**Introduction**

At the highest points of a catchment the stream network begins. When viewing a topographic map, one can see small grooves in the hillsides. These grooves, often enshrouded by a dense canopy of trees, conceal ribbons of water called headwater streams. These starting points of a fluvial network become a more apparent landscape feature as they flow downstream and coalesce into larger rivers. When viewed from their banks, headwater streams appear as modest rivulets, kept cool by the shade of the canopy above and constrained by steep banks so the water winds around large rocks and tree roots. Previous years’ leaves or needles litter the ground and many have accumulated in small pools in the stream (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 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).

. They are low the of many other streamsHeadwaters are vital components of a stream network because they contribute substantially to the water quality and biodiversity of downstream waterways (Alexander et al. 2007; Meyer et al. 2007). One way in which headwaters are important for downstream water quality is as sites of large subsidy input and transformation. As individuals, headwater streams are small and seem insignificant, but collectively they constitute almost 80% of a drainage network’s total stream length and drain more than 70% of the land surface (Colvin et al. 2019). This leads to a substantial amount of material entering these streams from the nearby landscape. The surface area to depth ratio in these small streams is relatively high compared to downstream reaches so this material will tend to travel less distance before it encounters the sediment or a biofilm where it may be stored, chemically altered, or assimilated into a living organism (Mulholland et al. 2000). Because headwaters have a tight connection to downstream reaches (Vannote et al. 1980), this rapid biogeochemical processing leads to large reductions in nutrients entering larger waterways (Peterson et al. 2001). This is important because excess nutrients in rivers degrades the water quality and hampers biodiversity through processes such as eutrophication (Carpenter et al. 1998).

(Warren et al. 2017) (Bilby and Bisson 1992) (Fry 1991)ecosystem (Warren et al. 2017)

Headwater streams 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 small 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).

Organisms need (Robbins et al. 2017) (Kaylor and Warren 2017a)

The activities of all of the aerobic organisms in a stream reach can be measured with the 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 Jr.,Robert O. and Hotchkiss, Erin R. 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).

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 (Hall and Hotchkiss 2017).

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.

in the stream ecosystem

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 stream metabolism and trout biomass.

*H*a1: Metabolism models with a gas exchange value based on stream slope will function correctly.

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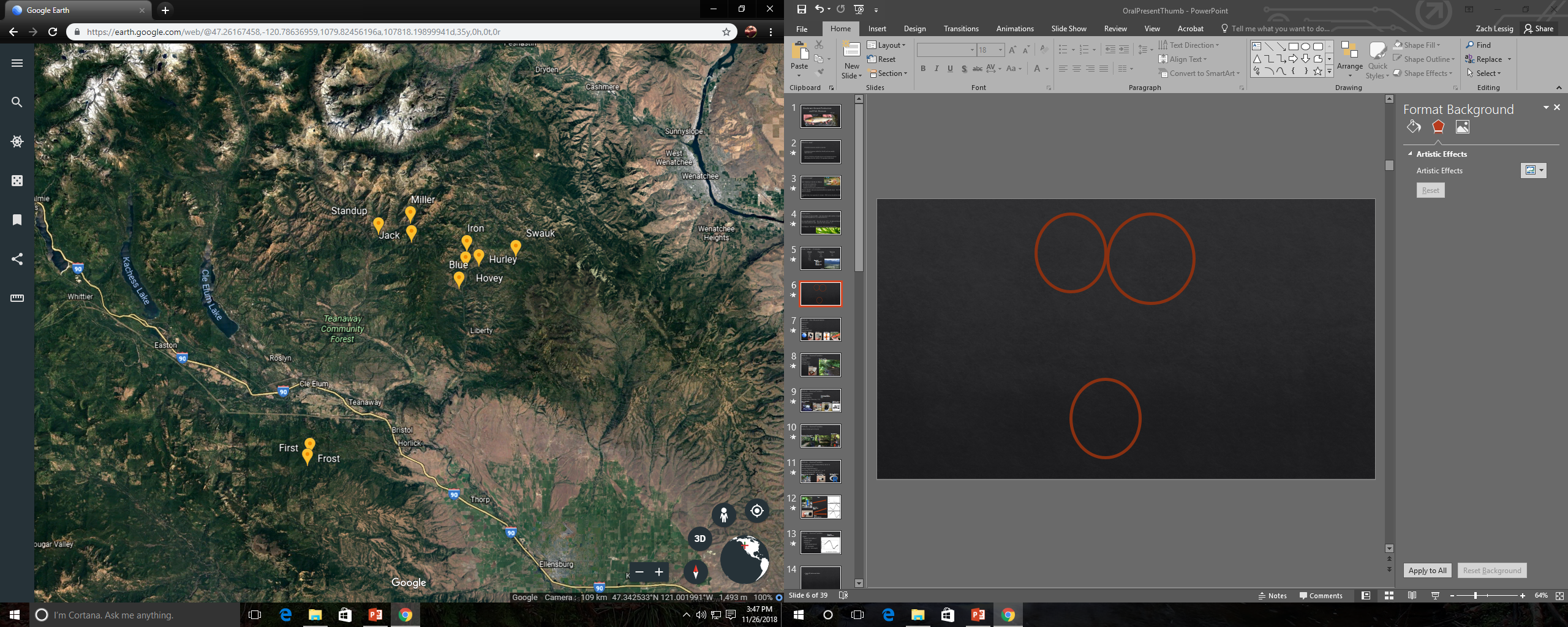
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*H*a5 Trout biomass will have a positive relationship with GPP.

*H*a6: Trout biomass will have a positive relationship with ER magnitude.

**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 with all streams as 1st through 3rd order (Figure 1). 

Taneum

Taneum

Teanaway

Swauk

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 19 July to 15 August, the second sampling period was in the fall of 2017 from 5 November to 16 November, and the final sampling was in the summer of 2018 from 26 Jun to 15 July.

At each site on these low order headwater streams, I collected site description data using 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. I also measured stream aspect (Lensatic compass, Engineer), stream slopes (Suunto PM-5 Clinometer) were from 2 to 10% and bank full widths ranged from 3.3 to 12.7 m. I conducted a Wolman Pebble Count (Wolman 1954) with 50 pebbles measured per stream where median pebble widths ranged from 35.5 to 92mm (Table 1).

Table 1. Site specific variables.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Catchment | Stream | Aspect | Elevation | Slope | Bank Full | Pebble Median |
|  |  |  | (m) | (%) | (m) | (mm) |
| Taneum | First | S | 880 | 5 | 5.07 | 42 |
| Taneum | Frost | N | 904 | 10 | 12.74 | 60 |
| Swauk | Hurley | W | 932 | 3 | 3.32 | 66 |
| Swauk | Hovey | E | 905 | 6 | 3.44 | 62 |
| Swauk | Blue | E | 869 | 3 | 5.20 | 35.5 |
| Swauk | Swauk | W | 1071 | 4 | 4.82 | 39 |
| Swauk | Iron | S | 950 | 2 | 5.16 | 43.5 |
| Teanaway | Jack | S | 954 | 3 | 3.62 | 68.5 |
| Teanaway | Miller | S | 981 | 5 | 6.08 | 55 |
| Teanaway | Standup | S | 871 | 8 | 4.42 | 92 |

For each sampling period I measured the following variables; stream discharge, riparian overstory density (canopy openness), light as photosynthetically active radiation (PAR), stream temperature, stream nutrients, and estimated fish biomass, and stream metabolism. I measured canopy openness with a densitometer (Spherical Crown Densiometer, Convex Model A, Forestry Suppliers). I measured stream discharge with a portable flow meter (Flo-Mate 2000, Marsh-McBirney) according to Rantz (1982). I conducted nutrient analysis, fish population/biomass estimates, and 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 ≤ 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) 25 m immediately upstream (35 m for Standup and 50 m for First 2017) of each site where DO probes were deployed and water samples were taken. The collected fish included mostly 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, assisted by a person 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. To analyze my catch, I anesthetized the fish using Tricaine Methanesulfonate to measure and weigh them 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. The values obtained were then multiplied by a previously determined correction factor to yield 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) from 4:00 p.m. on day one to 9:00 a.m. on day three so that n ≥ 41 hrs.

I used the diel DO and PAR curves to estimate stream metabolism with the statistical program R Version 3.5.2 (R Core Team 2013) by the single station open-channel method with inverse modeling using the supplemental R script from Supplemental File 34.3 from Hall and Hotchkiss (2017). 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 2. 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**

Seasonal Variables

Stream discharges ranged from 0.3 to 65.5 L s-1 (Figure 2.). Canopy openness values ranged from 4.9 % open for Frost Cr. in the Summer to 78.1% for the widest stream Standup Cr. during the fall (Figure 3.).

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Figure 2. Boxplot of stream discharge for all study sites at consecutive sampling periods. Means are not significantly different.

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Figure 3. Boxplot of riparian overstory density (canopy openness) for all study sites at consecutive sampling periods. Means are not significantly different.

Light as PAR ranged from 0.035 to 3.525 mols of photons m-2 d-1 (Figure 4.). The nitrate and ammonium values were calculated as negative in some instances and there were 2 large ammonium outliers, because of this, the values were shifted into the positive range, the outliers were dropped and the nitrate and ammonium were added together to produce a relative ordering of total dissolved inorganic nitrogen (DIN) where relative values ranged from 0.0021 to 0.178 mg N L-1 with the outliers as 0.1860 for Hovey Cr. In fall 2017 and 0.2559 mg N L-1 for Swauk Cr. in summer 2017(Figure 5.).

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Figure 4. Boxplot of light values as photosynthetically active radiation (PAR) for all study sites at consecutive sampling periods. Means with different letters are significantly different.

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Figure 5. Boxplot of nitrogen as relative values of dissolved inorganic nitrogen (DIN) (NH4+ + NO3-) for all study sites at consecutive sampling periods. Means with different letters are significantly different.

Phosphate as soluble reactive phosphorus (SRP) ranged from 0.0049 to 0.0610 mg P L-1 (Figure 6.) and carbon as dissolved organic carbon (DOC) ranged from 0.51 to 13.27 mg C L-1 (Figure 7.). The mean values of stream discharge and canopy openness did not change for consecutive samplings however PAR was lowest in the fall. All stream nutrients and DOC values stayed the same for the first two samplings and went up for the third sampling according to Tukey’s Honest Significant Difference (Tukey HSD) tests.

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Figure 6. Boxplot of phosphate as soluble reactive phosphate (SRP) for all study sites at consecutive sampling periods. Means with different letters are significantly different.

N:\Thesis\Rplot17.DOC.tiff

Figure 7. Boxplot of carbon as dissolved organic carbon (DOC) for all study sites at consecutive sampling periods. Means with different letters are significantly different.

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 8.).

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 8) and depth (Figure 9) 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 8. 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 9. 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 10). 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.

N:\Thesis\Rplot3.er.lit1.tiff

Figure 10. 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 11) and slope (Figure 12). ER magnitude did not appear to relate positively to nutrients (DOC, DIN, SRP) however ER did relate to GPP (Figure 13).

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Figure 11. 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 12. 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 13. 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 14). 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 15).

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Figure 14. 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 15. 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 16) and minimum daily temperature with an interaction on canopy openness (Figure 17). Trout biomass did not appear to relate to nutrients (DOC, DIN, SRP), light (PAR), or ecosystem metabolism (Figure 18).

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Figure 16. Boxplot of transformed trout biomass by catchment with the trout model p-value of 0.0007.

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Figure 17. 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 18. 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.

**Discussion**

GPP

A recent study by Mejia et al. (2018) estimated metabolism in streams in the same habitat as the present study and with similar methodology with the exception that the streams were not headwaters. Most of the GPP values I estimated (0.007 to 0.707 g O2 m-2 d-1 Figure 8.) were within the range reported by Mejia et al. (2018) (0.02 to 2.53 g O2 m-2 d-1). This however is not what would be expected given that the lowest solar radiation estimation by Mejia et al. (2018) per day (7.2 mol PAR m-2 d-1) was more than twice the highest value I determined for any of my sites (3.5 mol PAR m-2 d-1 Figure 4.) which appear consistent with other headwaters (e.g. Roberts et al. 2007) and GPP is heavily dependent on light (Odum 1956).

All of the streams in my study were almost certainly light limited with respect to GPP. Light limitation is the strongest factor controlling GPP below 3.5 mol PAR m-2 d-1 and GPP is severely limited below 2.2 mol PAR m-2 d-1 according to Warren et al. (2017) who also conducted their study in a similar environment to mine. All but one of my sites were at or below the 2.2 mol PAR m-2 d-1 threshold with the highest being 3.5 (Figure 4.). This suggests that very low GPP values should result from my low PAR values.

GPP is also heavily dependent on nutrients (Bernot et al. 2010) and the DIN in my study was relatively low with a mean of less than 0.02 mg N L-1 (Figure 5.) compared to 0.10 mg N L-1 for Mejia et al. (2018). A large study conducted in Great Britain found that primary production in headwaters is often limited by DIN concentrations (Jarvie et al. 2018) and another extensive study of temperate streams in the USA suggests that DIN below 0.04 mg N L-1 exhibits depressed levels of chlorophyll (Dodds et al. 2002). Chlorophyll is often used as a proxy for GPP since Ryther (1956). The mean SRP was much higher however in my sites (0.022 mg P L-1, Figure 6.) than Mejia et al. (2018) (0.003 mg P L-1) which may help to explain my unexpectedly high GPP values although this is appears unlikely based on the low light and nitrogen concentrations.

Additionally, Mejia et al. (2018) showed that GPP increases with increasing catchment area. The catchment area of the streams in my study were not determined, however the average stream discharge was far less (17.7 Figure 2. vs 420.2 L s-1 for Mejia et al. 2018) suggesting that the catchment area was also much less. The GPP values in my study again were not reflective of this.

ER

Ecosystem respiration values in my study also appear to be questionable. Mejia et al. (2018) reported that ER also increases with catchment area as well as discharge, PAR, and temperature. The values I have measured or estimated for all of these potential determinants were less for my study and yet the ER values I obtained (mean of -10.287 g O2 m-2 d-1 Figure 10.) were far greater in magnitude than what Mejia et al. (2018) reported (mean of -1.25 g O2 m-2 d-1).

Although it appears that the explicit values produced by the models that estimated metabolism may not be trustable, it remains a possibility that the relative order of values may be preserved. Assuming that the relative order of values was preserved, it would be expected that the relationships observed here would be similar to the relationships discovered in other studies. Small forested headwaters are known to display net heterotrophic metabolism meaning that the respired oxygen is much greater than the produced oxygen (Allan and Castillo 2007). My metabolism predictions displayed this relationship with R>>P (Figure 13.). It is also expected that GPP and ER will display a strong positive relationship (Hall et al. 2016) which my metabolism predictions also found. Although some of the expected relationships were indicated by the predictive inverse modeling, the environmental predictors found by the metabolism GLZM’s do not appear to affirm the ordering of values. Both of the GPP and ER models (GLZM’s) found stream depth to be a main effect while the ER model also found stream slope and the GPP model found sampling period. Stream depth is a variable that is put directly into the inverse modelling used to derive metabolism and slope is part of the equation used to derive a *K*600 which is also put directly into the inverse model. This leaves sampling period (when samples were taken) which is not suggestive of any relationship that may inform the reliability of the inverse modeling results. It is conceivable that although slope and depth were inverse model inputs, these physical parameters still had the most profound effect on metabolism, although this appears unlikely.

As discussed above, if metabolism was generally limited by low PAR and DIN it may be difficult to identify other drivers. Depth appears easier to rationalize as deeper streams may generate more metabolism simply because of the increase in physical dimensions of the stream. Slope presents itself with more difficulty though. If stream slope were a driver of ER, the mechanism seems obscure. Steeper slopes could lead to more soil erosion (Renard et al. 2017 Oct 19) and thus potentially more nutrients or carbon in the stream, however neither nutrients nor DOC were part of the GLZM outcomes. If increasing slope allows for more light penetration through the canopy then this would be expected to reveal itself as PAR, canopy openness, and/or increased temperature, a relationship which has not revealed itself in the data. This leads to the conclusion that although the relative ordering of metabolism values may reflect reality, this assumption is quite tenuous.

Trout

The trout GLZM found colder minimum daily water temperature and canopy openness to be important factors determining fish biomass (Figure 17.). Each of the trout biomass estimates fell within the range of a large data set compiled by Benjamin and Baxter (2012) who included data for the same subspecies of cutthroat and brook trout I sampled. The estimates I have provided are overwhelmingly due to cutthroat which are known to be a species heavily dependent on cold mountain streams (Isaak et al. 2016). Cutthroat are quite capable of existing in warmer water than where I found them but they are often outcompeted by rainbow trout at warmer temperatures (Bear et al. 2007) which may be part of the reason for this finding. The same relationship exists in my data with minimum, mean, and maximum water temperatures illustrating the robustness of this finding. The canopy openness effect in the GLZM was significant as an interaction with colder water. This finding is also well supported in the literature (Kaylor and Warren 2017a, Martens et al. 2019) with Kaylor and Warren (2017b) finding that the majority of vertebrate biomass in the streams they studied, including cutthroat trout, was accounted for by canopy openness alone.

No relationship was found between trout biomass and GPP (Figure 18.), ER or the PR ratio which may be substantive or an artifact of metabolism inverse modeling inaccuracies. The trout biomass estimation and GLZM appear to be consistent with previous empirical tests which is evidence indicating either the inverse modeling was flawed, the sample size was too small, or there was indeed no relationship with stream metabolism. Marcarelli et al. (2011) posit the idea that heterotrophic streams display a decoupling between ER and secondary productivity which may be why I did not find a connection here either. The authors did nonetheless, find a positive relationship between the PR ratio and secondary production in streams and suggest that carbon from GPP may be more responsible for supporting animal growth than allochthonous carbon. I did not detect this linkage and if there was a significant connection here, my data would depict a negative relationship (Figure 18.). Marcarelli et al. (2011) found this relationship with aquatic invertebrates and not fish though, perhaps this relationship is obscured at higher trophic levels. These conclusions are open to question however given the somewhat problematic metabolism estimations.

Future Studies

Future studies that attempt to estimate headwater whole stream metabolism using diel oxygen curves without using gas tracers to estimate the gas exchange may be better served by altering the methods presented here. Using the inverse modeling to estimate the gas exchange is likely a preferable technique although model results with a negative gas exchange, negative GPP, and positive ER will still need to be left out of the analysis. Increasing the initial sample size to compensate for this eventual loss of data may offset this. Increasing the sampling rate of the instruments to 1 minute or less is also recommended to increase the resolution of the data. These changes have the benefit of relatively simple methodology although it may still be limited to streams of lower slopes (Hall Jr. and Madinger 2018).

Another possibility may be to use an equation to derive the gas exchange value involving more parameters than slope. A meta-analysis by Palumbo and Brown (2014) which evaluated 18 different equations affirm that using equations that have slope as a parameter are less biased than equations which do not have slope as a parameter. They then suggest an equation from Thackston and Dawson (2001) for streams within the same depth and velocity range as the streams in my study which curiously does not include a slope component. This seeming contradiction may be because small steep streams behave uniquely or little effort has been put forth to extend predictive power to them and thus the meta-analysis had little to work with. Interestingly none of the equations include a component for stream bed roughness. Other studies including Ulseth et al. (2019) demonstrate that increasing the stream bed roughness to depth ratio causes large increases in gas exchange because of the increased turbulence which is typical of low order mountain streams. The same study also suggests that stream slope above 4% causes disproportionate increases in gas exchange because air bubbles begin to form and become entrained in the water column. This study does not suggest an equation to use for my application, however there appears to be much work attempting to extend equations for predictions of gas exchange rates to headwater mountainous streams and this may be expected in the near future.

Other techniques for estimating the gas exchange rate in headwaters likely exist for future studies of this kind. Pennington et al. (2018) found that the gas exchange rate can be calculated from the simultaneous measurement of both DO and CO2. This technique involves more instrumentation and more complex calculations but is uninvasive and produces a time-series of the gas exchange rate such that if environment conditions change which alter the gas exchange (e.g. flow variation, surface wind movement) this change will be accounted for. Another promising and creative avenue of research in this area makes use of sound. Morse et al. (2007) reasoned that turbulence drives gas exchange in steep streams (Chanson and Toombes 2003) and turbulence has acoustic properties (Leighton 2012). This led them to compare the sound coming from a stream at a standardized distance to measured gas exchange from gas injections. This study found a strong linear relationship between gas exchange and sound levels and has the benefit of using inexpensive equipment and simple methodology.

Conclusion

It appears that the mountainous headwater streams in Kittitas County I studied display characteristics that are fairly consistent with what would be expected. The streams are steep, cool, dark, low in nitrogen, and high in DOC. They are unexpectedly high in SRP. The cutthroat trout biomass is within expected ranges and the fish prefer colder water probably because of competition with rainbow trout. They seem to prefer more open canopies likely because of prey availability.

I was not able to reliably estimate metabolism because my methodology of determining gas exchange was flawed. Without reliable metabolism values I was not able to establish a relationship with trout biomass. Future research will likely produce methodology to easily and reliably estimate metabolism in headwaters whereupon trout biomass may be shown to exhibit a positive relationship with GPP.