HEADWATER STREAM METABOLISM AND FISH BIOMASS IN KITTITAS

COUNTY

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A Thesis

Presented to

The Graduate Faculty

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Zach Lessig

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ABSTRACT

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Headwater streams are important for the biological integrity of river systems because

they represent most of the length of the hydrological network, and control the

downstream flow of energy and nutrients to larger river systems.  Headwater streams are

culturally and economically important because they directly or indirectly support

recreationally important anadromous and resident fisheries.  Managing fish in these

systems often requires time-consuming population counts but fish biomass might be

related to overall stream productivity, which can be measured relatively easily using

models to estimate stream metabolism.  The goal of my study was to relate whole stream

metabolism to fish biomass in 10 different headwater streams on the eastern slopes of the

Cascade Mountains in Kittitas County, Washington.  I estimated fish biomass on two

occasions using a multiple pass removal population estimate multiplied by the average

fish mass, and I estimated ecosystem metabolism on 3 occasions using the single station

method with a diel oxygen curve and inverse modeling.  I estimated the critically

important air-water gas exchange values based on stream slope, using an empirical

relationship from a previously published study.  Gross primary production across sites

and sampling periods ranged from 0.01 to 0.71 g $O_2$ $m^{-2}$ $d^{-1}$, varied by sampling period,

and increased with stream depth.  Ecosystem respiration ranged from 4.55 to 24.29 g $O_2$

$m^{-2}$ $d^{-1}$, and increased with stream depth and slope.  Fish were mostly cutthroat trout (*Oncorhynchus clarkii lewisi*), and biomass ranged from 0 to 8.38 g $m^{-2}$, increasing with colder water especially under more open canopies, and differing by catchment, but there was no relationship with ecosystem metabolism.  Overall stream metabolism predictors were limited to model inputs, owing in part to extremely limiting levels of photosynthetically active radiation and dissolved inorganic nitrogen, and the air water gas-exchange estimations were likely inaccurate.  No relationships were found between metabolism metrics and trout biomass with photosynthetically active radiation, dissolved inorganic nitrogen, soluble reactive phosphorus, dissolved organic carbon, or other physical attributes of these streams.  Unfortunately, with the methods I used, stream metabolism cannot be used as a proxy for trout biomass in headwater streams.

TABLE OF CONTENTS

LIST OF FIGURES

## LIST OF TABLES

# LIST OF EQUATIONS

## 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 streams. 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 causing the water to wind around large rocks and tree roots. Previous years' leaves or needles litter the ground and stream bed, and 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 low order channels (i.e. streams that have not coalesced with many other streams; Strahler 1957) although some favor defining them as streams draining a catchment size of less than 100 ha (Gomi et al. 2002). In some cases, definitions involving a more quantitative characterization of stream size is favored where headwater streams are viewed as less than 3 m wide with an average annual discharge of less than 57 L s$^{-1}$ (Richardson and Danehy 2007). For the current study, headwater streams will be considered 1$^{st}$ through 3$^{rd}$ order streams as consistent with Vannote et al. (1980).

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 to the hydrological network (Vannote et al. 1980). Headwaters also exert substantial control on water quality to downstream waterways, mainly through their high surface area to depth ratio, which is higher than downstream reaches of increasing stream order (Alexander et al. 2007; Meyer et al. 2007). This high ratio causes material to travel shorter 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) primary 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 stream 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 are 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 material derived from terrestrial production and aquatic invertebrates, which can then 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 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.  The insect 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 anadromous salmonids such as coho (*Oncorhynchus kisutch*) and chum salmon (*Oncorhynchus keta*) , along with

steelhead (anadromous *Oncorhynchus  mykiss*), use headwaters extensively for rearing

habitat (Meyer et al. 2007).  The salmonid adults harbored by Pacific Northwest

headwaters, however, are trout (non-anadromous *Oncorhynchus spp.*) and char (non-

anadromous *Salvelinus spp.*) (Richardson and Danehy 2007).  The life histories of some

populations of cutthroat trout (*Oncorhynchus clarkii*), for example, may be played out

solely in headwaters.  In Washington, trout in are an important fish for recreational

angling, with an estimated annual net worth of $146 million (TCW Economics 2008;

Loomis and Ng 2012).  People also place an existence demand on trout because of

ethical, aesthetic, and historical reasons although this is difficult to relate directly to

economic value (Gresswell and Liss 1995).  The trout in small streams are not generally

targets for anglers however, these smaller ecosystems present themselves with a more

manageable size of stream to study, and smaller streams exhibit connectivity with larger

systems via downstream migration (Colvin et al. 2019). Cutthroat trout have experienced

massive declines in numbers in recent decades (Shepard et al. 2005), and some suggest

that decreased stream connectivity plays a major part in this because populations of

cutthroat may depend on one another for persistence in the hydrosystem through

metapopulation dynamics (Rieman and Dunham 2000).  Conversely, others suggest that

in isolated headwaters, these populations may experience protection from competition or

genetic admixture with other salmonids thereby preserving the species (Hilderbrand and

Kershner 2000).

   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).  Gross primary production by

photoautotrophs uses the energy in light to fix the carbon in $CO_2$ into organic hydrocarbons, releasing $O_2$. ER is the reverse process and is the mineralization of organic hydrocarbon to $CO_2$ which consumes $O_2$. This consumption of $O_2$ represents the aerobic use of energy by organisms in the stream (Hall and Hotchkiss 2017). Stream metabolism is therefore a reasonably comprehensive measure that sums the activity of the aerobic organisms in a stream (Mejia et al. 2018).

The determination of stream metabolism has long been of interest because of its all-inclusive scope, and researchers have developed various methods for its determination. Many methods try to estimate whole system production through subsampling. For example, net primary production (NPP) has been estimated through the difference in ash free dry weight of periphyton however, this method involves only limited subsamples of the benthos and does not include GPP (Sládečková 1962). 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 light and dark bottle method produces a measure of both production and respiration by measuring changes in $O_2$ in sealed containers over time, but this does not include organisms attached to the benthos and is better suited to lentic environments (Gaarder and Gran 1927). The recirculating chamber method whereby stream substrate in a closed chamber is held in the stream while oxygen measurements are taken at time intervals is better suited for lotic environments (Mclntire et al. 1964). Conditions however in the chamber do not necessarily reflect conditions in the rest of the stream and it may not scale well as an estimate for the entire stream (Tank et al. 2010).

Whereas the methods above estimate metabolism through subsamples, there are also whole-system methods to estimate metabolism. For example, a method currently receiving wide use 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 validated through comparison to the measured oxygen curve. To use this method, light measurements, oxygen saturation, and temperature must be measured frequently (e.g., 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 $K_{600}$ in $d^{-1}$ where 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, which is adequate for streams with low slope and high light availability. Alternatively gas exchange can be measured directly by diffusing a gas such as propane ($C_3H_8$) or sulfur hexafluoride ($SF_6$) into the stream at high volumes and measuring the decline in concentration at several distances downstream from the injection point. This process may however require permits, be cost prohibitive, and the gas may have undesirable effects in the environment (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 from 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 $K_{600}$ versus 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 to measure gas exchange which produced an $R^2$ of 0.68 when related to slope. Therefore, it may be possible to estimate $K_{600}$ from the slope of a high gradient stream for use in the inverse model to estimate stream metabolism.

Stream metabolism is frequently controlled by the availability of nutrients and energy sources. Dissolved organic carbon (DOC) often contains labile components which serve as an energy source that is readily metabolized by stream heterotrophic organisms (Findlay et al. 1993) and increases in labile DOC may stimulate ER (Bernhardt and Likens 2002). Dissolved organic carbon is also associated with increases in GPP however it is implicated as a result of GPP through cellular leakage (Robbins et al. 2017). Inorganic nitrogen (N) as ammonium ($NH_4^+$) or nitrate ($NO_3^-$), and inorganic phosphorus (P) as phosphate ($PO_4^{3-}$), 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 is also associated with ER due at least in part to photorespiration of autotrophs (Parkhill and Gulliver 1999). Trout biomass is also linked to light availability probably as a result of multiple mechanisms including increased prey availability because of open canopies, or trophic cascades (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 may be related to higher ER.  Presence of trout could also affect GPP via a trophic cascade (Young et al. 2008), which occurs when a change in the presence or activity of organisms at a higher trophic level affects the organisms of lower trophic levels through indirect pathways.  In the case of trout for example, if more fish consume more invertebrates which in turn consume less algae, this could cause higher rates of GPP.  It also remains a possibility that trout productivity is simply linked to environmental factors such as light or nutrients that also influence whole stream metabolism.  In other words, productive streams are more productive at all trophic levels.  To that end, 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 could 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 could 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.

$H_{a1}$: GPP will have a positive relationship with stream nutrients.

$H_{a2}$: ER will have a positive relationship with stream nutrients.

$H_{a3}$: Trout biomass will have a positive relationship with stream nutrients.

$H_{a4}$ Trout biomass will have a positive relationship with GPP.

$H_{a5}$: Trout biomass will have a positive relationship with ER.

**Methods**

<u>Study Design</u>

I selected ten study sites on first through $3^{rd}$ order headwater streams in the Taneum (n=2), Swauk (n=5), and Teanaway (n=3) 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).
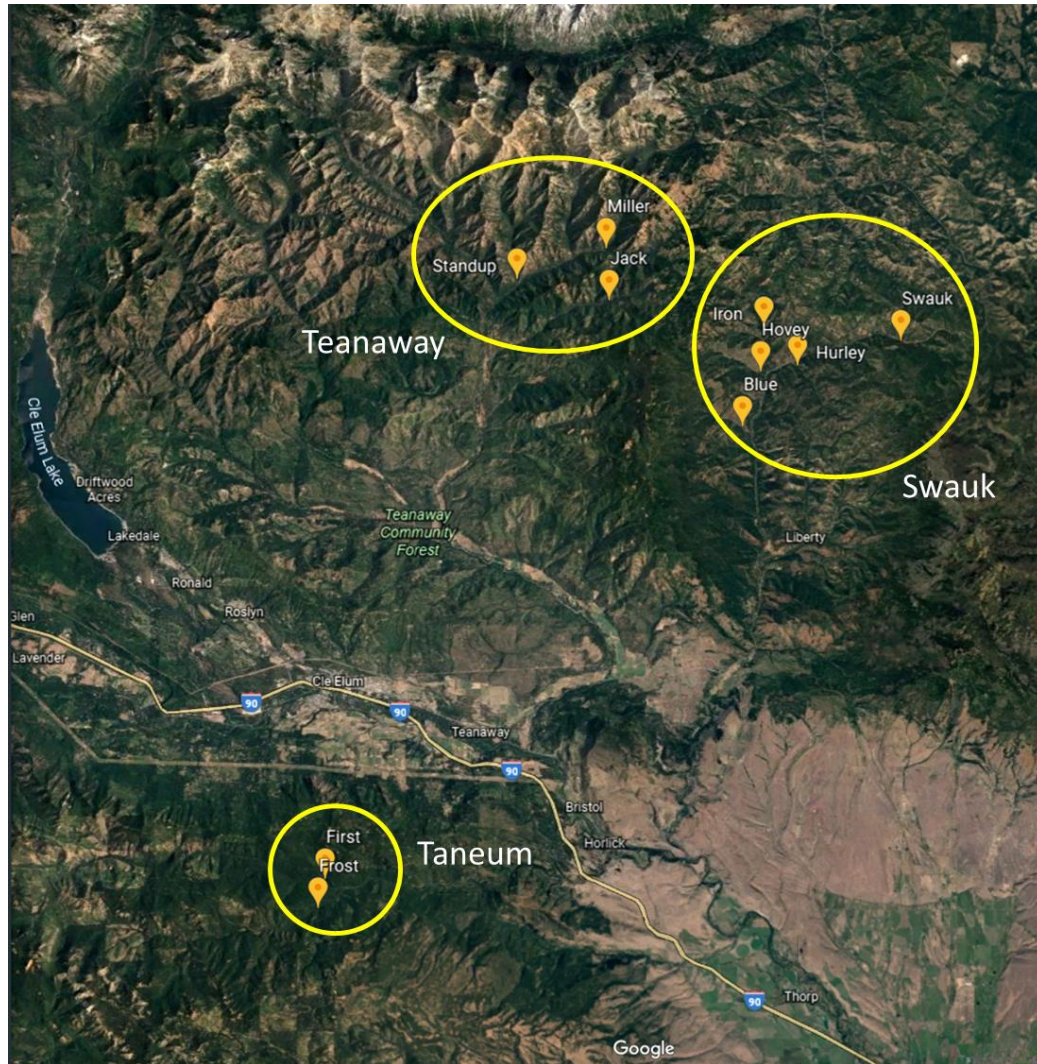
**Figure 1** Map showing Taneum, Swauk, and Teanaway, catchments with respective study sites.

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), and stream slope (Suunto PM-5 Clinometer).  I also conducted a Wolman Pebble Count (Wolman 1954)

with 50 pebbles per stream (Table 1).  Despite an intervening spring snowmelt, the

pebble count was conducted just one time

**Table 1** Site characteristics.

| Catchment | Stream | Aspect | Elevation (m) | Slope (%) | Bank Full (m) | Pebble Median (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 | 5.2 | 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 (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, recorded diel $O_2$ curves, recorded diel water

temperature curves, measured photosynthetically active radiation, and stream metabolism

estimates in the lab, and according to the methods described in detail below.

<u>Stream Nutrients</u>

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 ammonium ($NH_4^+$) 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 $NH_4^+$ was added to the sample to ensure concentrations were above the detection limit.  The added $NH_4^+$ was then subtracted.  I analyzed nitrate and nitrite ($NO_3^- + NO_2^-$) in the sample, hereafter referred to as $NO_3^-$, using the cadmium reduction method (Morris and Riley 1963) 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 ($PO_4^{3-}$), 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 $NH_4^+$, $NO_3$, and SRP were all run on an AQ1 Discrete Analyzer (Seal Analytical).  The acidified DOC sample was measured using the infrared method (APHA 2017) with a Shimadzu TOC-L (TOC-L Total Organic Carbon Analyzer, Shimadzu) with techniques outlined in the administrators manual.

<u>Fish Biomass Estimates</u>

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 size 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 (Lockwood and Schneider 2000). 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) and calculated the fish population

(Equation 1) and standard error (Equation 2) according to Lockwood and Schneider (2000).

$$N = \frac{C_1{}^2}{(C_1 - C_2)}$$

**Equation 1** Where, $C_1$ is the number of fish removed in the first pass, $C_2$ is the number of fish removed in the second pass, and N is the population estimate in numbers of fish.

$$SE = \sqrt{\frac{C_1{}^2 C_2{}^2 (C_1 + C_2)}{(C_1 - C_2)^4}}$$

**Equation 2** Where, $C_1$ is the number of fish removed in the first pass, $C_2$ is the number of fish removed in the second pass, and SE is the standard error of N from Equation 1.

This population estimate (N from Equation 1) 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 divided by the stream width (m), final unit of g $m^{-2}$. The error associated with fish biomass came from multiplying the standard error of the population estimate (SE from Equation 2) by the average mass of the fish. The average fish mass came from the combination of the fish caught in both passes ($C_1 + C_2$ from Equation 1).

## 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 ($\mu$mol 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 minimum 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 in 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 ($K_{600}$ - 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 where $K_{600}$ is considered a free parameter, a method that works well for low gradient streams with high GPP (Hall and Madinger 2018). 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 $K_{600}$ 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 $K_{600}$.

The first method I used was to estimate $K_{600}$ as a free parameter. For each modeled metabolism estimate (n=30), I regressed measured diel oxygen data vs modeled oxygen data to obtain an $R^2$ value for each estimate. After modelling all metabolism

16

estimates, I selected the $K_{600}$'s from the subset of the regression models that had four

characteristics: a positive $K_{600}$ and GPP, a negative ER, and an $R^2 > 0.95$. Model output is

erroneous if the GPP is negative or if the ER is positive (Hall and Hotchkiss 2017), and a

negative $K_{600}$ can not be trusted (Demars et al. 2015). Using this subset of models, I

explored relationships between $K_{600}$ and the data I collected that should be related to $K_{600}$

(i.e., discharge, velocity, depth and slope), and found that mean stream velocity had the

strongest relationship. I then used $K_{600}$ vs stream velocity to derive an equation to

estimate the $K_{600}$ values for the models rejected due to erroneous values of GPP, ER, or

$K_{600}$ (Equation 3)

$$\ln(K_{600}) = 1.6703 \ln(velocity) + 3.2482$$

**Equation 3** where $K_{600}$ is the general gas exchange rate in units of $d^{-1}$ and velocity is the average stream velocity in m s$^{-1}$. This was derived from a sample size (n) of 14, with $R^2 = 0.27$, and p=0.07.


The rejected metabolism models were re-run with these 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, resulting in 21 of 30 possible models retained.

The second method of estimating $K_{600}$ I investigated was to derive $K_{600}$ 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 (Equation 4) which was

used to derive $K_{600}$ values for all of the models which ultimately produced 26 retained

models with positive GPP and negative ER out of 30 possible.

$$\ln(K_{600}) = 0.9557 \ln(\text{slope}) + 3.0667$$

**Equation 4** where $K_{600}$ is the general gas exchange rate in units of $d^{-1}$ and slope is the stream slope in %. This was derived from Hall and Madinger (2018) from a sample size (n) of 8, with $R^2$=0.68, and p=0.01.

I chose to continue analysis with the model output produced by the literature-derived $K_{600}$ values because inverse modeling that estimates $K_{600}$ as a free parameter is intended for streams that generally have a lower gradient, and high gradient, low order streams such as those I studied have unexpectedly high $K_{600}$ 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 $R^2$ 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 repeated measures analysis of variance (rmANOVA) 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

| Responses | Random Effects |
|---|---|
| GPP (g $O_2$ m$^{-2}$ d$^{-1}$) | Catchment (total of 3) |
| ER (g $O_2$ 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) | Nitrogen (DIN mg L$^{-1}$) |
| Wetted Width (m) | Phosphorus (SRP mg L$^{-1}$) |
| Depth (m) | Carbon (DOC mg L$^{-1}$) |
| Stream Velocity (m s$^{-1}$) | |

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 "drop1" 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 ($p \leq 0.05$, $\alpha = 0.05$). 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 (for a flow diagram of this process, see Figure 2).
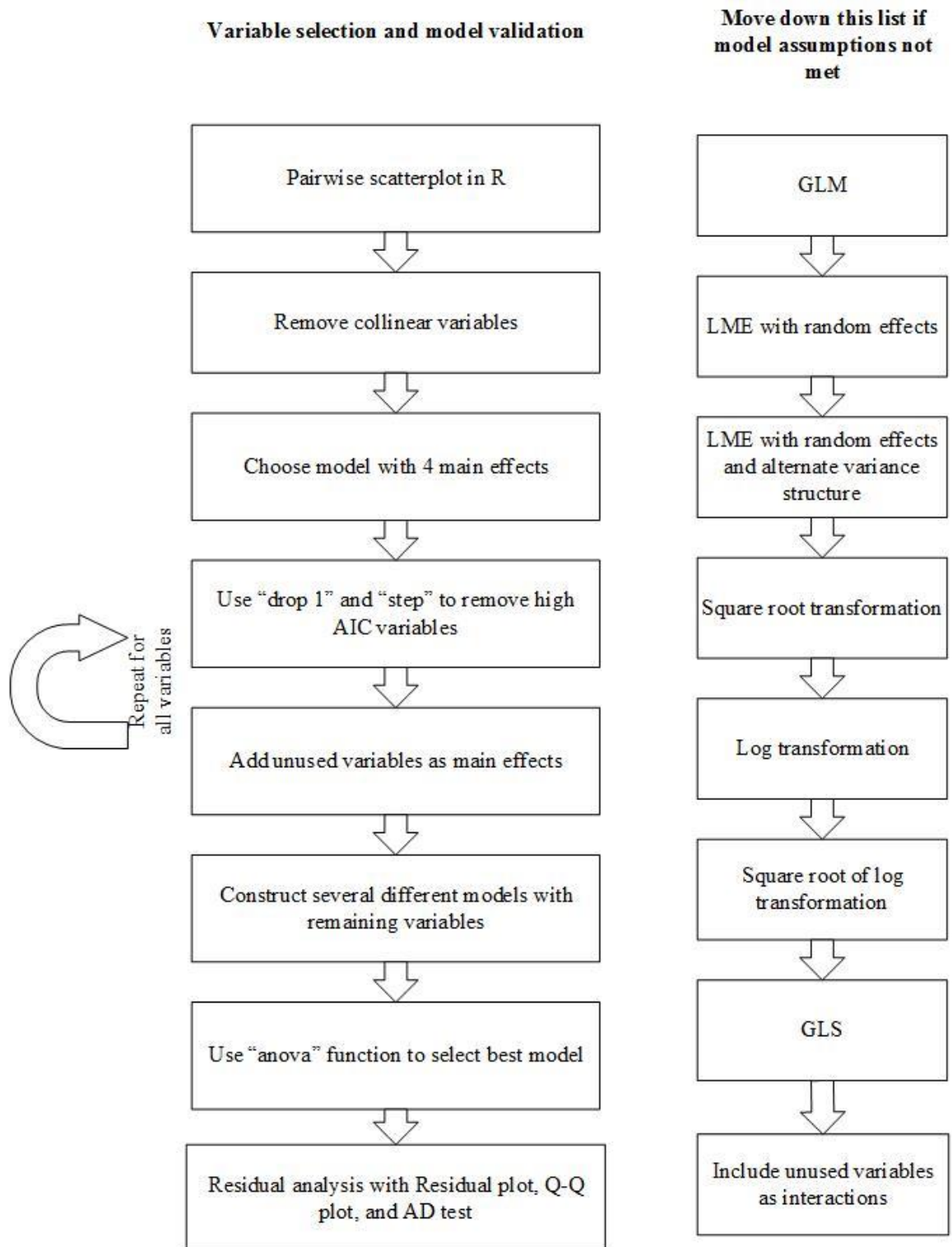
**Variable selection and model validation**

**Move down this list if model assumptions not met**

| Pairwise scatterplot in R | GLM |
|---|---|
| Remove collinear variables | LME with random effects |
| Choose model with 4 main effects | LME with random effects and alternate variance structure |
| Use "drop 1" and "step" to remove high AIC variables | Square root transformation |
| Add unused variables as main effects | Log transformation |
| Construct several different models with remaining variables | Square root of log transformation |
| Use "anova" function to select best model | GLS |
| Residual analysis with Residual plot, Q-Q plot, and AD test | Include unused variables as interactions |

Repeat for all variables

**Figure 2** Flow diagram of model selection process

## Results

<u>Seasonal Variables</u>

Stream discharges ranged from 0.3 to 65.5 L s$^{-1}$, and they were 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 they were 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 sampling period (ANOVA, p=0.001; Figure 3C).
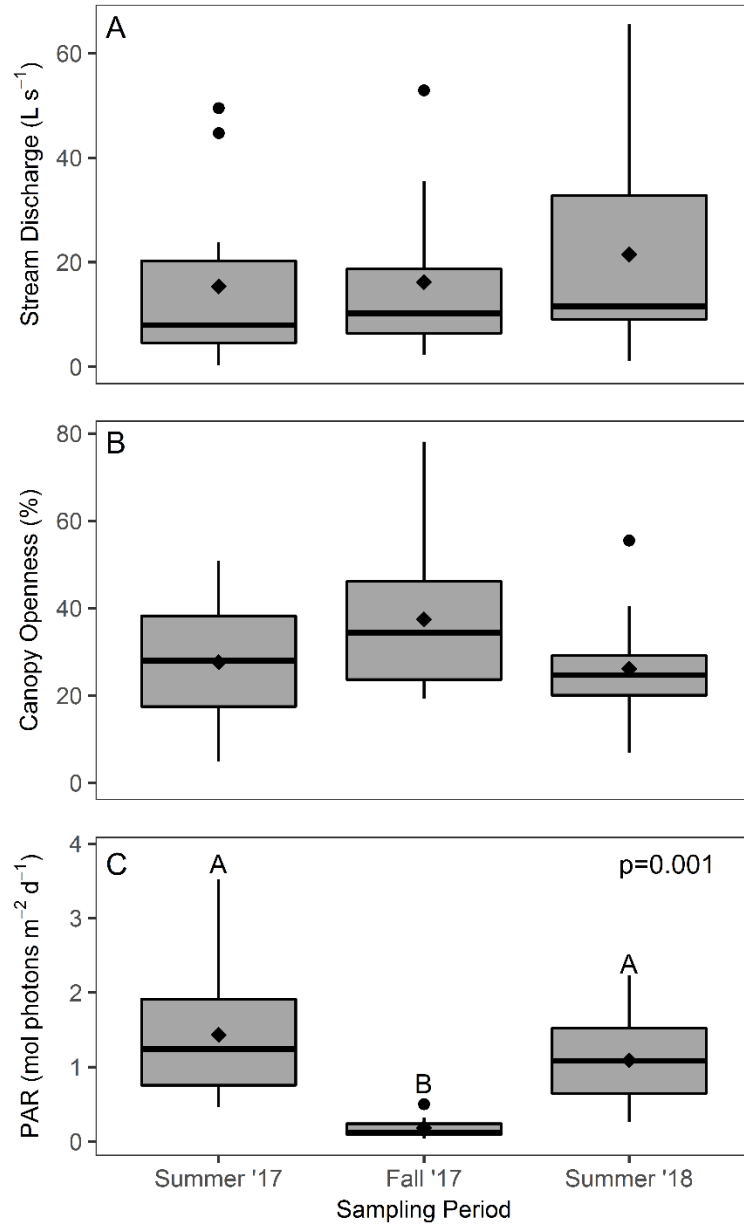
**Figure 3** Boxplot of selected seasonal variables at consecutive sampling periods. Means are represented by black diamonds. Means with different letters are significantly different according to Tukey's Honest Significant Difference Test. **A.** Stream discharge ($L\ s^{-1}$) with no difference among 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 $NH_4^+$ and $NO_3^-$ concentration, and despite spiking $NH_4^+$ analyses, some $NH_4^+$ and $NO_3^-$ values were calculated as a negative concentration. Because of this, I linearly shifted values into a positive range,

23

and then added $NH_4^+$ and $NO_3^-$ 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 0.0178 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).
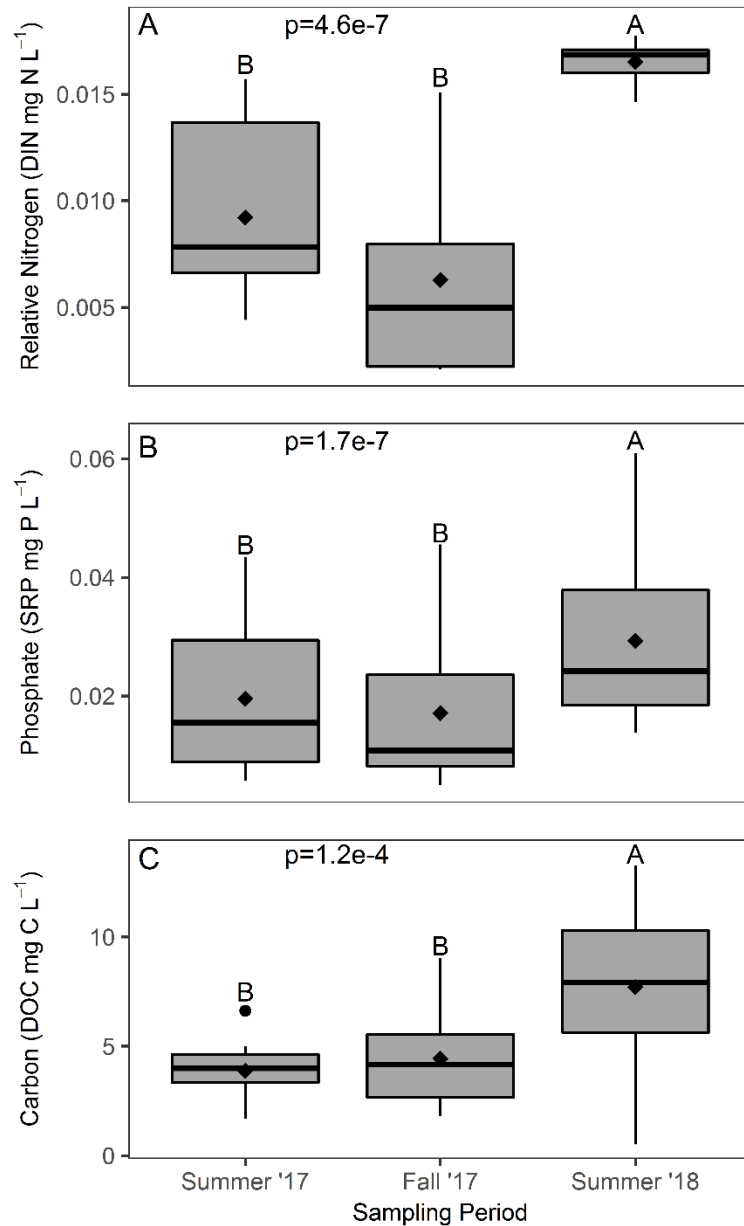
**Figure 4** Boxplot of nutrient concentrations at consecutive sampling periods. Means are represented by black diamonds. Means with different letters are significantly different according to Tukey's Honest Significant Difference Test. All nutrient concentrations were not different for summer 2017 and fall 2017 and then rose in summer 2018. **A.** Nitrogen as relative values of dissolved inorganic nitrogen (DIN; $NH_4^+ + NO_3^-$; mg N L$^{-1}$) with summer 2018 significantly different at p=4.6e-7. **B.** Phosphate as soluble reactive phosphate (SRP; $PO_4^{3-}$; 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.

The final linear mixed effects model for GPP included sampling period

(p<0.0001; Figure 5) and depth ($R^2_{adj}$=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 $O_2$ $m^{-2}$ $d^{-1}$ compared to means of 0.12 and 0.15 g $O_2$ $m^{-2}$ $d^{-1}$ for fall 2017

and summer 2017 respectively.  GPP was not related to daily PAR or nutrient
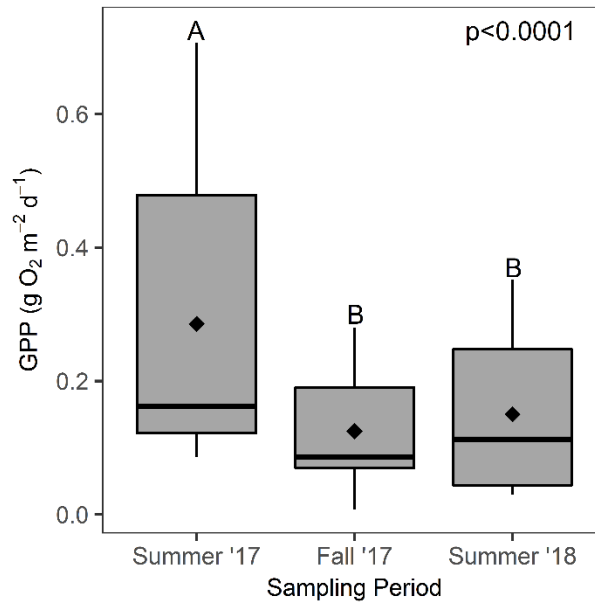
concentrations (DIN, SRP, and DOC).



**Figure 5** Boxplot of  GPP (g $O_2$ $m^{-2}$ $d^{-1}$) for all study sites at consecutive sampling periods with associated linear mixed effects p<0.0001 from the GPP model.  Means are represented by black diamonds. 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.
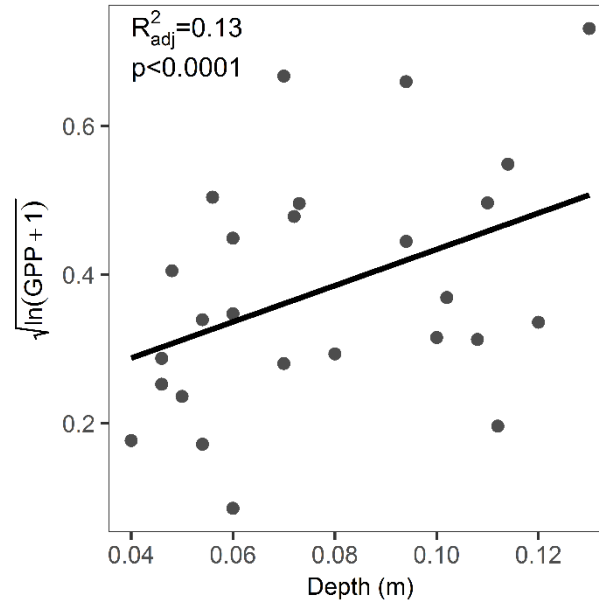
**Figure 6** Regression of log transformed GPP and stream depth (m) with associated linear mixed effects p<0.001 and $R^2_{adj}$ of 0.13 from the GPP model.

## 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 to facilitate modeling and conceptualization. Ecosystem respiration was not significantly related to seasonality in the final model (Figure 7).
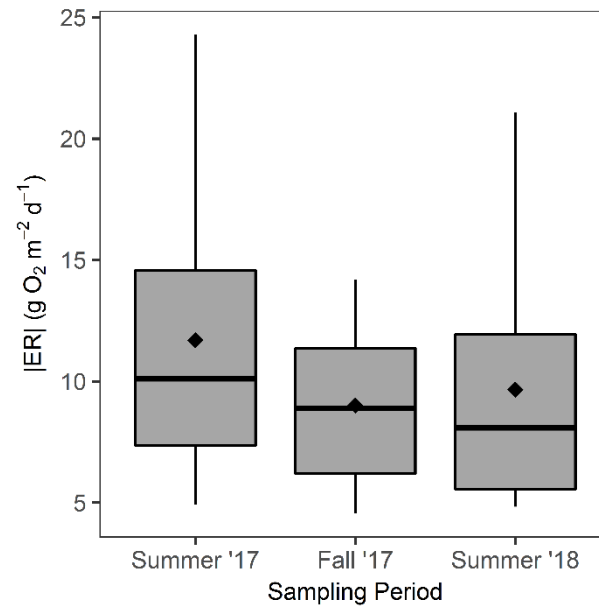
**Figure 7** Boxplot of the absolute value of ER (g O$_2$ m$^{-2}$ d$^{-1}$) for all sites at consecutive sampling periods. Means are represented by black diamonds and are not significantly different.

The final linear mixed effects model relating ER to environmental variables included depth (R$^2_{adj}$=0.36, p<0.0001; Figure 8) and slope (R$^2_{adj}$=0.57, p<0.0001; Figure 9) as main effects and site as a random effect.
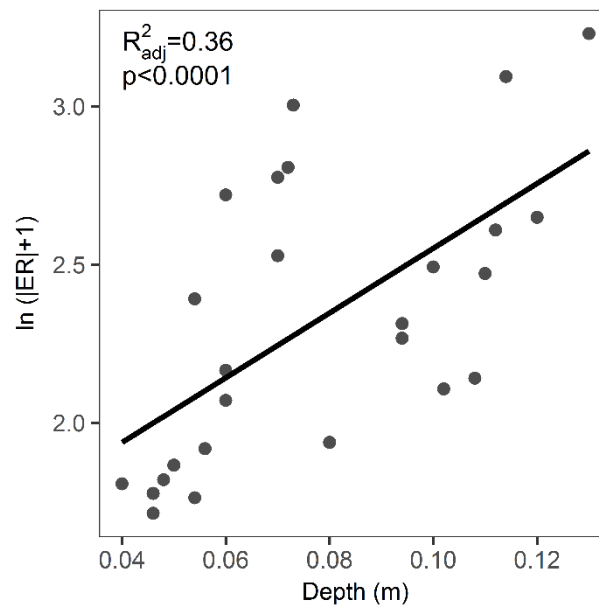


**Figure 8** Regression of log transformed ER and stream depth (m) with an associated linear mixed effects model. R$^2_{adj}$ of 0.36 and p< 0.0001 from the ER model.
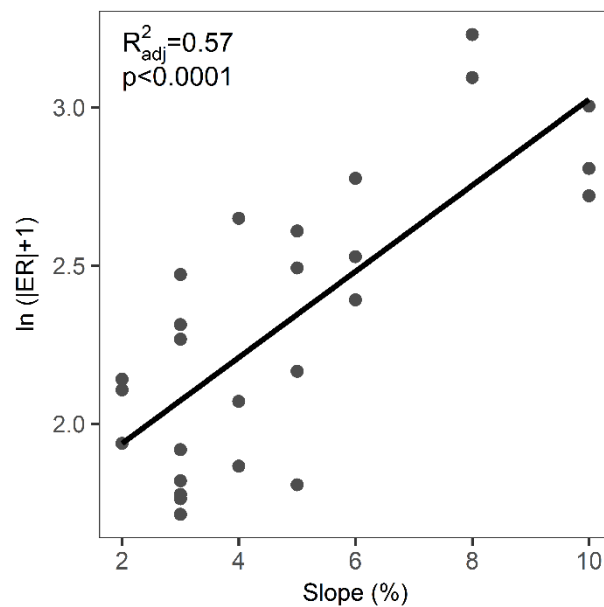
**Figure 9** Regression of log transformed ER and Slope (%) with an associated linear mixed effects model $R^2_{adj}$ of 0.57 and p< 0.0001 from the ER model.

As with GPP, ER was not related to nutrient concentrations (DIN, SRP, DOC),

and ER and GPP were positively related to each other ($R^2_{adj}$=0.41, p=2.6e-4; Figure 10).
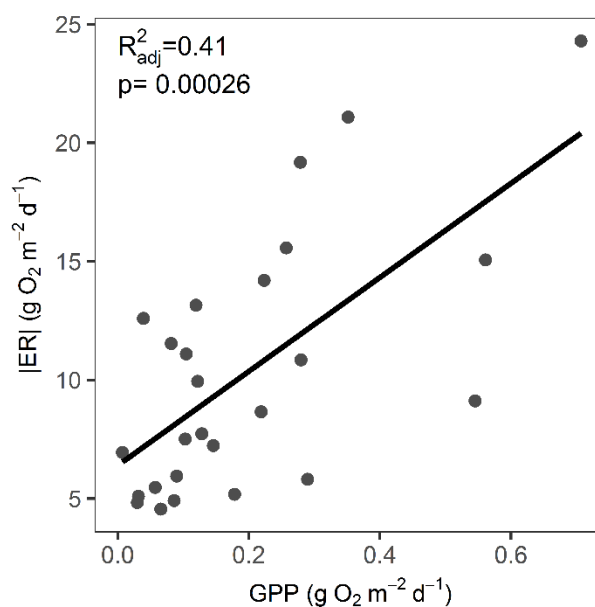


**Figure 10** Regression of absolute value of ER and GPP (g $O_2$ m$^{-2}$ d$^{-1}$) with an $R^2_{adj}$ of 0.41 and p=2.6e-4.

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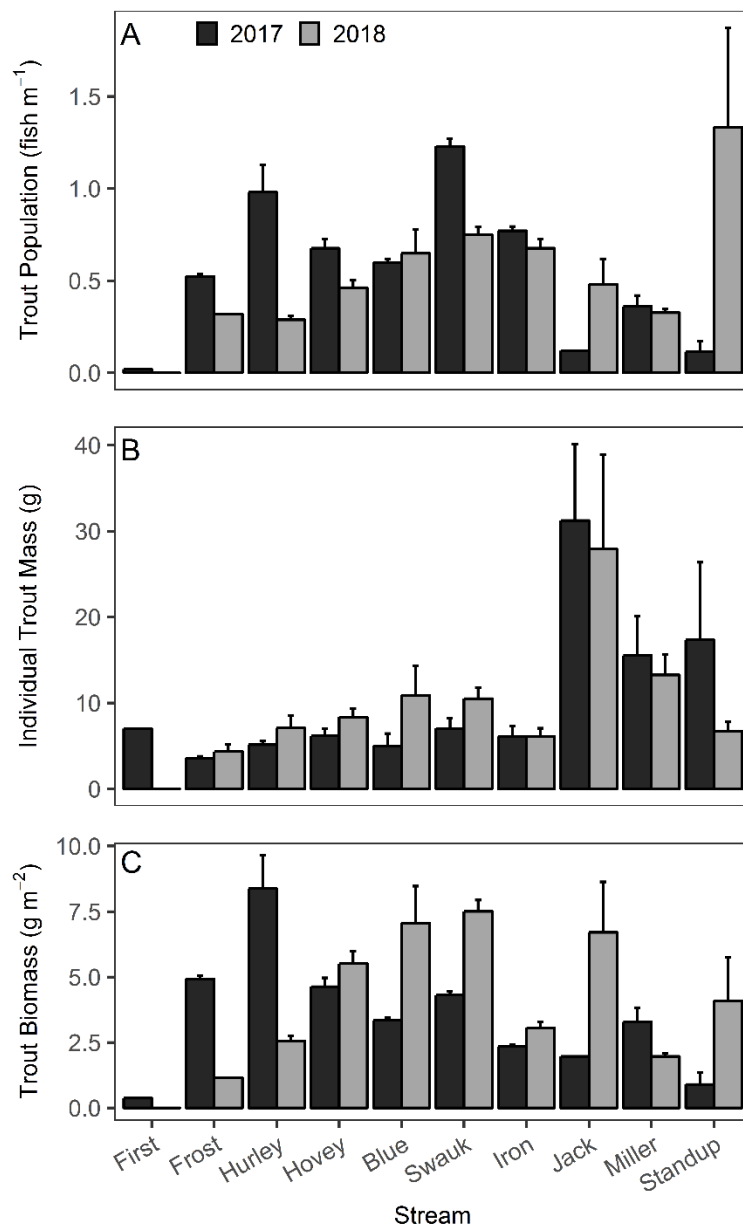
**Figure 11** Metrics of trout by stream and year arranged by increasing wetted width and grouped by catchment **A.** Trout population (fish $m^{-1}$ of stream length; ± standard error) **B.** Mean mass of individual fish per stream (g; ± standard error). **C.** Mean trout biomass (g $m^{-2}$ of stream;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

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) such that there was more trout biomass in colder water and under

more open canopies (Figure 13).  The interaction shown in Figure 13 shows boxplots of

transformed trout biomass values grouped into 3 equal intervals of water temperature and

2 canopy openness categories.  These canopy openness categories were chosen because

trout biomass values appeared to diverge below 26% open canopies which facilitated

graphical representation.  Trout biomass had no relationship with stream nutrients (DOC,

DIN, and SRP), light (PAR), or ecosystem metabolism (Figure 14).
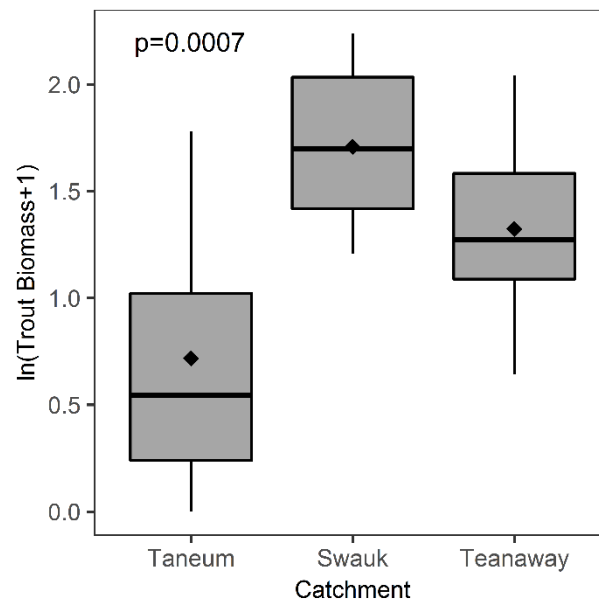


**Figure 12** Boxplot of log transformed trout biomass by catchment with an associated
general least squares model.  Means are represented by black diamonds with p=0.0007
from the trout biomass model.
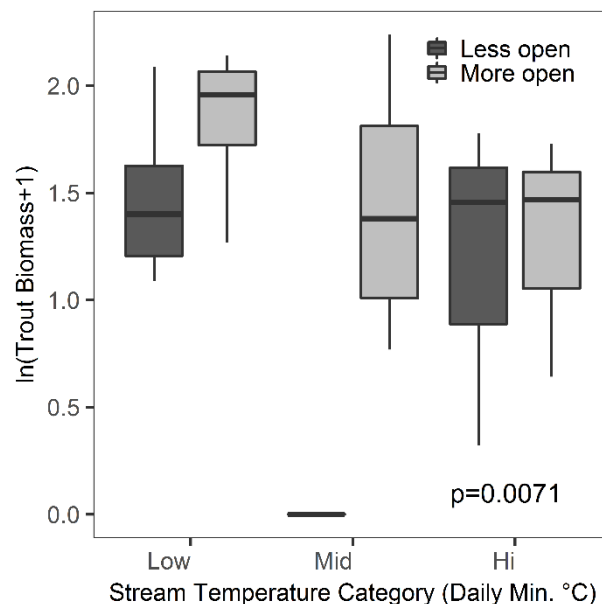
**Figure 13** Boxplot of log transformed trout biomass by water temperature interacting with canopy openness. Temperature categories are defined as Low (6.8 – 8.1°C), Mid (8.2 – 9.5°C), and Hi (9.6 – 10.9°C). Openness categories are defined as Less open (5 – 25% open), and More open (26 - 56% open). There is an associated general least squares p=0.0071 from the trout biomass model for the interaction of stream temperature with canopy openness. Overall there is more biomass at lower temperatures with more biomass under open canopies.
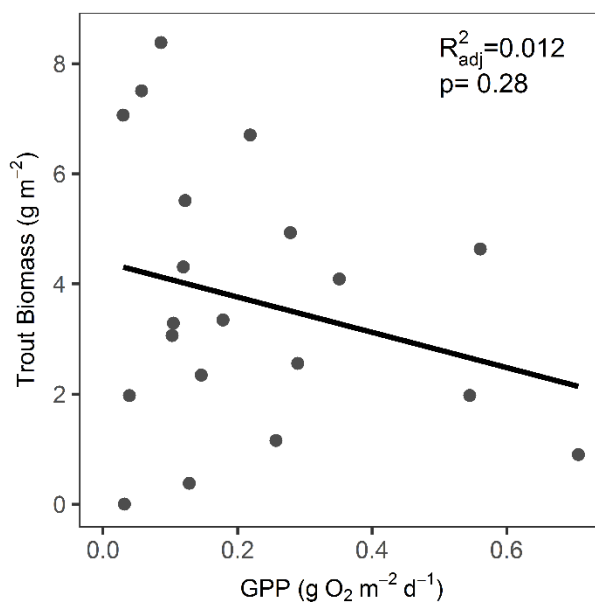


**Figure 14** Regression of GPP (g $O_2$ m$^{-2}$ d$^{-1}$) and trout biomass (g m$^{-2}$) showing no significant relationship. Trout biomass also showed no significance with ER or the PR ratio.

**Discussion**

The primary goal of this study was to explore the relationship between stream metabolism and trout biomass. Despite finding no relationship between trout biomass and metabolism, I did find that GPP varied by sampling period and increased with stream depth while ER also increased with stream depth and slope but did not vary by sample period. Trout biomass was positively associated with colder water, more open canopies and different among catchments. Although metabolism metrics are frequently related to PAR, DIN, SRP, and DOC, I did not find relationships with those potential predictor variables.

<u>GPP</u>

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 GPP is severely light-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. A recent study of stream metabolism by Mejia et al. (2018) in a similar environment to my study sites and using similar methodology as this study found mean GPP of 0.67 g $O_2$ $m^{-2}$ $d^{-1}$ associated with mean PAR of 18.6 mol $m^{-2}$ $d^{-1}$ and with no values below the light limitation threshold. In comparison, the mean GPP value I estimated across sites was 0.20 g $O_2$ $m^{-2}$ $d^{-1}$ (Figure 5) with a mean PAR of 0.9 mol $m^{-2}$ $d^{-1}$ and with all but one value at or below the severe light limitation threshold. Another study of small forested headwater streams found annual GPP rates of 1.38 g $O_2$ $m^{-2}$ $d^{-1}$ although during the

period of deciduous canopy closure, the rates were lower, around 0.5 g $O_2$ m$^{-2}$ d$^{-1}$ (value inferred from graph; Roberts et al. 2007). Larger, more open rivers may have GPP as high as 22.1 g $O_2$ m$^{-2}$ d$^{-1}$ but some rates may be as low as 0.3 g $O_2$ m$^{-2}$ d$^{-1}$ which may be due to large amounts of turbidity blocking light from reaching the stream bed (Hall et al. 2015; Hall et al. 2016). The PAR and GPP values from my data generally indicate light limitation.

Whereas I found a positive relationship between stream depth and GPP (Figure 6), others have found that GPP increases with catchment area (Lamberti and Steinman 1997; Finlay 2011; Mejia et al. 2018). Given that stream depth generally increases with catchment area (Pedersen 2019), my findings are congruent with theirs, however, my study streams likely had much smaller catchment areas overall considering mean stream discharge in my sites was 237x less (17.7 vs 420.2 L s$^{-1}$) than those in Mejia et al. (2018). The relationship with stream depth might suggest that bigger streams have more light penetration to relieve light limitation, but this pattern was not reflected in PAR or canopy openness in my data (Mejia et al. 2018).

GPP is also frequently limited by inorganic N availability (Bernot et al. 2010; Jarvie et al. 2018). An extensive study of temperate streams in the USA suggests that DIN below 0.04 mg N L$^{-1}$ suppresses chlorophyll (Dodds et al. 2002), which is often used as a proxy for GPP (Ryther 1956). The mean DIN I report was 0.02 mg N L$^{-1}$ (Figure 4A), but because my reported DIN was artificially inflated to account for poor detection limit the actual mean was less. This suggests that DIN concentrations in these streams are also limiting GPP despite the likelihood of primary limitation by PAR, similar to what others have found in the Klamath River (Genzoli and Hall 2016). Moreover,

metabolism estimates and nutrient limitation analysis in some of the same streams indicates a combination of light and N limitation of GPP (Arango et al. unpublished). Co-limitation by DIN and PAR may explain the lack of relationship between GPP and PAR in my dataset.

While N is often important, some studies have found a positive relationship between SRP and GPP (Mulholland et al. 2001) although SRP may limit GPP only at very low concentrations (Bernot et al. 2010). In eastern Washington, SRP may be less important for GPP given some sites in the Methow River have reasonably high GPP (2.53 g $O_2$ $m^{-2}$ $d^{-1}$) at low SRP concentrations (0.002 mg P $L^{-1}$; Mejia et al. 2018). It is also unclear at what point SRP becomes limiting because the mechanism likely involves the ratio of DIN:SRP (Kominoski et al. 2018). For example, DIN in my study streams was very low as was GPP despite reasonably high SRP concentrations (0.022 mg P $L^{-1}$) matching what other studies have found in headwaters (Johnson et al. 2009). Overall, the balance of evidence suggests that GPP in my study streams was limited by low light and/or inorganic N availability.

Comparing the headwater streams in my study to those from a larger river system (e.g., Mejia et al. 2018) may not be entirely warranted however there is little else to compare with my measured values. The majority of studies involving stream metabolism are conducted in much larger systems or very different habitats. 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). There are investigations of headwaters in similar environments to mine but they use

chlorophyll *a* instead of primary production (e.g. Warren et al. 2017). Considering the

prevalence of small coniferous streams in the montane west, there have been very few

metabolism studies of these economically and culturally important habitats.

### ER

The ER values I calculated had an overall mean of 10.29 g $O_2$ $m^{-2}$ $d^{-1}$ across sites

and sample periods (Figure 7). Consistent with other headwaters, these streams displayed

strong heterotrophic metabolism, with the magnitude of ER far exceeding GPP (Bott et

al. 2006; Roberts et al. 2007). The magnitude of these ER estimates however, does not

agree with previous studies. For example, the mean in my study was 8.2x greater in

magnitude than what Mejia et al. (2018) reported (1.25 g $O_2$ $m^{-2}$ $d^{-1}$) and higher than most

large rivers (Hall et al. 2016). Consistent with many other studies, GPP and ER were

highly correlated (Roberts et al. 2007; Bernot et al. 2010; Hall et al. 2016; Mejia et al.

2018; Figure 10) indicating that organic matter dynamics generally scale together, which

is common in small forested headwaters (Allan and Castillo 2007). Despite the strong

correlation between GPP and ER, I did not find seasonal variation in ER whereas I did

for GPP. One expectation might have been higher ER in fall due to a peak in organic

matter inputs to the stream (Roberts et al. 2007), but the relative lack of streamside

deciduous vegetation and dominance of coniferous vegetation might have dampened any

seasonal pattern.

The environmental predictors found by my optimized statistical models suggested

stream depth was an important determining factor, with deeper streams displaying greater

ER (Figure 6). Stream slope was also implicated, with steeper streams displaying greater

ER (Figure 9), which may be a unique finding. The relationship with stream depth is consistent with other findings (Mejia et al. 2018), and appears easy to rationalize as a driver of ER because deeper streams may generate more metabolism simply because of the increase in physical dimensions of the stream. Slope presents itself with some difficulty however. If stream slope were a driver of ER, the mechanism seems obscure. Steeper slopes could lead to more soil erosion (Renard et al. 1991) and thus potentially more nutrients or carbon in the stream but neither nutrients nor DOC were part of the GLZM outcomes. If greater 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, but I did not find relationships with those variables. 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 coarse 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 to accumulate it (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, so that particular relationship may be spurious. Other factors that have been identified as controls on ER include DIN and DOC (Bernot et al. 2010). However I found no relationships between those variables and my estimates of ER.

Overall, metabolism in these streams was likely limited by low PAR and low

DIN, and the significant predictors of depth, slope and sampling period do not appear to

yield insight into drivers of stream metabolism. The fact that stream depth is a variable

used by the inverse model to derive metabolism, and that slope is part of the equation

used to derive $K_{600}$ which is also used by the inverse model, might be the only reason

those factors are significant. Moreover, the ER values are unrealistically high suggesting

that the magnitudes of the values produced by my modeled estimates may be

questionable, though it remains a possibility that the relative order of values may be

preserved.

<div align="center">Trout Biomass</div>

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 daily 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 other trout

species 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 colder temperatures

had higher biomass under open canopies but higher temperatures had uniformly lower

trout biomass. This finding is also well supported in other studies which found that trout

biomass was higher in reaches where trees had been removed (Kaylor and Warren 2017a,

Martens et al. 2019).  Additionally, Kaylor and Warren (2017b) found that canopy

openness alone accounted for the majority of vertebrate biomass in the streams they

studied, including cutthroat trout, possibly due to increased light which leads to more

effective feeding for visual predators or which may cause bottom-up trophic pathways.

Although I found a similar relationship with canopy openness, I did not find a

relationship with PAR.  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.  This lack of relationship may be because heterotrophic streams display a

decoupling between whole stream respiration and animal respiration implying that

organic matter breakdown by microbes drives whole stream ER (Marcarelli et al. 2011).

However that same study found a positive relationship between the P/R ratio and insect

secondary production, suggesting that carbon from aquatic GPP may be more responsible

for supporting animal growth than carbon from terrestrial GPP (Marcarelli et al. 2011).  I

did not sample macroinvertebrates, and I could not detect this linkage in my trout data

(Figure 14), or perhaps this relationship is obscured at higher trophic levels.  These

conclusions are open to question however given the relatively low sample size and

possibly problematic metabolism estimations.

## Future Studies

Future studies that attempt to estimate whole stream headwater 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 than using a linear equation based on stream slope which I have done (examples in methods). Model results with a negative gas exchange, negative GPP, and positive ER will still need to be left out of the analysis though, so increasing the initial sample size to compensate for this eventual loss of data may offset this. Also, increasing the sampling rate of the DO meter to 1 minute or less might increase the resolution of the data to improve results and decrease the number of sites that would need to be dropped due to spurious estimates. 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, but there appears to be much work attempting to extend equations for predictions of gas exchange rates to mountainous headwater streams, so 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 $CO_2$.  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) can be dynamically modeled.  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 somewhat high in SRP. The cutthroat trout biomass is high but within expected ranges and these fish have a tendency to inhabit colder water, probably because of competition with rainbow trout in warmer water. They seem to be found under more open canopies, likely because of prey availability or higher foraging efficiency.

I was not able to establish a relationship between stream metabolism and trout biomass. This may be because this relationship does not exist in the streams I studied, the relationship is too weak to be detected by my methodology, or my metabolism estimation methodology needs refinement. Future research will likely produce methodology to more easily and reliably estimate metabolism in headwaters whereupon this question may be revisited.

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