**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 accumulate in small pools in the stream. Some of these leaves will appear sturdy and intact while others show the invariable signs of decomposition where fungi, bacteria, and aquatic insects have left little but 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 or other food particles drifting downstream (Hughes 1992).

A succinct definition for headwater streams has not been completely agreed on although they are broadly understood as 1st or 2nd order channels (i.e. a stream that has coalesced with at least one other 1st order stream) (Strahler 1957) although some favor defining it as a stream draining a catchment size of less than 100 ha (Gomi et al. 2002). In the Pacific Northwest (USA) however, a definition involving a more quantitative characterization of stream size is favored. In this region and for the current study, headwater streams are defined as less than 3 m wide with an average annual discharge of less than 57 L s-1 (Richardson and Danehy 2007).

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 to fuel biological activity, making headwaters sites of energy input from terrestrial sources to streams (Vannote et al. 1980). Headwaters also contribute substantially to water quality in downstream waterways mainly through their high surface area to depth ratio relative to downstream reaches of higher stream order (Alexander et al. 2007; Meyer et al. 2007). This high ratio causes material to travel less distance before encountering a storage site in sediment or biofilm where it can be 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 substantial reductions in nutrients entering larger waterways (Peterson et al. 2001), with implications for downstream processes such as eutrophication (Carpenter et al. 1998).

A small forested headwater stream ecosystem sustains an integrated community of organisms distinctly structured by differing energy inputs. These energy inputs are differentiated by their origin, either from terrestrial (i.e., allochthonous) or aquatic (i.e., autochthonous) production. The amount of light reaching the stream in headwaters is often much less than in downstream reaches where the channel is more open, so sparse solar radiation typically limits autochthonous production (Warren et al. 2017). When the canopy is closed however, an abundance of plant matter often enters the stream in the form of foliage or wood (Bilby and Bisson 1992). This allochthonous plant material, often serves as the energetic foundation for headwater ecosystem food webs (Fry 1991). Because these ecosystems often depend on allochthonous energy subsidies from the surrounding environment rather than energy produced in the aquatic ecosystem, they are considered net heterotrophic.

When allochthonous matter enters streams, aquatic fungi and bacteria colonize and consume it, forming a thin, slimy biofilm as they metabolize the hydrocarbons. Headwater streams often have scant inorganic nutrients such as phosphorus or nitrogen (Warren et al. 2017) so these nutrients rapidly assimilated for critical cellular processes while the hydrocarbons are used for biofilm structure or mineralized as an energy source, releasing carbon dioxide through respiration. The metabolism of allochthonous organic matter by biofilms also provides a critical link between difficult to digest terrestrial production and aquatic invertebrates, which then can become a food source for fishes.

Aquatic invertebrates are frequently characterized by what they eat rather than their taxonomic name. Some known as “shredders” eat biofilm-laden leaves whereas those called “collectors” wait for particles of food to be delivered to them by the current or they actively gather small scraps from the stream bed. A few are predatory, spending their time hunting for other invertebrates while still others called “scrapers” eat algae or biofilm directly from rock or other surfaces. 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.

Headwater streams sustain certain species of culturally and economically important salmonid fishes (Family Salmonidae). Many salmonids such as coho, and chum salmon, along with steelhead use headwaters extensively for rearing habitat (Meyer et al. 2007). The salmonids that the Pacific Northwest headwaters harbor as adults however, are trout (Richardson and Danehy 2007). The life histories of some populations of cutthroat trout, for example, may be played out solely in headwaters. Cutthroat have experienced massive declines in numbers recently (Behnke 1992) and in isolated headwaters, these populations may experience protection from competition or genetic admixture with other salmonids thereby preserving the species (Hilderbrand and Kershner 2000). In the western USA, trout in general 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 harvest (Gresswell and Liss 1995).

The activities of all of the aerobic organisms in a stream reach can be described by measuring 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, releasing O2. ER is the reverse 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 determination of stream metabolism has been of interest because of its all-inclusive scope and various methods for its determination have been developed. GPP has been measured directly through the difference in ash free dry weight of periphyton however, this method involves only limited subsamples of the benthos and does not include ER (Naiman and Sedell 1980). Chlorophyll *a* extraction from stream autotrophs followed by spectrophotometric measurement has been used as a proxy for GPP, but this too is limited in application and does not include ER (Lorenzen 1967). The recirculating chamber method involves placing stream substrate in a closed chamber which is held in the stream and oxygen measurements are taken at time intervals from the chamber (Mclntire et al. 1964). This method has the advantage of producing measurements of both GPP and ER however conditions in the chamber do not necessarily reflect conditions in the rest of the stream and it probably does not scale well as an estimate for the entire stream (Tank et al. 2010). Another method for estimating stream metabolism that is currently receiving a lot of attention is the single station open channel diel oxygen method (Hall and Hotchkiss, 2017). This method assumes that oxygen saturation in the open stream at any particular time is a function of GPP, ER, and the oxygen exchange rate between the air and water (Odum 1956). Inverse modeling is used to solve for GPP and ER where GPP is assumed to be proportional to the amount of light and the remaining oxygen deficit is assumed to be a result of ER. This produces a modeled oxygen curve which can be compared to the measured oxygen curve for accuracy. To use this method, light measurements, oxygen saturation, and temperature must be measured frequently (commonly 5-15 minute intervals), while a single measurement of barometric pressure is used to calculate 100% saturation. The last remaining parameter required is the gas exchange or reaeration rate, often reported as K600 in d-1 where 600 refers to Schmidt number scaling used for comparison between different gasses. The K600 may be estimated as a free parameter in the inverse modeling technique or measured directly. Estimating K600 as part of the model is adequate for streams with low slope and high light availability. Directly 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, and Hall et al. (2016) report a K600 to stream slope relationship with an R2 of 0.89. Similarly, in a later study, Hall and Madinger (2018) include data from gas injections in small headwater streams which produced an R2 of 0.68. Using this relationship it may be possible to calculate a K600 from the slope of the stream which can then be used in the inverse modeling to estimate stream metabolism.

Stream metabolism is frequently controlled by the availability of nutrients and energy sources. Dissolved organic carbon (DOC) often serves as an energy source and is readily metabolized by stream heterotrophic organisms (Findlay et al. 1993). DOC is associated with moderate increases in GPP (Robbins et al. 2017) and larger increases in ER (Bernhardt and Likens 2002). Nutrients containing nitrogen (N) and phosphorus (P), usually as ammonium (NH4+), nitrate (NO3-) and phosphate (PO43-), are also known to increase the metabolism of headwater microbes (Benstead et al. 2009) via increases in 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 2017a).

The presence of trout in a headwater stream may also relate to overall stream metabolism. For example, the respiration of trout will be included directly in the stream ER estimate (Hall 1972), so more trout could be related to higher ER. Presence of trout could also affect GPP via a trophic cascade (Young et al. 2008). A trophic cascade occurs when a change in the presence or activity of organisms at a higher trophic level affects the organisms of other trophic levels through indirect pathways. In the case of trout for example, more fish consume more invertebrates which could in turn consume less algae, allowing for higher rates of 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 in the stream ecosystem. There appears to be a lack of research directly investigating the relationship of whole stream metabolism to higher trophic levels (Marcarelli et al. 2011), and if a study can show they are linked, there may be management implications. For example, knowing fish population numbers is important for management, but measuring population size is also resource intensive (Quist et al. 2009). If a relationship between stream metabolism and fish can be established, the need for time consuming fish population estimates could be reduced.

The ultimate 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.

*H*a2: GPP will have a positive relationship with stream nutrients.

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

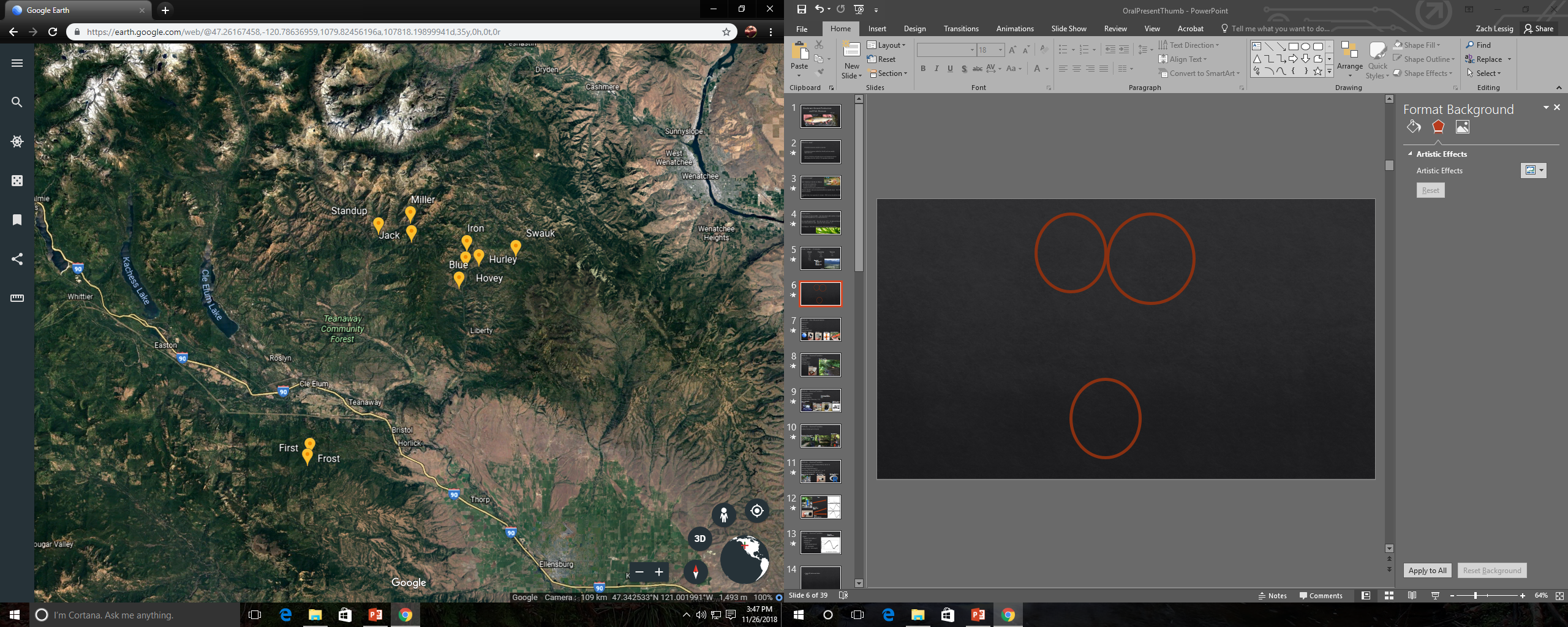
*H*a4: Trout biomass will have a positive relationship with stream nutrients.

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

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

**Methods**

Study Design

I selected ten study sites on first through 3rd order headwater streams in the Taneum (n=2), Teanaway (n=3), and Swauk (n=5) catchments 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 2 sites in Taneum were on First, and Frost creeks; the 5 sites in Swauk were on Hurley, Hovey, Blue, Swauk, and Iron creeks; and the 3 sites in Teanaway were on Jack, Miller, and Standup creeks and (Figure 1.). 

Taneum

Taneum

Teanaway

Swauk

Figure 1 Map showing Taneum, Swauk, and Teanaway, 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 study site, I collected site description data once. These descriptors included GPS coordinates (MotionX-GPS version 24.1, Fullpower Technologies on Apple iPhone 5), stream aspect (Lensatic compass, Engineer), elevation (Google Earth), stream slope (Suunto PM-5 Clinometer), and I also conducted a Wolman Pebble Count (Wolman 1954) with 50 pebbles per stream (Table 1).

Table 1. Site characteristics.



For each sampling period (n=3) I measured or estimated the following variables: stream discharge, riparian canopy openness, stream nutrients (ammonium, nitrate, phosphate and DOC), fish biomass, stream metabolism (GPP and ER), photosynthetically active radiation, and stream temperature.

I measured stream discharge with a portable flow meter (Flo-Mate 2000, Marsh-McBirney) according to Rantz (1982), and canopy openness with a densitometer (Spherical Crown Densiometer, Convex Model A, Forestry Suppliers). I conducted nutrient analysis, fish biomass estimates, stream metabolism estimates and measured photosynthetically active radiation and stream temperature according to the methods described in detail 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). In the field, I acidified one of these samples intended for DOC analysis with 100 µL of 0.5N HCl to ensure pH ≤ 2. All samples were transported in a cooler out of the field and stored in a freezer within 24 h until analyses could be performed.

I analyzed the samples for nitrogen in the form of ammonium (NH4+) using the phenol-hypchlorite method (Solórzano 1969) in a methodology adapted from EPA-103-B Rev. 1 (2012) with the exception that 0.025 mg/L NH4+ was added to the sample to ensure concentrations were above the detection limit. The added NH4+ was subtracted before data analysis. I analyzed nitrogen in the form of nitrate and nitrite (NO3- + NO2-), hereafter referred to as NO3-, using the cadmium reduction method ) according to a methodology adapted from EPA-127-B Rev. 1 (2016). I ultimately added the ammonium and nitrate concentrations together to obtain a concentration of total dissolved inorganic nitrogen (DIN). I meausured phosphate (PO43-), referred to here as soluble reactive phosphorus (SRP), using the molybdate method (Murphy and Riley 1962) according to EPA-155-B Rev. 0 (2016) . The samples of NH4+, NO3, and SRP were all run on an AQ1 Discrete Analyzer (Seal Analytical). The acidified DOC sample was measured using the infrared method with a Shimadzu TOC-L (TOC-L Total Organic Carbon Analyzer, Shimadzu) with techniques outlined in the administrators manual.

Fish Population Estimates

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 water samples were taken and DO probes were deployed for metabolism estimates. The collected fish included native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) with some 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 collected in Jack Cr. 2018, and they were included in the population and biomass estimate. 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 (35 m for Standup and 50 m for First 2017), 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 to estimate population and did not include block-nets (Lockwood and Schneider 2000). 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 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 (Lockwood and Schneider 2000). 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 (g) and then dividing by the stream width (m). The error associated with fish biomass came from multiplying the standard error of the population estimate by the average mass of the fish. 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 (mg L-1) and temperature (°C). 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 PAR as pulses s-1, a proprietary measure that can be converted to PAR (µmol photons m-2 s-1) (Shaffer and Beaulieu 2012). These two instruments were synchronized to collect data every 10 minutes (first sampling period only) or every 5 minutes (second and third samplings) from 4:00 p.m. on day one to 9:00 a.m. on day three (≥ 41 h per deployment).

I used the diel DO and PAR curves to estimate stream metabolism using the supplemental R script for the single station open-channel method with inverse modeling from Supplemental File 34.3 from Hall and Hotchkiss (2017) in the statistical program R Version 3.5.2 (R Core Team 2013). 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 (K600 - explained below).

Included in the R script is the option to estimate metabolism (e.g. GPP and ER) and K600 directly from the oxygen, temperature and light data where K600 is considered a free parameter, a method that works well for low gradient streams with high GPP (Hall Jr. and Madinger 2018). Another option is to supply a K600 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 estimating K600.

One method I investigated for estimating K600 was to run the model with the option to estimate both metabolism and K600 for all samples. From this data I used a linear regression to model the diel oxygen data vs the modeled oxygen data to obtain an R2 value for each sample. From these I selected the K600s from the subset of the regression models that had four characteristics: a positive K600 and GPP, a negative ER, and an R2>0.95. Model output is erroneous if the GPP is negative or if the ER is positive (Hall and Hotchkiss 2017), and a negative K600 can not be trusted (Demars et al. 2015) . Using this subset of models, I explored relationships between K600 and data I collected that should be related to K600 (discharge, velocity, depth and slope), and found that mean stream velocity had the strongest relationship. I then used K600 vs stream velocity to derive an equation that I used to estimate the K600 values for the models that were rejected due to erroneous values of GPP, ER, or K600:

N=14, R2=0.27, P=0.07

Where K600 is the general gas exchange rate in units of d-1 and velocity is the average stream velocity in m s-1. The rejected metabolism models were re-run with these derived K600 values and metabolism was estimated again. The metabolism estimates from all of these models were then kept if they had +GPP and –ER, resulting in 21 retained models of 30 possible models.

The other method I investigated was to derive K600 values from relationships found in literature data. Hall and Madinger (2018) suggest there is a strong relationship between stream slope and gas exchange 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 d-1 and slope is the stream slope in %. This equation was used to derive K600 values for all of the models which ultimately produced 26 retained models with positive GPP and negative ER out of 30 possible.

I chose to continue analysis with the model output produced by the literature-derived K600 values because inverse modeling that estimates K600 as a free parameter is intended for streams that generally have a lower gradient, and high gradient streams have unexpectedly high K600 values when measured directly (Hall and Madinger 2018). Although this technique used an equation based on a relationship with a lower sample size (n=8 vs n=14), it had a larger R2 and smaller p-value compared to the equation I derived from my own data, and it produced 5 more usable model output values (26 vs 21).

Statistical Analysis

Seasonal Variables

The seasonal variables including GPP, ER, trout biomass, stream discharge, canopy openness, PAR, DIN, SRP, and DOC were analyzed with a one way analysis of variance (ANOVA) using the command ‘aov’ in the statistical program R Version 3.5.2 (R Core Team 2013). A Tukey Honest Significant Difference (Tukey HSD) post hoc test was conducted on each of these to identify which mean was significantly different using the R package agricolae Version 1.3.1 (Mendiburu 2019) and the command ‘HSD.test’.

Model Selection Process

I used R Version 3.5.2 (R Core Team 2013) and the ‘lme4’ package (Bates et al. 2015) to develop a generalized linear model for each of the response variables (GPP, ER, and trout biomass) using the predictor variables (site, hydrologic, and nutrient data) I measured (Table 2.).

Table 2 Response and predictor variables shown as random or fixed effects



Prior to model selection, I used a pairwise scatterplot of all the response and predictor variables to assess collinearity and to reduce predictor variables. When variables shared a collinearity value of 0.6 or greater, I kept the variable that had the best relationship with the response and removed the other variable from further analysis (Zuur et al. 2009). I then chose a general linear model (GLM) with several predictors and no interactions and used the “drop 1” and “step” functions in R to return AIC values associated with each predictor variable. Variables that performed poorly were removed, other unused variables were added, and the process was repeated. After working through the list of variables, a small subset remained with which I constructed several different GLMs for each response variable and its remaining predictors. I used R’s “anova” function to compare these GLMs with each another to evaluate the most explanatory model from among the possible models (model with the lowest p-value). From the best of these models, I then constructed a Q-Q plot, a residual plot, and performed an Anderson-Darling test for normality on the residuals (p≤0.05, α=0.05). 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 2. 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 using alternate variance structures. 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 square root transformed and the process of model selection was started again. I proceeded with model selection in this way working iteratively with alternate transformations of the response variables until a model was produced that best met assumptions. I then returned 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.

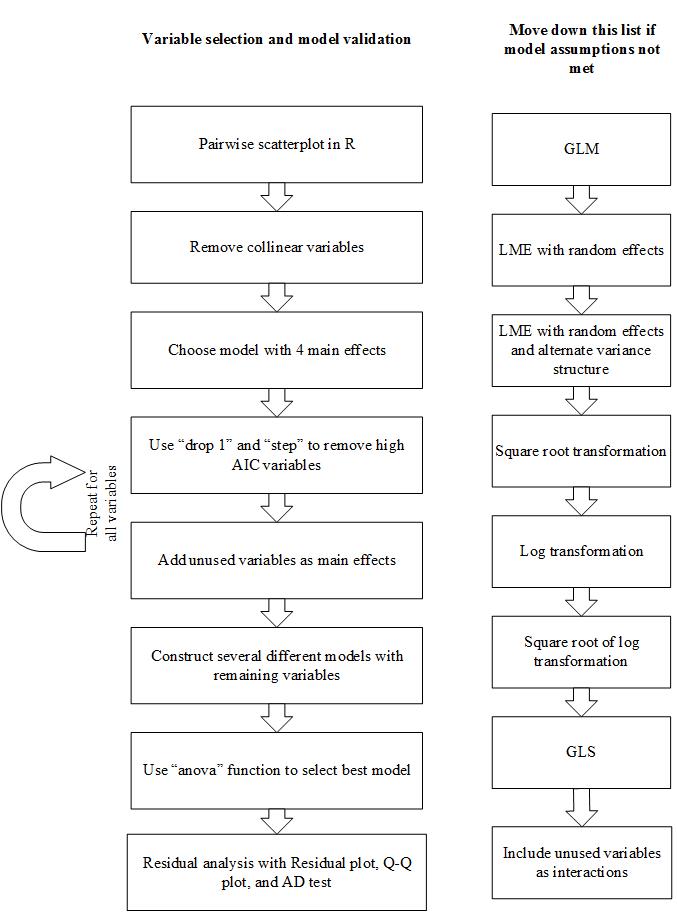


Figure 2 Flow diagram of model selection process

**Results**

Seasonal Variables

Stream discharges ranged from 0.3 to 65.5 L s-1, and was not significantly different among seasons (ANOVA, p=0.082) (Figure 3A). 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 and was not significantly different among seasons (ANOVA, p=0.065) (Figure 3B). Light as PAR ranged from 0.035 to 3.525 mols of photons m-2 d-1 where the Fall 2017 sampling period had a significantly lower mean than either summer (ANOVA, p=0.001) (Figure 3C).

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Figure 3 Boxplot of selected seasonal variables at consecutive sampling periods. Means with different letters are significantly different according to Tukey’s Honest Significant Difference Test. A. Stream discharge (L s-1) with no difference among sampling periods. B. Canopy openness (% open) with no difference among sampling periods. C. Light values as photosynthetically active radiation (PAR; mols of photons m-2 d-1). Fall 2017 mean is significantly less than either summer at p=0.001.

Due to relatively high detection limits and very low NH4+ and NO3-concentration, and despite spiking NH4+ analyses, some NH4+ and NO3- values were calculated as a negative concentration. Because of this, I linearly shifted values into a positive range, and then added NH4+ and NO3- together to produce a relative measure of total dissolved inorganic nitrogen (DIN). I then removed two unreasonably high DIN outliers (0.1860 for Hovey Cr. in fall 2017 and 0.2559 mg N L-1 for Swauk Cr. in summer 2017). Relative DIN values ranged from 0.0021 to 0.178 mg N L-1 with the last sampling period showing significantly higher relative concentrations than the previous 2 sampling periods (ANOVA, p=4.6e-7) (Figure 4A). SRP ranged from 0.0049 to 0.0610 mg P L-1 with the last sampling period showing significantly higher concentrations than the previous 2 sampling periods (ANOVA, p=1.7e-7) (Figure 4B). Dissolved organic carbon (DOC) ranged from 0.51 to 13.27 mg C L-1 with the last sampling period also showing significantly higher relative concentrations than the previous 2 sampling periods (ANOVA, p=1.2e-4) (Figure 4C).

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Figure 4 Boxplot of nutrient concentrations at consecutive sampling periods. Means with different letters are significantly different according to Tukey’s Honest Significant Difference Test. All nutrient concentrations were not different for summer 2017 and fall 2017 and then rose in summer 2018. A. Nitrogen as relative values of dissolved inorganic nitrogen (DIN; NH4+ + NO3-; mg N L-1) with summer 2018 significantly different at p=4.6e-7. B. Phosphate as soluble reactive phosphate (SRP; PO43-; mg P L-1) with summer 2018 significantly different at p=1.7e-7. C. Carbon as dissolved organic carbon (DOC; mg C L-1) with summer 2018 significantly different at p=4.6e-7.

Factors Related to GPP

The final linear mixed effects model for GPP included sampling period (p<0.0001) (Figure 5) and depth (R2adj=0.13, p<0.0001) (Figure 6) as main effects and had site as a random effect. Among sites, the highest GPP occurred in summer 2017 with a mean of 0.29 g O2 m-2 d-1 compared to means of 0.12 and 0.15 g O2 m-2 d-1 for fall 2017 and summer 2017 respectively. GPP was not related to daily PAR or nutrient concentrations (DIN, SRP, and DOC).

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Figure 5 Boxplot of GPP (g O2 m-2 d-1) for all study sites at consecutive sampling periods with associated linear mixed effects p<0.0001 from the GPP model. Means with different letters are significantly different with summer 2017 higher than the following two sampling period means according to Tukey’s Honest Significant Difference Test.

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Figure 6. Regression of log transformed GPP and stream depth (m) with associated linear mixed effects p<0.001 and R2adj of 0.13 from the GPP model.

Factors Related to ER

Ecosystem respiration is a negative number because it represents subtraction of oxygen from the environment, but it will be discussed here in terms of its absolute value (positive) to facilitate modeling and conceptualization. The final linear mixed effects model relating ER to environmental variables included depth (R2adj=0.36, p<0.0001) (Figure 8) and slope (R2adj=0.57, p<0.0001) (Figure 9) as main effects and site as a random effect. As with GPP, ER was not related to nutrient concentrations (DIN, SRP, DOC), and although ER was not significantly related to seasonality in the final model, ER and GPP were positively related to each other (R2adj=0.41, p=2.6e-4) (Figure 10)

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Figure 7 Boxplot of the absolute value of ER (g O2 m-2 d-1) for all sites at consecutive sampling periods. Means with different letters are significantly different according to Tukey’s Honest Significant Difference test.

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Figure 8. Regression of log transformed ER and stream depth (m) with an associated linear mixed effects R2adj of 0.36 and p< 0.0001 from the ER model.

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Figure 9. Regression of log transformed ER and Slope (%) with an associated linear mixed effects R2adj of 0.57 and p< 0.0001 from the ER model.

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Figure 10. Regression of absolute value of ER and GPP (g O2 m-2 d-1) with an R2adj of 0.41 and a p=2.6e-4.

Factors Related to Trout Biomass

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 for each site and sampling period combination to range from 0 in First Cr. 2018 (Taneum catchment) to 1.33 fish m-1 in Standup Cr. 2018 (Teanaway Catchment) (Figure 11A). The mean trout mass per individual fish ranged from 3.58 g in Frost Cr. 2017 (Taneum Catchment) to 31.23 g in Jack Cr. 2017 (Teanaway Catchment) (Figure 11B). I estimated trout biomass in g m-2 to range from 0 in First Cr. 2018 (Taneum Catchment) to 8.38 g m-2 in Hurly Cr. 2017 (Swauk Catchment) (Figure 11C), and it was not significantly different among seasons (ANOVA, p=0.30).

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Figure 11 Metrics of trout by stream and year arranged by increasing wetted width and grouped by watershed A. Trout population (fish m-1 of stream length; ± standard error) B. Mean mass of individual fish per stream (g; ± standard error). C. Mean trout biomass (g m-2 of stream; ± standard error from population).

The final model relating trout biomass in g m-2 was a general least squares model with exponential variation. There were main effects of catchment (p=0.0007) (Figure 12) and minimum daily temperature which had a significant interaction with canopy openness (p=0.0071) (Figure 13). Trout biomass had no relationship with stream nutrients (DOC, DIN, and SRP), light (PAR), or ecosystem metabolism (Figure 14).

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Figure 12. Boxplot of log transformed trout biomass by catchment with an associated general least squares p=0.0007 from the trout biomass model.

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Figure 13. Boxplot of log 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 an associated general least squares p=0.0071 from the trout biomass model for the interaction of stream temperature with canopy openness. Stream temperature is significant by itself whereas canopy openness is not. Overall there is more biomass at lower temperatures with more biomass under open canopies.

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Figure 14. Regression of GPP (g O2 m-2 d-1) and trout biomass (g m-2) showing no significant relationship. Trout biomass also showed no significance with ER or the PR ratio.

**Discussion**

The primary goal of this study was to explore the relationship between stream metabolism and trout biomass. Despite finding no relationship between trout and metabolism I did find positive relationships between GPP, ER, stream depth and slope while trout biomass was positively associated with colder water and more open canopies.

GPP

Light as PAR is generally the most critical factor for determining GPP (Bernot et al. 2010). Limitation of PAR in forested headwater systems is the strongest factor controlling GPP below a threshold of 3.5 mol m-2 d-1 and is severely limited below 2.2 (Warren et al. 2017). All but one of my sites were at or below 2.2 mol PAR m-2 d-1 with the highest being at the 3.5 mol threshold (Figure 3C). In this regard, all of the streams in my study were almost certainly light limited with respect to GPP. This suggests that very low GPP values should result from these low PAR values, and it appears that the GPP values I estimated may be unreasonably high. A recent study by Mejia et al. (2018) of stream metabolism conducted in streams of similar habitat and with similar methodology as this study found mean GPP to be 0.67 g O2 m-2 d-1 with a mean PAR of 18.6 mol m-2 d-1 with no values being below the light limitation threshold. The mean GPP value I estimated across sites was 0.20 g O2 m-2 d-1 (Figure 5.) with a mean PAR of 0.9 mol m-2 d-1 with virtually no values being above the light limitation threshold. This suggests that although the sites in their study were not light limited and had 20.6x more PAR than the sites I studied, the GPP they estimated was only 3.4x higher. Additionally, they found that GPP increases with increasing catchment area and although the catchment areas of the streams in my study were not determined, the mean stream discharge was 237x less (17.7 vs 420.2 L s-1) suggesting that the catchment area was also far less. The GPP values in my study again were not reflective of this. Comparing the headwater streams in my study to this larger system may not be entirely warranted however there is little else to compare these values to. There are investigations of headwaters in similar environments to mine but they use chlorophyll *a* instead of g of O2 (e.g. Warren et al. 2017), some studies are of forested headwaters and use similar methodology but the region is very dissimilar (e.g. deciduous forest in Tennessee; Roberts et al. 2007), or the region and methodology is similar but the local habitat is not comparable (e.g. pasture/urban; Bernot et al. 2010).

GPP is also frequently limited by nitrogen availability (Bernot et al. 2010), and a large study conducted in Great Britain found that primary production in headwaters is often limited by DIN concentrations (Jarvie et al. 2018). 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), which is often used as a proxy for GPP (Ryther 1956). The DIN in my study is represented on a relative basis because the numbers were artificially inflated to make the scale of comparison positive. The mean that was calculated after this was 0.02 mg N L-1 (Figure 4A.) which suggests that the actual mean was likely less. This means that the nitrogen concentrations in these streams would most likely limit GPP even if PAR was increased. Mejia et al. (2018) found a mean of 0.10 mg N L-1 in their streams which is above the GPP limitation threshold.

Some studies also find a positive relationship with SRP and GPP (Mulholland et al. 2001) although a closer look may reveal that SRP is limiting only at very low concentrations (Bernot et al. 2010). Mejia et al. (2018) did find low SRP in their streams (0.003 mg P L-1) while I found much higher concentrations (0.022 mg P L-1) matching what other studies have found in headwaters (Johnson et al. 2009). It therefore remains a possibility that GPP in the streams studied my Mejia et al. (2018) was limited by SRP whereas the GPP in my study streams was limited by light and DIN. This does not however give an indication of how close the GPP values from these two studies should be to each other. It is also not clear at what point SRP becomes limiting because the mechanism likely involves the ratio of DIN:SRP and yet manipulations of stream nutrient ratios may not even produce detectable differences in GPP (Kominoski et al. 2018). Overall, the balance of evidence suggests that GPP in my study streams was limited by low light and/or inorganic N availability.

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. Other factors that have been identified in studies include DIN and DOC (Bernot et al. 2010). The values I have measured or estimated for all of these potential determinants, except DOC which they did not report, were less for my study and yet the mean ER values I obtained (10.29 g O2 m-2 d-1; Figure 7.) were 8.22x greater in magnitude than what they reported (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 such as how GPP and ER relate to one another. Small forested headwaters are known to display net heterotrophic metabolism, meaning that respired oxygen is greater than produced oxygen (Allan and Castillo 2007). Consistent with other headwater streams, my metabolism estimates showed R far exceeding P (Figure 10.). 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 these relationships were expected, the environmental predictors found by my optimized statistical models do not yield any additional insight. In both of the GPP and ER models stream depth was a significant predictor; in contrast the ER model also found stream slope and the GPP model found sampling period to be significant predictors respectively. 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 K600 which is also put directly into the inverse model. This leaves sampling period (when samples were taken) which is not suggestive of any additional environmental driver of stream metabolism given that the first summer was significantly different than the second summer, thus season itself was not consistently different.

Because metabolism in these streams was likely limited by low PAR and low 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 either. Increasing slope is associated with an increase in stream step pool morphology (Chartrand and Whiting 2000) and one may expect this to have an effect on respiration. It might be expected that course particulate matter (CPOM) such as leaves, needles, and sticks may accumulate more in pools than in other stream features such as riffles and the majority of stream ER is associated with the breakdown of this material (Marcarelli et al. 2011). Unexpectedly however, the reverse of this appears to be the case. CPOM tends to accumulate less in pools because there is less physical structure which it tends to accumulate behind (Quinn et al. 2007). It might be expected then that, if anything, ER may decrease with increasing step pool morphology. Aside from this, I am aware of no other study that posits an increase in ER with slope.

Trout

The trout biomass estimates (Figure 11C.) were relatively high yet still fell within the range of biomass estimates found in streams across the historical range of westslope cutthroat trout (Benjamin and Baxter 2012). Colder minimum daily water temperature was a significant predictor of trout biomass during my study (Figure 13.), and the same relationship existed with minimum, mean, and maximum water temperatures. Cutthroat trout depend on cold mountain streams (Isaak et al. 2016), and although they can live in warmer water than I sampled, they are often outcompeted by rainbow trout in warmer environments (Bear et al. 2007).

Higher canopy openness was also a significant predictor of trout biomass (Figure 13.), but canopy openness interacted with water temperature such that at colder temperatures had higher biomass under open canopies and at higher temperatures there was uniformly lower trout biomass. This finding is also well supported in other studies (Kaylor and Warren 2017a, Martens et al. 2019) that found…. Additionally, Kaylor and Warren (2017b) found that the majority of vertebrate biomass in the streams they studied, including cutthroat trout, was accounted for by canopy openness alone. They suggest that this is likely due to increased light which leads to more effective feeding for predators that hunt visually as well as bottom-up trophic pathways. I however was not able to establish that relationship with PAR values. Studinski and Hartman (2015) suggest that open canopies lead to an increase in terrestrial insect subsidies which may be the leading cause for increased fish biomass.

No relationship was found between trout biomass and GPP (Figure 14.), ER, or the P/R ratio which may either be substantive or an artifact of metabolism inverse modeling inaccuracies or relatively low sample size. According to a meta-analysis, heterotrophic streams display a decoupling between ER and secondary productivity (i.e. the respiration is almost entirely due to organic matter breakdown) which may be why I did not find a relationship (Marcarelli et al. 2011). The same study did however find a positive relationship between the P/R ratio and secondary production in streams, suggesting that carbon from GPP may be more responsible for supporting animal growth than allochthonous carbon (Marcarelli et al. 2011). I did not detect this linkage and if there was a significant connection here, my data would depict a negative relationship (Figure 14.). 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 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, so increasing the sampling rate of the DO meter to 1 minute or less might increase the resolution of the data to improve results. These changes have the benefit of relatively simple methodology although the technique 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% (slopes in my study range 2-10%; Table 1.) 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 changing environmental conditions that alter the gas exchange rate (e.g. flow variation, surface wind movement) 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 may have been 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.