**Introduction**

When viewing mountainous regions from high elevation small grooves can be seen in the hillsides which often conceal narrow headwater streams. These almost impossibly thin ribbons of water are regularly enshrouded by a dense canopy of trees and their presence is only inferred based on the steep concave nature of the terrain. These are the very starting points of a fluvial network that becomes a much more apparent feature of the landscape as these dendritic channels coalesce into larger rivers. When viewed from their banks, headwater streams present themselves as modest rivulets with steep banks winding around large rocks and tree roots. They are often kept cool and shaded by the canopy above, where previous years’ leaves or needles litter the ground and have accumulated in small pools (personal observations). Most of these leaves will appear sturdy and intact but some may show the invariable signs of decomposition where fungi, bacteria and aquatic insects have left little but the skeletonized remains (Suberkropp and Klug 1980). Occasionally small fish can even be seen darting around and jostling for positions within the current, seeking the best position to feed on small insects and other morsels that are unlucky enough to be caught drifting downstream (Hughes 1992).

Headwaters are vital components of stream networks which contribute substantially to the water quality and biodiversity of larger, more conspicuous waterways (Alexander et al. 2007; Meyer et al. 2007). Although individually these streams may be meager, en-masse they form almost 80% of the system’s total length and more than 70% of the land surface is drained by them (Colvin et al. 2019). This leads to a substantial amount of material entering these streams where it begins to be transformed by the biological, physical, and chemical processes endemic to headwaters and conveyed downstream where it impacts the inhabitants and processes of much wider channels (Vannote et al. 1980).

Headwaters sustain certain species of culturally and economically important fish. The life histories of some populations of trout are played out solely in headwaters while others use them as rearing habitat for their young. A few species seek them out as refuges from heat and predation (Meyer et al. 2007). In the Pacific Northwest headwaters, these fish are generally trout (Family Salmonidae) (Richardson and Danehy 2007) and in the western USA trout are an important fish for recreational angling which has a sizable economy surrounding it (TCW Economics 2010; Loomis and Ng 2012). Although the trout in first order streams are not generally the target of anglers, these smaller systems present themselves with a more manageable size of stream to study and smaller streams exhibit connectivity with larger systems (Colvin et al. 2019). Trout are also valued simply for their presence regardless of harvesting (Gresswell and Liss 1995).

A succinct definition for headwater streams has not been completely agreed on although they are broadly understood as less than 3 m wide, and have an average discharge of less than 57 L s-1, being generally 1st order streams (not yet confluenced with another stream) and drain a catchment of less than 100 ha (Richardson and Danehy 2007).

A small forested headwater stream ecosystem contains an integrated community of organisms which displays a distinct structure peculiar to that habitat. The amount of light reaching the stream is often much less than downstream reaches where the channel width is sufficient to part the canopy of trees. Little solar radiation is typically left available to stimulate large amounts of photosynthesis in the primary producers or photoautotrophs, such as algae, that inhabit the stream. There is however, usually an abundance of plant matter mostly in the form of leaves or pine needles. This plant material known as allochthonous (from the outside) organic matter, serves largely as the energetic foundation for headwater ecosystems. Consequently, these ecosystems are dependent on subsidies from the surrounding environment often making them net heterotrophic (not producing their own food). The plant matter is typically first colonized by aquatic fungi which begin to consume the leaves and spread throughout. This is shortly followed by bacteria which start to form a thin slimy biofilm. The leaves are composed almost entirely of hydrocarbons which the biofilm slowly breaks down and liberates through digestion. The scant nutrients such as phosphate and nitrate are absorbed for critical cellular processes while some of the hydrocarbons are used for the structure of the biofilm. Some of these hydrocarbons are also broken down completely and used as an energy source while the residual carbon dioxide is exhausted to the aquatic environment through respiration. Many aquatic invertebrates seek out these biofilm laden leaves and begin shredding them to ingest the most nutritious and soft parts. Other invertebrates may patiently wait for discarded particles of food to be delivered to them by the current or actively collect small scraps from the stream bed. A few are predatory which spend their time hunting for other invertebrates which have found themselves vulnerable. This whole food web is overshadowed by the presence of fish which regularly occupy the top trophic level and continuously monitor the water column for anything that may fit in their mouth.

The activities of all of the aerobic organisms in a stream reach can be evaluated with a metric of stream metabolism. Stream ecosystem metabolism is the combination of gross primary production (GPP) and ecosystem respiration (ER). GPP by photoautotrophs uses the energy in light to fix the carbon in CO2 into organic hydrocarbons which releases O2. ER is the reverse of this process and is the mineralization of organic hydrocarbon to CO2 which consumes O2. This consumption of O2 represents the use of energy by organisms in the stream (Hall and Hotchkiss 2017). Stream metabolism is therefore a comprehensive measure which sums the activity of virtually all of the organisms in a stream (Mejia et al. 2018).

The presence of trout in a headwater stream may relate to overall stream metabolism. The respiration of trout will be included directly in the stream ER estimate (Hall 1972) and may also affect GPP due to a trophic cascade (Young et al. 2008). A trophic cascade occurs when a change in the presence or activity of organisms at a particular trophic level affects the organisms of other trophic levels through indirect pathways. In the case of trout for example, more fish may relate to more GPP. More fish could consume and put more pressure on invertebrates which will in turn consume less algae which will allow for more algal growth and thus GPP. It also remains a possibility that ER, GPP and trout may relate to one another due to mechanisms that either increase or decrease production and metabolism of most trophic levels.

Organisms need an energy source and certain nutrients to maintain activity levels, growth and reproduction. Dissolved organic carbon (DOC) occurs in varying concentrations in streams and is readily absorbed and metabolized by stream microbial organisms (Findlay et al. 1993). DOC is associated with moderate increases in GPP (cite) and larger increases in ER (Bernhardt and Likens 2002) but may decrease fish production at least in lakes (Benoît et al. 2016). Nutrients such as nitrate and phosphate are also known to increase the metabolism of headwater microbes (Benstead et al. 2009) and increase overall GPP (Mulholland et al. 2001), ER (Pascoal et al. 2005), and trout biomass (Artigas et al. 2013). Light availability is the major stimulant of GPP (Warren et al. 2017) and may also be associated with ER (Parkhill and Gulliver 1999), and trout (Kaylor and Warren 2017).

A method for estimating stream metabolism that is currently receiving a lot of attention is the single station open diel oxygen method (Hall and Hotchkiss 2017). This method assumes that oxygen saturation at any particular time is a function of GPP, ER and the oxygen exchange rate between the air and water (Odum 1956). GPP and ER are often solved for using inverse modeling where the amount of light is assumed to be proportional to GPP and the remaining oxygen deficit is assumed to be ER. This will produce a modeled oxygen curve which can be compared to the measured oxygen curve for accuracy. To do this, light measurements and oxygen saturation must be measured frequently (commonly 5-15 minute intervals) along with temperature, salinity, and barometric pressure to calculate 100% saturation. The last remaining parameter required is the gas exchange or reaeration rate often reported as *K*600 in d-1 (600 refers to Schmidt number scaling used for comparison between different gasses).

The *K*600 may be estimated as a free parameter in the inverse modeling technique or measured directly. Estimating *K*600 as part of the model is adequate for streams with low slope and high light availability, however it is more accurate to measure gas exchange directly in shaded streams with higher slopes which are typical of headwater streams. Measuring gas exchange is done by diffusing a gas of choice into the stream at high volumes and measuring concentrations downstream from the injection point. This process may however require permits, be cost prohibitive, and the gas may have undesirable effects.

An alternative to measuring the gas exchange directly in headwater streams may be to estimate this value from physical attributes of the stream and relationships reported in the literature. Palumbo and Brown (2014) suggest that stream slope is the most accurate variable to include when predicting gas exchange in this way and Hall et al. (2016) reports a *K*600 to stream slope relationship with an *R*2 of 0.89. Similarly in a later study Hall and Madinger (2018) include data from gas injections in small headwater streams which produces an *R*2 of 0.68. Using this relationship it may be possible to calculate a *K*600 from the slope of the stream which can then be used in the inverse modeling to estimate stream metabolism.

The goal of this study was to use estimates of stream metabolism with a derived gas exchange value to predict trout biomass in headwater streams and to investigate what water quality parameters best predict both stream metabolism and trout biomass.

*H*a1: Trout biomass will have a positive relationship with GPP.

*H*a2: Trout biomass will have a positive relationship with ER magnitude.

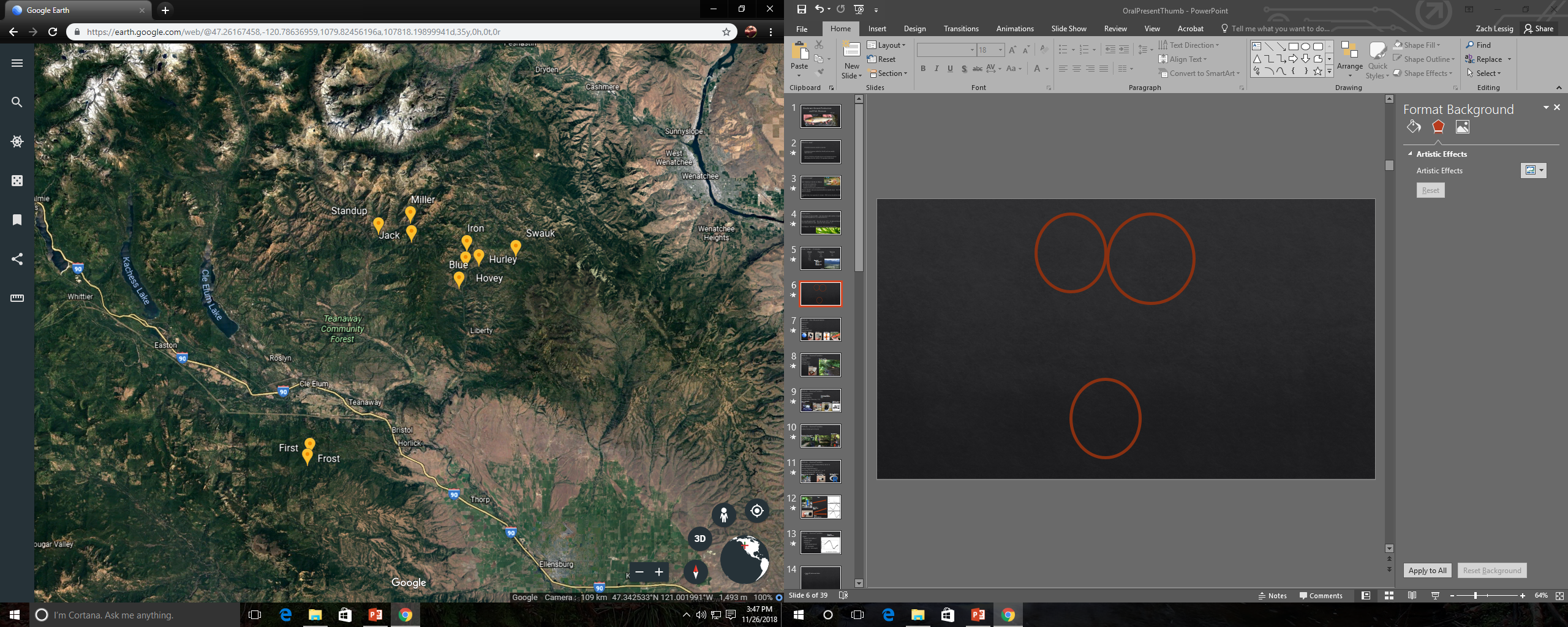
*H*a3: Trout biomass will have a positive relationship with stream nutrients.

*H*a4: GPP will have a positive relationship with stream nutrients.

*H*a5: ER will have a positive relationship with stream nutrients.

**Methods**

Study Design

My ten study sites were located on first and second order headwater streams in the Swauk (n=5), Teanaway (n=3), and Taneum (n=2) catchment areas in Kittitas County, WA. These sites, on the east slope of the Cascade Mountains in the Yakima River Basin, have a hydrograph mainly driven by snowmelt with peak runoff in May and baseflow at the end of July to beginning of October (US Bureau of Reclamation 2019). The 5 sites in Swauk were on Blue, Hovey, Hurley, Iron, and Swauk creeks. The 2 sites in Taneum were on First and Frost creeks, and the 3 sites in Teanaway were on Jack, Miller and Standup creeks (Figure 1). 

Taneum

Swauk

Teanaway

Figure 1. Map showing Teanaway, Swauk, and Taneum catchments with respective study sites.

I sampled these sites 3 times between 2017 and 2018 to capture seasonal variation in stream conditions. The first sampling period was in the summer of 2017 from July 19 to August 15, the second sampling period was in the fall of 2017 from November 5 to November 16, and the third and final sampling was in the summer of 2018 from Jun 26 to July 15.

At each site on these low order headwater streams I collected site description data one time; GPS coordinates (MotionX-GPS version 24.1, Fullpower Technologies on Apple iPhone 5), and elevation (Google Earth) with sites ranging from 869 to 1071 m. Stream aspect (Lensatic compass, Engineer) which was converted to a value of degrees south facing (e.g. 180º is directly south and 0° is north). Stream slopes (Suunto PM-5 Clinometer) were from 2 to 10% and bank full widths ranged from 3.3 to 12.7m. I conducted a Wolman Pebble Count (Wolman 1954) with 50 pebbles measured per stream where median pebble widths ranged from 35.5 to 92mm.

For each sampling period I took data on the following variables; riparian overstory density (canopy openness) with a densitometer (Spherical Crown Densiometer, Convex Model A, Forestry Suppliers) according to methods written on the instrument where values ranged from 4.9 % open for one of the most narrow streams (Frost) in the Summer to 78.1% for the widest stream (Standup) during the fall. I measured stream discharge with a portable flow meter (Flo-Mate 2000, Marsh-McBirney) according to Rantz (1982) with discharges ranging from 0.3 to 65.5 L/s. For each sampling period I also sampled stream water for nutrient analysis, conducted fish population/biomass estimates and estimated stream metabolism according to the methods below.

Stream Nutrients

I collected stream water in acid washed HDPE bottles using 1 µm glass fiber syringe filters (Type A/E Glass Fiber Filter, Pall Corporation). I acidified one of these samples intended for dissolved organic carbon (DOC) analysis with 100 µL of 0.5N HCl to ensure pH was at or below 2. These samples were transported in a cooler out of the field and stored in a freezer until analysis.

I analyzed the samples for ammonium (NH4+) using methodology according to EPA-103-B Rev. 1 (2012) with the exception that 0.025 mg/L ammonium was added to the sample to ensure concentrations were within the detection limit and later subtracted. I analyzed nitrate + nitrite (NO3- + NO2-; hereafter referred to as NO3-) according to EPA-127-B Rev. 1 (2016) and added the ammonium and nitrate concentrations together to obtain a concentration of total dissolved inorganic nitrogen (DIN). I analyzed the sample for phosphate (PO43-) referred to here as soluble reactive phosphorus (SRP) according to EPA-155-B Rev. 0 (2016) . These samples were run on an AQ1 Discrete Analyzer (Seal Analytical). The acidified DOC sample was analyzed on a Shimadzu TOC-L (TOC-L Total Organic Carbon Analyzer, Shimadzu) with techniques outlined in the administrators manual.

Fish

I conducted a population estimate of stream salmonids (Family Salmonidae) immediately upstream of each site where DO probes were deployed and water samples were taken. The fish included in the estimate were almost all native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) with some fish displaying signs of hybridization with the native Columbia Basin redband rainbow trout (*Oncorhynchus mykiss gairdneri*) (Weigel et al. 2002). A few non-native eastern brook trout (*Salvelinus fontinalis*) were included in the Jack Cr. 2018 population and biomass estimate as well. Some young-of-the-year (YOY) salmonids and sculpin (*Cottus spp.*) were also encountered but not included in the estimates.

I used a backpack electrofisher (LR-20B Electrofisher, Smith Root) to collect fish from a 25 m length of stream and an assistant who caught the salmonids 50 mm or more in length with a dip net and placed them in a 5 gallon bucket. I used the two-pass depletion method (Lockwood and Schneider 2000) to estimate populations and did not include block-nets. Block-nets to prevent migration were not used because these streams were relatively small and the time elapsed between the first and second pass was only a few minutes. The assumptions are met for this estimate as long as migration is negligible. I anesthetized the fish to measure and weigh them using Tricaine Methanesulfonate according to Central Washington University Institutional Animal Care and Use Committee (IACUC protocol #A041710). I calculated the fish population with the two-pass depletion method from Lockwood and Schneider (2000) as follows:

Where, C1 is the number of fish removed in the first pass, C2 is the number of fish removed in the second pass, N is the population estimate in numbers of fish and SE is the standard error of N. This population estimate was then divided by the length of stream sampled to provide a measure of fish population in fish m-1.

I estimated fish biomass by multiplying the population estimate by the average mass of the fish and then dividing by the stream width. The average fish mass came from the combination of the fish caught in both passes.

Stream Metabolism

At each site and for each sampling period I deployed a dissolved oxygen (DO) probe (miniDOT Submersible Water Logger, Precision Measurement Engineering) in the stream to measure DO in mg L-1 and temperature. I also deployed a photosynthetically active radiation (PAR) logger (Odyssey Photosynthetic Active Radiation Logger, Dataflow Systems) on the stream bank within 2 meters of the DO probe to measure light as PAR in µmol photons m-2 s-1. These two instruments were synchronized to collect data every 10 minutes (first sampling period only) or every 5 minutes for (successive samplings) at least 36 hours per stream from ~4:00 p.m. on day one to ~9:00 a.m. on day three. After this deployment, I immediately moved the loggers to a different site until all sites had been sampled.

I used the diel DO and PAR curves to estimate stream metabolism with the statistical program R Version 3.4.3 (R Core Team 2013) by the single station open-channel method with inverse modeling from (Hall and Hotchkiss 2017) using the supplemental R script from Supplemental File 34.3. Additional data needed to complete the calculation included barometric pressure calculated from elevation using the same R script, stream depth obtained from flow measurements and the air-water general gas exchange rate (*K*600) explained below.

Included in the R script is the option to estimate metabolism (e.g. GPP and ER) and *K*600 directly from the oxygen, temperature and light data which works well for low gradient high GPP streams (Hall Jr. and Madinger 2018) where *K*600 is considered a free parameter. Another option is to supply a *K*600 value and use the model to estimate only GPP and ER. It is recommended that in headwater streams this method is used where the K600 is measured using tracer gas additions (Hall and Hotchkiss 2017). I did not have the tracer gas method available to me so I investigated alternative methods of deriving a K600.

One method I investigated for deriving *K*600 involved running the model with the option to estimate both metabolism and *K*600 for all samples. From this data I ran a linear model in R of the diel oxygen data vs the modeled oxygen data and obtained an R2 value for each sample. From these I selected the *K*600’s from a subset of the models that had + *K*600, +GPP, –ER and *R*2>0.95 to run regressions with. Hall and Hotchkiss (2017) assert that the model output is erroneous if the GPP is negative or if the ER is positive and Demars et al. (2015) indicate a negative *K*600 can not be trusted. I ran regressions with these *K*600 values against all of the relevant data I had collected and found that average stream velocity had the highest *R*2. I used the stream velocity vs *K*600 to derive an equation that was then used to estimate the *K*600 values for the models that were thrown out:

N=14, R2=0.27, P=0.07

Where *K600* is the general gas exchange rate in units of 1/d and *velocity* is the average stream velocity in m s-1. These models were re-run with the derived *K*600 values and metabolism was estimated again. The metabolism estimates from all of these models were then kept if they had +GPP and –ER which resulted in 21 models.

The other method I investigated was to derive *K*600 values from literature data. Hall and Madinger (2018) suggest there is a strong relationship between stream slope and gas exchange and include slope data along with *K*600 values as determined by Argon gas injections to the stream. I used their data to derive an equation:

N=8, R2=0.68, P=0.01

Where *K600* is the general gas exchange rate in units of 1/d and *slope* is the stream slope in %. This equation was used to derive *K*600 values for all of the models which ultimately produced 26 models displaying +GPP and –ER after the inverse modeling was run.

I chose to continue analysis with the model output produced by the literature derived *K*600 values. I did this because the inverse modeling that estimates *K*600 as a free parameter is intended for streams that generally have a lower gradient and Hall and Madinger (2018) suggest that *K*600 values are unexpectedly high when measured directly in high gradient streams. Although this technique used an equation based on a relationship with a lower sample size (n=8 vs n=14) it had a larger *R*2, smaller p-value and 5 more usable model output values (26 vs 21).

Statistical Analysis

I developed a generalized linear model for each of the response variables GPP, ER, and trout biomass. The predictor variables were the above mentioned site, hydrologic, and nutrient data (Table 1). I conducted this analysis with R and the ‘lme4’ package (Bates et al. 2015).

Table 1. Response and predictor variables shown as random or fixed effects

|  |  |
| --- | --- |
| **Responses** | **Random Effects** |
| GPP (g O2 m-2 d-1) | Catchment (total of 3) |
| ER (g O2 m-2 d-1) | Site (total of 10) |
| Trout Biomass (g m-2) | Sampling Period (total of 3) |
| **Fixed Effects** | |
| Elevation (m) | Discharge (L s-1) |
| Aspect (°South Facing) | Canopy Openness (%) |
| Slope (%) | Daily PAR (mol photons m-2 d -1) |
| Bank Full (m) | Stream Temp. (°C) |
| Pebble Median (mm) | Carbon (DOC mg L-1) |
| Wetted Width (m) | Nitrogen (DIN mg L-1) |
| Depth (m) | Phosphorus (SRP mg L-1) |
| Stream Velocity (m s-1) |  |

I started model selection with a pairwise scatterplot of the response and all predictor variables. Where variables shared a collinearity value of 0.6 and greater, I kept the variable that had the best relationship with the response and removed the other variable from further analysis. I then chose a general linear model (GLM) with several predictors and no interactions and used R’s “drop 1” and “step” functions which returned AIC values associated with each predictor variable. Variables that performed poorly were removed and other unused variables were added in and the process was repeated. After I had worked through the list of variables I had a small subset remaining with which I constructed several different GLM’s. I used R’s “anova” function to compare these GLM’s with one another which returned p-values associated with the comparison. From the best of these models, I then constructed a Q-Q plot, a residual plot and ran an Anderson-Darling test for normality on the residuals. If these results showed evidence of heteroscedasticity or non-normal residuals I moved to a generalized linear model (GZLM). A different GZLM was constructed with the variables in question for each of the random effects listed in Table 1. These were then analyzed with residual plots and the anova function and based on the weight of evidence, the best of these was used in a GZLM that allowed for alternate variance structures. This process of residual analysis and comparison was then repeated for models with variance that was constant, varied by power, exponential, and constant power. If the best of these models (based on p-values and residual analysis) did not appear to meet the model assumptions, the response variable was then transformed with a mild transformation and the process of model selection was started again. I proceeded with model selection in this way working iteratively with stronger response transformations until a model was produced that best met assumptions. I then went back to the non-collinear variables that were not included in the current model and included them as an interaction term one by one and compared these to each other while analyzing the residuals. The best of these was then considered the final model.

**Results**

GPP

I estimated the mean Gross Primary Production (GPP) across all sites and sampling periods to be 0.196 g O2 m-2 d-1 (Figure 2).

The model I determined for GPP was a linear mixed effects model with a square root of a log transformation with site as a random effect. The main effects were determined to be sampling period (Figure 2) and depth (Figure 3) both with a model p-value<<0.05. GPP did not appear to be related to daily amounts of light (PAR) or concentrations of nutrients (DOC, DIN, SRP) or was weakly negatively associated.

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Figure 2. Mean gross primary production (GPP) (± 1 standard error) for all study sites at consecutive sampling periods with associated linear mixed effects model (LME) p-value. Means with different letters are significantly different.

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Figure 3. Regression of transformed GPP and stream depth with associated adjusted *R*2 and model p-value.

ER

I estimated the mean ER across all sites and sampling periods to be -10.29

g O2 m-2 d-1 (Figure 4). Although ecosystem respiration is noted as a negative number because it is thought of as a subtraction of oxygen from the environment, it will be discussed here in terms of its absolute value (positive) to facilitate modeling and conceptualization.

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Figure 4. The absolute value (magnitude) of mean stream ecosystem respiration (ER) values for all sites at consecutive sampling periods (±1 standard error). Means with different letters are significantly different according to Tukey’s Honest Significant Difference (Tukey HSD) test.

The model I determined for ER was a linear mixed effects model with a log transformation of the absolute value and site as a random effect. The main effects were determined to be depth (Figure 5) and slope (Figure 6). ER magnitude did not appear to relate positively to nutrients (DOC, DIN, SRP) however ER did relate to GPP (Figure 7).

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Figure 5. Regression of transformed ER and stream depth (m) with an adjusted R2 of 0.36 and p< 0.0001 from the ER model.

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Figure 6. Regression of transformed ER and Slope (%) with an adjusted R2 of 0.57 and p< 0.0001 from the ER model.

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Figure 7. Regression of ER and GPP with an adjusted *R*2 of 0.41 and a p-value of 0.00026.

Fish

I sampled a total of 230 westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and 4 eastern brook trout (*Salvelinus fontinalis*) with a minimum fish length of 50 mm, median 79 mm and a maximum length of 215 mm (8.5 inches). I estimated the trout population in fish per meter of stream length of all sites and both fish sampling periods to range from 0 in First Cr. (Taneum Catchment) 2018 to 1.33 fish m-1 in Standup Cr. (Teanaway Catchment) 2018. The mean trout mass per individual fish ranged from 3.58 g in Frost Cr. (Taneum Catchment) 2017 to 31.23 g in Jack Cr. (Teanaway Catchment) 2017 (Figure 8). I estimated trout biomass in g m-2 to range from 0 in First Cr. (Taneum Catchment) 2018 to 8.38 g m-2 in Hurly Cr. (Swauk Catchment) 2017 (Figure 9).

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Figure 8. Mean mass of individual fish per stream (± standard error) and year of sampling with streams arranged by increasing wetted width and grouped by watershed.

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Figure 9. Mean trout biomass per stream and year of sampling (± standard error from population) with streams arranged by increasing wetted width and grouped by watershed. These values were arrived at by multiplying the fish population per meter of stream by the mean weight of the individual fish, the result was then divided by the stream wetted width.

The model I determined for trout biomass in g m-2 of stream surface area was a generalized least squares with a log transformation. The variance was allowed to change based on an exponential function and the main effects were catchment (Figure 10) and minimum daily temperature with an interaction on canopy openness (Figure 11). Trout biomass did not appear to relate to nutrients (DOC, DIN, SRP), light (PAR), or ecosystem metabolism (Figure 12).

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Figure 10. Boxplot of transformed trout biomass by watershed with the trout model p-value of 0.0007.

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Figure 11. Boxplot of transformed trout biomass by water temperature category (1.4 °C range for each category) and canopy openness category (25.3 % range for each category). There is a p-value of 0.0071 for the interaction of stream temperature and canopy openness. Stream temperature is significant by itself whereas canopy openness is not. The low and mid temperature ranges have more trout biomass in the open canopy category. Overall there is more biomass at lower temperatures.

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Figure 12. Regression of GPP and trout biomass showing no significant relationship. The relationship of trout biomass was further from significance when compared to ER or the PR ratio.