera Families that had larger sample sizes, which suggests that for certain Trichoptera Families (ex. Polycentropodidae, Rhyacophilidae) more data must be collected to determine accurate size to mass relationships for the particular families.

When considering nonlinear models, a lower standard error of regression is preferred because it suggests that the data points are closer to the model line. In every instance with the alcohol-preserved specimens,the standard error of regression is lower for the nonlinear model than the linear model (Table 1). However, certain nonlinear models do not represent significant relationships between the predictor and response variables. For example, the nonlinear model for the relationship between surface area and dry mass for Polycentropodidae Family is nonsignificant, while the linear model is (Table 1). This tended to occur when fewer data points were available for the macroinvertebrate family. For example, the Phryganeidae Family is not included in the table provided in this study because both the linear and nonlinear models relating size and mass were not significant. Only three members of this family were found when sampling, which shows that a certain number of specimens must be collected and analyzed in order to build an accurate model for relating size and mass.

It is also important to consider the efficiency and ease of linear versus nonlinear models. Though the standard error of regression for all of the nonlinear models for the Trichoptera Families listed in Table 1 was lower than the standard error of regression for the linear model, the errors of regression are not that different. In most cases, both models are significant and suggest there is a relationship between the size measurement and the dry mass of the macroinvertebrate. It must be considered whether a slight decrease in standard error of regression is worth the extra work of applying a nonlinear model and potentially transforming the data. Subsequent studies and additiions to the data set should include more specimens from the lower-represented Trichoptera Families, and other transformations such as log transformation could be applied to the data for potentially an even better fit to the data collected.

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