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Quantifying spatial differences in metabolism in headwater streams

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Abstract: Stream functioning includes simultaneous interaction among solute transport, nutrient processing, and metabolism. Metabolism is measured with methods that have limited spatial representativeness and are highly uncertain. These problems restrict development of methods for up-scaling biological processes that mediate nutrient processing. We used the resazurin–resorufin (Raz-Rru) tracer system to estimate metabolism at different spatial scales (habitat, subreach, and reach) in 2 headwater streams of the H. J. Andrews Experimental Forest (Oregon, USA), and present a mathematical framework for its application. We investigated the relationship between metabolism and hydrodynamics, i.e., geomorphic units (e.g., pool–riffle, pool–cascade), bed materials (i.e., alluvium vs bedrock channels), and type of transient storage (i.e., pure hyporheic exchange, pure surface transient storage, and a combination of both). The metabolic hotspots detected by the Raz-Rru system in both watersheds were related to hydrodynamic conditions known to increase biological processing. Higher respiration rate coefficients were found in subreaches with extensive hyporheic flow and flow through large woody-debris complexes, and higher reaeration rate coefficients were found in subreaches with intensive respiration activity and higher flow velocities. Because such hydrodynamic conditions and their effects on stream processing are difficult to quantify in headwater streams without the use of tracer techniques, the Raz-Rru system proved to be a good integrator of solute transport and stream metabolism processes.

Key words: resazurin, smart tracer, aerobic respiration, metabolism, headwater streams, transient storage, hotspots

Stream functioning includes simultaneous interaction among solute transport, metabolism, and nutrient processing (Cummins 1974, Young and Huryn 1999, Peterson et al. 2001, Allan and Castillo 2007, Aristegi et al. 2009). Stream metabolism accounts for the fluxes of O₂ and C through primary production and community respiration. Therefore, estimates of metabolism are necessary to quantify stream C budgets. They also provide useful information about the ‘health’ of the ecosystem (Young et al. 2008), and routine measures of respiration and primary production are being incorporated in monitoring programs to assess the success of restoration projects and the effects of perturbations (Bunn et al. 2010).

Stream metabolism is commonly estimated with O₂ mass balances, which depend on metabolic processes, stream–atmosphere mass transfer (reaeration), and stream–groundwater interactions (McCutchan et al. 2002, Bott 2007, Reichert et al. 2009). Quantifying stream O₂ fluxes is difficult because of the elevated background O₂ concentrations in the stream and in the atmosphere, and because of the multiple

existing pathways for production and consumption (Reichert et al. 2009, Riley and Dodds 2013). Moreover, logistical challenges restrict the use of stable isotopes (e.g., ¹⁸O) for routine estimations of stream metabolism, either for technical or scientific applications (Tobias et al. 2007).

All other hydrodynamic and thermodynamic conditions being equal, respiration primarily drives stream–atmosphere O₂ mass transfer in headwater streams (respiration occurs both at day- and nighttime). Therefore, respiration should be the targeted process for estimating metabolism, instead of estimation of reaeration rate coefficients (status quo). Use of theoretical approaches to estimate reaeration rate coefficients generally has resulted in highly uncertain predictions of stream–atmosphere mass transfer (Kilpatrick et al. 1989, Genereux and Hemond 1992, Melching and Flores 1999, Jha et al. 2004, Aristegi et al. 2009). Measuring reaeration can be technically challenging, and therefore, numerous empirical methods based on geomorphic parameters have been proposed (Grace and Imberger 2006, Raymond et al. 2012, Demars and Manson 2013). In an

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extensive review, Aristegi et al. (2009) studied 21 streams with a wide range of hydrodynamic conditions in northern Spain and measured metabolism using several methods to estimate reaeration rates, i.e., nighttime regression, lag between noon and peak O_2 concentration, and 10 empirical equations based on stream geomorphic conditions. These analyses showed that uncertain estimates of reaeration rate coefficients could result in uncertainties in the estimation of gross primary production (GPP), community respiration (R), and net metabolism (NM = GPP – R) with magnitudes even higher than the estimated values (e.g., GPP = 16.6 ± 19.6 , R = 18.6 ± 18.0 ; NM = -2.0 ± 9.4 g $O_2/m^2/d$; see table 6 in Aristegi et al. 2009). Altogether, these observations suggest that C budgets and assessments of human impacts on stream metabolism and nutrient processing are hindered by uncertain estimations of reaeration rates.

To address some of these challenges, we have introduced the resazurin–resorufin (Raz-Rru) system as an alternative technique to estimate metabolism in headwater streams (Haggerty et al. 2008, 2009, Argerich et al. 2011, González-Pinzón et al. 2012, Stanaway et al. 2012, González-Pinzón and Haggerty 2013). Some of the advantages of this ‘smart’ tracer system are: 1) the irreversible transformation of Raz to Rru is proportional to cellular respiration, and it neither stimulates nor inhibits respiratory activity (González-Pinzón et al. 2012); 2) Raz is a tracer that can be used to investigate metabolism in situ and in vivo at different spatial scales; 3) processing Raz-Rru samples can be as easy as deploying a fluorometer in the field or in the laboratory to record highly accurate changes in tracer concentrations (Lemke et al. 2013c); and 4) the Raz-Rru system can be coupled easily with other biogeochemical processes of interest, e.g., nutrient processing, environmental effects of emerging contaminants, and biochemical O_2 demand from wastewater discharges. However, the Raz-Rru system is a proxy measurement for respiration. Therefore, it comes with its own set of uncertainties, unknowns, and nonideal behaviors. The disadvantages of the system are: 1) Raz and Rru sorb to sediment (Haggerty et al. 2008, Lemke et al. 2013a, b), 2) the proportion of O_2 consumption with respect to Raz-to-Rru transformation (molar processing ratio of dissolved O_2 [DO] to Raz = K_{DO}^{Raz}) is ecosystem-dependent (González-Pinzón et al. 2012), 3) Raz and Rru are thought to undergo transformations that are independent of the Raz-to-Rru transformation.

Here we describe how the Raz-Rru system can be used to quantify metabolism and to detect hotspots in stream ecosystems. We apply the system in 2 headwater streams of the H. J. Andrews Experimental Forest (Oregon, USA) to investigate the relationship between metabolism and hydrodynamics, i.e., geomorphic units (e.g., pool–riffle, pool–cascade), bed materials (alluvium vs bedrock channels), and type of transient storage (pure hyporheic exchange, pure surface transient storage, and a combination of both).

METHODS

Estimating rate coefficients and fluxes of metabolism from the transformation of Raz

In this section, we describe how the transformation of Raz can be used to estimate rates and fluxes of respiration, reaeration, and primary production. First, we estimate respiration rates and fluxes using Raz as a proxy tracer for DO. Then, we estimate reaeration and primary production using nighttime and daytime DO mass balances.

Estimating respiration rate coefficients González-Pinzón and Haggerty (2013) developed an efficient method that can be used to estimate aerobic respiration rate coefficients (λ_{resp}), using the transformation rate coefficient of Raz ($\lambda_{\theta,sz}^{Raz}$). We depart from the transient-storage-model equations and assume that respiration in the main channel and dispersion are negligible (Bencala and Walters 1983, Wörman et al. 2002, Runkel 2007, Argerich et al. 2011) to estimate λ_{resp} (per unit time [1/T]) as

$$\lambda_{\theta,sz}^{Raz} = \frac{\ln(m_{0,Raz}^{up}/m_{0,Raz}^{dn})}{\tau} \quad (\text{Eq. 1})$$

$$\lambda_{resp} = \begin{cases} [\lambda_{\theta,sz}^{Raz} K_{DO_{min}}^{Raz}, \lambda_{\theta,sz}^{Raz}], & \text{if } K_{DO}^{Raz} = [< 1, < 1] \\ [\lambda_{\theta,sz}^{Raz} K_{DO_{min}}^{Raz}, \lambda_{\theta,sz}^{Raz} K_{DO_{max}}^{Raz}], & \text{if } K_{DO}^{Raz} = [< 1, > 1] \\ [\lambda_{\theta,sz}^{Raz}, \lambda_{\theta,sz}^{Raz} K_{DO_{max}}^{Raz}], & \text{if } K_{DO}^{Raz} = [> 1, > 1] \end{cases} \quad (\text{Eq. 2})$$

where $\lambda_{\theta,sz}^{Raz}$ (1/T) is the volume-averaged transformation rate coefficient of Raz in the storage zone. K_{DO}^{Raz} is the molar processing ratio of DO to Raz [moles DO processed/moles Raz processed], which should be experimentally estimated (e.g., González-Pinzón et al. 2012); $m_{0,Raz}^{up}$ and $m_{0,Raz}^{dn}$ (MT/L³, where M is unit mass and L is unit length) are the 0th temporal moments of the upstream and downstream breakthrough curves (BTCs) of Raz; and τ (T) is the mean travel time in the stream reach. All quantities expressed in square brackets indicate minimum and maximum values (i.e., [min, max]). Eq. 2 is interesting because it shows that estimating in-stream λ_{resp} considering the coupling of hydrology and biological O_2 demand can be attained using a simple algebraic equation. Moreover, the uncertainty in the estimation of the transport parameters is less significant than the uncertainty in K_{DO}^{Raz} (see full demonstration of this concept in González-Pinzón and Haggerty 2013).

The absolute temporal moments of order n (m_n) can be estimated from experimental BTCs following the methods described by Das et al. (2002) as

$$m_n = \sum_{k=1}^r \left(\frac{t_k + t_{k+1}}{2} \right)^n \left(\frac{C_k + C_{k+1}}{2} \right) (t_{k+1} - t_k) \quad (\text{Eq. 3})$$

where C (M/L^3) is the measured concentration at time t (T), k is an index, and r is the total number of observations. The mean travel time τ between 2 sampling locations can be estimated with the conservative tracer BTCs as

$$\tau = \frac{m_{1,cons}^{dn} - m_{1,cons}^{up}}{m_{0,cons}^{dn} - m_{0,cons}^{up}}. \quad (\text{Eq. 4})$$

Estimating rate coefficients of reaeration and primary production Once λ_{resp} is estimated through the transformation of Raz, reaeration and primary production rate coefficients and fluxes can be estimated from nighttime and daytime O_2 mass balances. The O_2 mass balance for a parcel of water flowing between 2 stations can be described as (McCutchan et al. 2002, McCutchan and Lewis 2006)

$$\frac{dm}{dt} = C_g Q_g + (GPP - R)\bar{A} + \lambda_{reae}(Sv - m) \quad (\text{Eq. 5})$$

$$\frac{dv}{dt} = Q_g \quad (\text{Eq. 6})$$

where m (M) is the DO parcel mass, C_g (M/L^3) is the DO concentration in the incoming ground water, Q_g (L^3/T) is the net groundwater discharge, GPP and R ($M/L^2/T$) are the primary production and community respiration fluxes, $\bar{A}$ (L^2) is the planar area of the channel covered by the parcel of water ($\bar{A} \approx L \times \text{width}$), λ_{reae} ($/T$) is the reaeration rate coefficient, S (M/L^3) is the DO saturation concentration, v (L^3) is the volume of the parcel at time t (T). This mass balance assumes that: 1) the parcel is well mixed, 2) the temperature, barometric pressure, and groundwater inputs are spatially homogeneous, and 3) the channel is approximately rectangular.

Equation 5 can be rearranged to estimate rate coefficients of metabolism explicitly as

$$\frac{dm}{dt} = C_g Q_g + (\lambda_{pp} - \lambda_{resp})m + \lambda_{reae}(Sv - m) \quad (\text{Eq. 7})$$

where λ_{pp} and λ_{resp} ($/T$) are the primary production and respiration rate coefficients, respectively.

The analytical solution for the downstream reach concentration $O_2(t)$ (solving Eqs 6 and 7), with initial upstream concentration $O_{2,up}$ is

$$O_2(t) = \frac{\exp(-\Lambda t) V_{ini}}{\Lambda^2 (V_{ini} + Q_g t)} \left[\Lambda \left(\frac{C_g Q_g}{V_{ini}} (\exp[\Lambda t] - 1) + O_{2,up} \Lambda \right) + \frac{\lambda_{resp} S}{V_{ini}} \left(Q_g - \Lambda V_{ini} + \exp[\Lambda t] (\Lambda V_{ini} + Q_g (\Lambda t - 1)) \right) \right] \quad (\text{Eq. 8})$$

$$\Lambda = \lambda_{reae} + \lambda_{resp} - \lambda_{pp} \quad (\text{Eq. 9})$$

$$V_{ini} = \bar{A} z_0 \quad (\text{Eq. 10})$$

where z_0 is the initial average depth of the parcel. When groundwater inputs are negligible ($Q_g \rightarrow 0$), Eq. 8 simplifies to

$$O_2(t) = O_{2,up} \exp[-\Lambda t] + \frac{\lambda_{reae} S}{\Lambda} (1 - \exp[-\Lambda t]). \quad (\text{Eq. 11})$$

If DO is recorded with probes, $O_2(t)$, $O_{2,up}$ and S are known quantities. λ_{resp} can be estimated independently from the Raz-Rru system with Eq. 2. Therefore, Eq. 8 or Eq. 11 can be used to estimate λ_{reae} when nighttime DO signals are used (i.e., when $\lambda_{pp} = 0$).

If λ_{reae} estimated from the nighttime mass balance can be assumed to be representative of the daytime reaeration patterns, λ_{pp} would be the only unknown when daytime DO signals are subsequently used. Otherwise, the net effects of reaeration and primary production would have to be integrated into a single rate coefficient ($\lambda_{input} = \lambda_{reae} + \lambda_{pp}$). Also, if 2 separate Raz injections are done, i.e., nighttime and daytime conditions, independent values for λ_{resp} can be estimated (λ_{resp}^{day} , λ_{resp}^{night}). If only 1 injection is done, respiration at daytime and nighttime would have to be assumed to be equivalent (status quo).

Estimating fluxes of metabolism Once λ_{resp} and λ_{pp} have been estimated, the NM flux ($NM = GPP - R$) can be computed by equating the analytical solutions of Eqs 5 and 7:

$$GPP - R = \frac{\exp[-\Lambda t] z_0}{(\exp[-\lambda_{reae} t] - 1) \Lambda^2} \cdot \left[\exp[\Lambda t] \Lambda^2 \left(-\lambda_{reae} O_{2,up} + \frac{Q_g}{V_{ini}} (C_g - S) + \lambda_{reae} S \right) + \exp[\Lambda t] \lambda_{reae} \left(\Lambda \left(\Lambda O_{2,up} - \frac{Q_g C_g}{V_{ini}} \right) + \frac{\lambda_{reae} S}{V_{ini}} (Q_g - \Lambda V_{ini}) \right) + \exp[(\lambda_{reae} + \Lambda) t] \frac{\lambda_{reae} - \Lambda}{\lambda_{ini}} \left(\Lambda Q_g (C_g - S) + \lambda_{reae} S (Q_g (\Lambda t - 1) + \Lambda V_{ini}) \right) \right] \quad (\text{Eq. 12})$$

When groundwater inputs are negligible ($Q_g \rightarrow 0$), Eq. 12 simplifies to

$$GPP - R = \frac{\exp[-\Lambda t] z_0 \lambda_{reae}}{(\exp[\lambda_{reae} t] - 1) \Lambda} \left((\exp[(\lambda_{reae} + \Lambda) t]) S (\lambda_{reae} - \Lambda) - \exp[\Lambda t] \Lambda (O_{2,up} - S) + \exp[\lambda_{reae} t] (O_{2,up} \Lambda - \lambda_{reae} S) \right). \quad (\text{Eq. 13})$$

The respiration flux R can be estimated at nighttime conditions ($GPP = 0$) with Eqs 12 and 13, replacing Λ ($\Lambda = \lambda_{reae} + \lambda_{resp} - \lambda_{pp}$) by $\phi = \lambda_{reae} + \lambda_{resp}$.

Estimating DO longitudinal profiles Once λ_{resp} , λ_{reae} , and λ_{pp} are estimated, Eq. 8 or Eq. 11 can be used to generate longitudinal profiles of DO concentrations following the

same parcel of water, i.e., moving downstream with the mean travel time along the DO stations. Longitudinal DO profiles with reaeration subtracted can be estimated using actual DO concentrations for nighttime conditions. Mathematically, we can set $\lambda_{\text{reae}} = 0$, and estimate $O_2(t)$ for each subreach between consecutive DO stations. Starting at the most upstream station (station 1 [S1]), $O_{2,up}$ is a known (measured) O_2 concentration. From the 2nd station on, the initial concentration $O_{2,up}$ is the $O_2(t)$ previously estimated, e.g., to estimate the DO concentration at S3 ($O_{2(@3)}$), $O_{2,up} = O_{2(@2)}$, and so forth thereafter. We refer to these concentrations hereafter as 'DO after respiration'. The effects of reaeration can be estimated in the actual DO concentrations observed at nighttime conditions, by subtracting DO after respiration from the 'DO actual reading' values. These concentrations are referred to as 'DO reaeration'.

Following the same procedure described above for nighttime conditions, we also can outline DO profiles for daytime conditions. Again, $\lambda_{\text{reae}} = 0$ and $\lambda_{pp} = 0$ to solve for $O_2(t)$ for each subreach within consecutive DO stations (starting at S1). These concentrations are referred to as 'DO after respiration'. Afterward, we can estimate the combined effects of reaeration and primary production with the actual DO concentrations by subtracting DO after respiration from the DO actual reading values. These concentrations reflect the net input of O_2 to the stream and are referred to as 'DO GPP and reae'.

Research sites

We conducted field experiments in watersheds 1 (WS01) and 3 (WS03) in the H. J. Andrews Experimental Forest in the western Cascade Mountains (lat 44°20'N, long 122°20' W). WS01 and WS03 are 2nd-order tributaries of Lookout Creek and drain areas of 95.9 and 101.1 ha, respectively. The average flow rates during the experiments were 1.2 L/s and 3.9 L/s, respectively. Flow rates were estimated using weirs maintained by the US Forest Service.

Kasahara and Wondzell (2003), Gooseff et al. (2003), and Wondzell (2006) presented detailed descriptions of the geomorphology of these 2 watersheds, and we revisit only the most relevant characteristics here: 1) stream channel and valley-floor morphology are mainly shaped by infrequent debris flows, and therefore, gross geomorphology does not change significantly from year to year; 2) large-scale heterogeneities in the surface and subsurface (i.e., buried and exposed bedrock, boulders, and large woody debris) trap sediments and obstruct the stream channel by forming steps; 3) the valley floor of WS01 is relatively unconstrained and is $\sim 3.5\times$ wider than the active channel in the study reach; 4) the valley floor of WS03 is more constrained by bedrock and is $\sim 2.3\times$ wider than the active channel; 5) the slope of the 2 watersheds is ~ 0.13 m/m with steps accounting for $>50\%$ of the elevation change along the longitudinal profile of the stream; and 6) both streams contain short secondary channels. WS01 and WS03

are <2 km apart, and the reaches investigated were 81 and 160 m long, respectively.

Previous research has been done in these streams to investigate the influence of topography in hydrologic exchange patterns. Kasahara and Wondzell (2003) used data from stream, well, and piezometer heads and found that pool-step sequences were the primary drivers of hyporheic exchange and created lateral flowpaths with relatively short residence times that were captured by down-valley flows and that secondary channels were not important drivers of hyporheic exchange because of their proximity to the main channel. Our study reaches were not exactly the same as those analyzed by these authors (i.e., our reaches were within their reaches and had similar discharges), but we highlight their estimates that $\sim 76\%$ (WS01) and $\sim 100\%$ (WS03) of stream discharge would flow through the hyporheic zone in a 100-m reach. In WS01 and WS03 at high and low baseflow discharge (<12 L/s), 1) "spatial patterns, exchange fluxes, and residence-time distributions of hyporheic exchange flows are little affected by stream discharge, at least over the range of baseflow discharges and in the types of mountain stream channel examined" (Wondzell 2006, p. 282), 2) at both flow conditions, the size of the hyporheic zone and the residence times were greater where large woody debris were present and formed steps than in smaller size and even more frequent step-pool sequences, and 3) the in-channel component of transient storage in these headwater streams is small compared to hyporheic exchange processes (Wondzell 2006).

We defined sampling points to characterize distinct types of geomorphic units, bed materials, and type of transient storage (Table 1). We also measured flow depths and longitudinal distances by the methods presented by Bott (2007).

Stream tracer injections

We continuously coinjected Raz and NaCl as a conservative tracer for 39 (WS01) and 48 h (WS03). We attempted to increase Raz concentration in the stream from 0 to 300 $\mu\text{g/L}$ and to increase background specific conductance by ~ 250 $\mu\text{S/cm}$. We collected background samples to characterize ambient conditions at all sampling sites before the injection started. We sampled the breakthrough curves of Raz-Rru and NaCl at 6 surface water stations (SA-SF) (Table 1) to quantify stream metabolism at the subreach and reach scales (Eq. 2).

We used specific conductance (EC) as a proxy for Cl concentration and measured it at 1-min intervals with either YSI-6000 MS V2 multiparametric sondes (Yellow Springs Instruments, Yellow Springs, Ohio) or Campbell Scientific EC probes (Logan, Utah) in the surface sampling sites. We calibrated all sondes prior to the injection and cross-checked them for 3 h before the injection began and at the end of the experiment. We collected samples for Raz-Rru analyses ($n > 60$ for each breakthrough curve) either manually or with ISCO samplers (Teledyne ISCO, Lin-

Table 1. Geomorphic characteristics of the subreaches studied in WS01 and WS03, H. J. Andrews Experimental Forest (Oregon, USA). Inj = injection site, SP = step–pool, PR = pool–riffle, BP = bedrock pools and riffles, R = runs, SsF = subsurface flow, L = large woody debris.

Subreach	Geomorphic units	Length (m)	Mean water depth (cm)
WS01			
Inj–SA	SP, R, L	22	9.0
SA–SB ^a	PR	15	18.0
SB–SC	SP, PR, L	6	5.0
SC–SD ^a	R	21	3.5
SD–SE ^a	SP, BP	8	3.0
SE–SF ^a	BP	9	10.0
WS03			
Inj–SA ^b	PR, R	38	8.1
SA–SB	R	18	9.9
SB–SC ^b	SP, PR, L	36	4.3
SC–SD	SP, PR, L	21	6.6
SD–SE ^b	R	16	8.7
SE–SF ^b	SsF, PR	31	3.3

^a Station where propane gas evasion was measured in WS01

^b Station where propane gas evasion was measured in WS03

coln, Nebraska). Sampling times were informed by real-time measurements of EC, and we took extended samples of Raz–Rru past the recovery time of EC background conditions to capture the tail. We filtered (0.7 μ m) and refrigerated samples in the field. We measured the fluorescence signals of the Raz–Rru samples (proxy for concentration) in the laboratory with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, California) after standardizing the samples to pH 8.5 (Haggerty et al. 2008). We did the tracer injections in WS01 from 9 to 15 August 2011 and in WS03 from 16 to 21 August 2011. Stream surface water temperature ranged from 12.5 to 14°C in WS01 and 11.8 to 16.2°C in WS03. Minimum streamwater temperatures generally occurred between 0800 and 1000 h, and maximum temperatures occurred between 1800 and 2000 h.

Estimates of in-stream metabolism

We estimated stream metabolism using the Raz–Rru system and the traditional 2-station diel technique (e.g., Bott 2007). We deployed YSI-6000 MS V2 multiparametric sondes in each stream to measure O₂ concentration (mg/L), O₂ saturation (%), temperature (°C), and specific conductance (μ S/cm) every minute. The sondes were immediately upstream of the injection point and in the BTC locations. We deployed the sondes in the stream 22 h before the injection was started and removed them 76 h after the injection was stopped. We used this information to constrain

the O₂ mass balance from which in-stream rate coefficients of metabolism were estimated with both the Raz–Rru system and the 2-station diel technique. The main difference between the 2 techniques is the approach followed to close the mass balance. In the Raz–Rru system, λ_{resp} is estimated first through the transformation of Raz to Rru (Raz transformation is used as a proxy of DO consumption), whereas in the 2-station diel technique λ_{reae} is estimated first through, e.g., mass balance of a tracer gas (e.g., SF₆ or propane is used as a proxy of DO stream–atmosphere transfer).

We estimated λ_{reae} for the 2-station diel technique with the propane gas evasion and nighttime regression methods (Young and Huryn 1996, Fellows et al. 2001, Grace and Imberger 2006, Bott 2007). We measured propane gas concentrations at 4 randomly chosen stations to compare estimates of metabolism (Table 1). The use of other techniques to estimate λ_{reae} was restricted because the hydrodynamic conditions of our streams were significantly different than those where most empirical approaches have been developed (Melching and Flores 1999, Aristegi et al. 2009, Raymond et al. 2012). Next, we estimated stream R and GPP with the methods described by Bott (2007) for each of the 2 techniques used to estimate λ_{reae} .

We used a 1-way analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test to compare λ_{reae} and NM estimated by each of the 3 techniques (Raz–Rru, propane evasion, and nighttime regression) at the 3 (WS01) and 4 (WS03) sites at which these methods were used (see Discussion). We compared the spatial variability of the λ_{resp} estimated with the Raz–Rru system with the coefficient of variation (CV).

Calibration of the molar processing ratio K_{DO}^{Raz} at the field sites

We conducted chamber experiments to calibrate the molar processing ratio K_{DO}^{Raz} (M O₂/M Raz) at each watershed, as described in González-Pinzón et al. (2012). We took sediment samples (smaller than pebbles) by hand every ~10 m along the reaches and incubated them for >5 d in recirculating chambers ($n = 2$ for each watershed) closed to the atmosphere. Raz was injected instantaneously to generate a concentration of ~300 μ g/L upon complete mixing. We measured O₂ concentrations inside the chambers with YSI ProODO (Yellow Springs Instruments) and Hach HQ40D probes (Hach, Loveland, Colorado) every time we took a sample for Raz–Rru analysis. We collected, filtered, and refrigerated Raz–Rru samples at 4°C and then read them within 72 h of sampling.

RESULTS

Stream solute transport

We used information from the tracer experiments to estimate: 1) the proportion of conservative mass recovered at the end of each subreach in relation to the mass in-

jected (i.e., the conservative mass recovery [CMR]), 2) the mass of Raz recovered after correcting by CMR (RMRC), and 3) the mean travel times and mean velocities in the subreaches. We did not find considerable changes in CMR along the reaches, i.e., $\text{CMR} \approx 1.0$, except at the end of subreach SF in WS03 (station SF; Table 2), where we did not completely recover the tail of the BTC (i.e., $\text{CMR} = 0.86$), even though we collected data for 70 h after the injection stopped. Mean flow velocities through the subreaches clearly reflected the different hydrodynamic conditions present in the streams. For instance, mean velocities as low as ~ 2 m/h were found in subreaches dominated by pools, large woody debris, and hyporheic flow, whereas mean velocities of ~ 30 m/h were found in subreaches where riffles prevailed (Tables 1, 2).

Stream metabolism from the transformation of Raz

Respiration rate coefficients (λ_{resp}) Volume-averaged transformation rate coefficients ($\lambda_{0,\text{sz}}^{\text{Raz}}$) estimated using the mean travel times (τ) for the conservative tracer and the 0th Raz temporal moments (Eq. 1, Table 2) were used in conjunction with the values of $K_{\text{DO}}^{\text{Raz}}$ reported by González-Pinzón et al. (2012) (Table 2, note that $K_{\text{DO}}^{\text{Raz}} = 1/K_{\text{Raz}}^{\text{DO}}$) to estimate λ_{resp} and the associated confidence bounds (Eq. 2). λ_{resp} ranged between 7.1×10^{-2} and 3.0×10^{-1} /h in WS01 (Fig. 1A), and between 1.4×10^{-2} and 2.7×10^{-1} /h in WS03 (Fig. 1C). In WS01, we estimated slightly smaller λ_{resp} at the end of the reach, where the bedrock pools and riffles sequences were present (Fig. 2C). In WS03, the highest λ_{resp} occurred in subreaches SC–SD and SE–SF, which had conditions favoring microbial metabolism (Fig. 2A, B). SC–SD contained many step–pool and pool–riffle bedforms with stream water flowing through large woody debris complexes, which increase retention times, provide rich substrata for microbial communities, and increase hyporheic exchange (Sedell et al. 1988, Nakamura and Swanson 1993, Bilby and Bisson 1998, Kasahara and Wondzell 2003). SE–SF had almost completely subsurface (hyporheic) flow, and stream water reemerged only ~ 10 m upstream of the end of the reach.

Reaeration (λ_{reae}) and primary production (λ_{pp}) rate coefficients The conservative tracer revealed negligible groundwater inputs, so we solved Eq. 11 (no groundwater inputs) to estimate λ_{reae} from a nighttime O_2 mass balance ($\lambda_{\text{pp}} = 0$ and λ_{resp} as described above; Fig. 1B, D), and then estimated λ_{pp} from a daytime O_2 mass balance (Fig. 3A, C). We assumed negligible changes from day to night for λ_{resp} and λ_{reae} to estimate λ_{pp} .

λ_{reae} ranged between 1.9×10^0 and 2.3×10^1 /h in WS01 (Fig. 1B) and between 5.5×10^{-3} and 5.9×10^0 /h in WS03 (Fig. 1D). In WS01, the highest λ_{reae} was obtained for subreach SE–SF, characterized by a sequence of pools and riffles formed over a bedrock outcrop (Fig. 2C). In WS03, the

Table 2. Conservative and reactive solute transport characteristics. In WS01, mean $Q \approx 1.2$ L/s, $K_{\text{DO}}^{\text{Raz}} = 0.58 \pm 0.03$. In WS03, mean $Q \approx 3.9$ L/s, $K_{\text{DO}}^{\text{Raz}} = 0.70 \pm 0.04$. CMR = conservative mass recovered at the end of the subreach (estimated from the 0th temporal moments), RMRC = resazurin (Raz) mass recovered at the end of the subreach, corrected from conservative mass recovered, Q = discharge, $K_{\text{DO}}^{\text{Raz}}$ is the molar processing ratio of dissolved O_2 (DO) to Raz (M DO processed/M Raz processed), τ = travel time, u = reach mean velocity, inj = injection site.

Station	τ (h)	u (m/h)	CMR	RMRC
WS01				
Inj–SA	4.4	5.0	1.00	1.00
SA–SB	2.0	7.5	1.08	0.58
SB–SC	0.8	7.5	1.04	0.47
SC–SD	1.3	16.2	1.04	0.39
SD–SE	3.4	2.4	1.03	0.26
SE–SF	0.8	11.3	1.04	0.22
WS03				
Inj–SA	2.6	14.6	1.00	1.00
SA–SB	0.6	30.0	1.02	0.99
SB–SC	4.4	8.2	1.05	0.62
SC–SD	1.2	17.5	1.00	0.45
SD–SE	2.1	7.6	1.00	0.41
SE–SF	8.2	3.8	0.86	0.11

highest λ_{reae} was obtained for subreach SC–SD, characterized by a sequence of pools, riffles, and debris complexes (Fig. 2A).

λ_{pp} ranged between 1.0×10^{-3} and 9.2×10^{-2} /h in WS01 (Fig. 3A), and between 1.0×10^{-3} and 6.0×10^{-2} /h in WS03 (Fig. 3C). In WS01, the smallest λ_{pp} occurred in the subreaches where water flowed over bedrock pools and riffles (i.e., SD–SE and SE–SF; Fig. 2C). In WS03, the smallest λ_{pp} occurred in the subreach with almost complete subsurface flow (i.e., SE–SF; Fig. 2B).

DO longitudinal profiles The DO longitudinal profiles (Figs 4A–D, 5A–D) highlight hotspots of metabolic activity, indicated by the steep slopes of the DO after respiration lines. These profiles also show that in the hypothetical absence of reaeration, DO concentrations would have fallen because of R to ~ 1.0 to 2.0 mg/L in the reach in WS01 and to ~ 1.5 to 2.7 mg/L in the reach in WS03. In streams with significant groundwater inputs, the DO reaeration profile in nighttime conditions would be representative of reaeration after respiration and low-DO-concentration groundwater inputs. At daytime conditions, the DO GPP and reae profile also would incorporate the groundwater input effects.

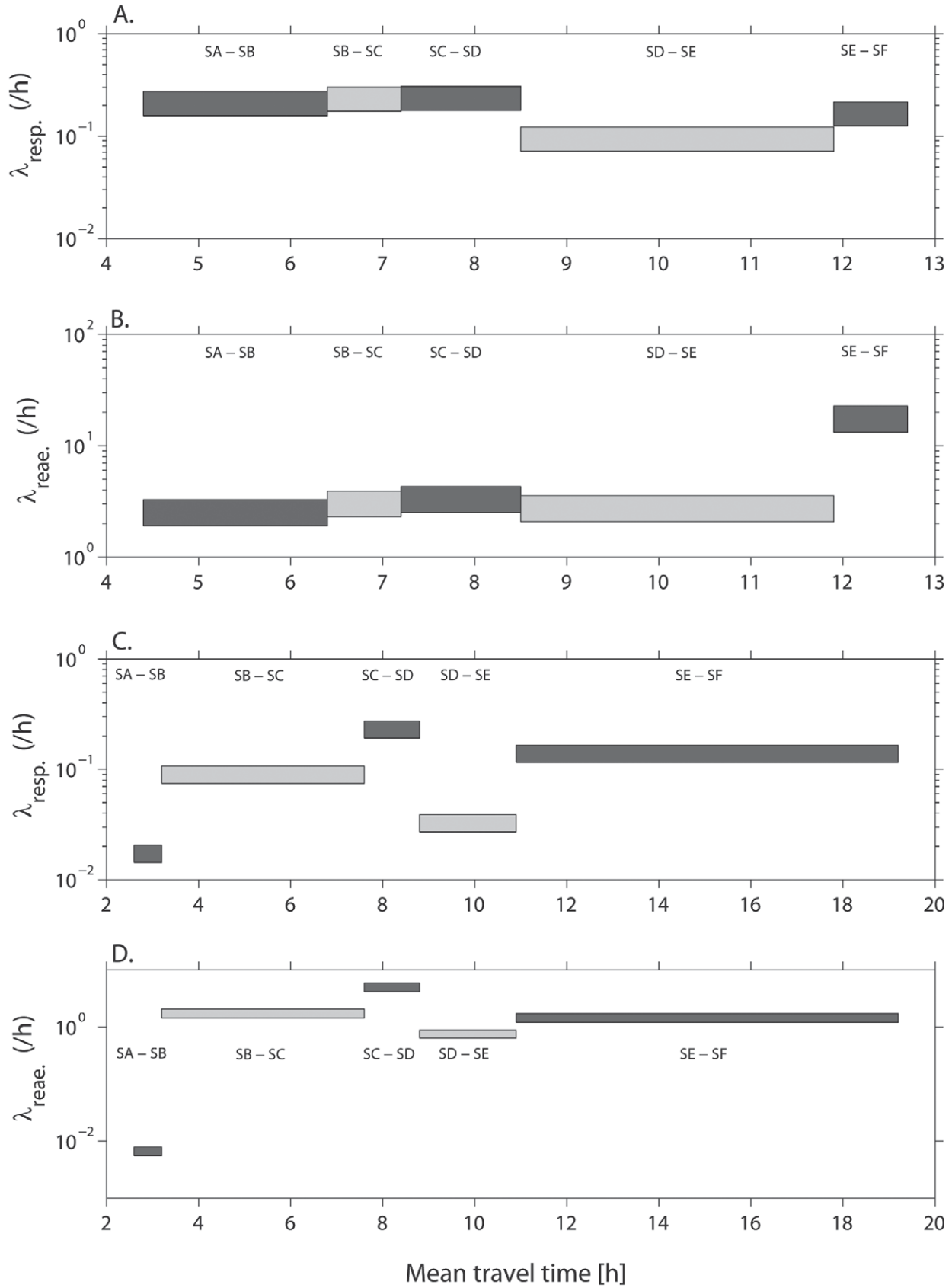


Figure 1. Respiration ($\lambda_{resp.}$) (A, C) and reaeration ($\lambda_{reac.}$) (B, D) rate coefficients estimated from the resazurin–resorufin (Raz-Rru) system for different subreaches in WS01 (A, B) and WS03 (C, D). Rate coefficients were computed using nighttime O_2 histories. Lower and upper bounds (indicated by the height of each bar) describe the uncertainty in the estimates (Eq. 2).

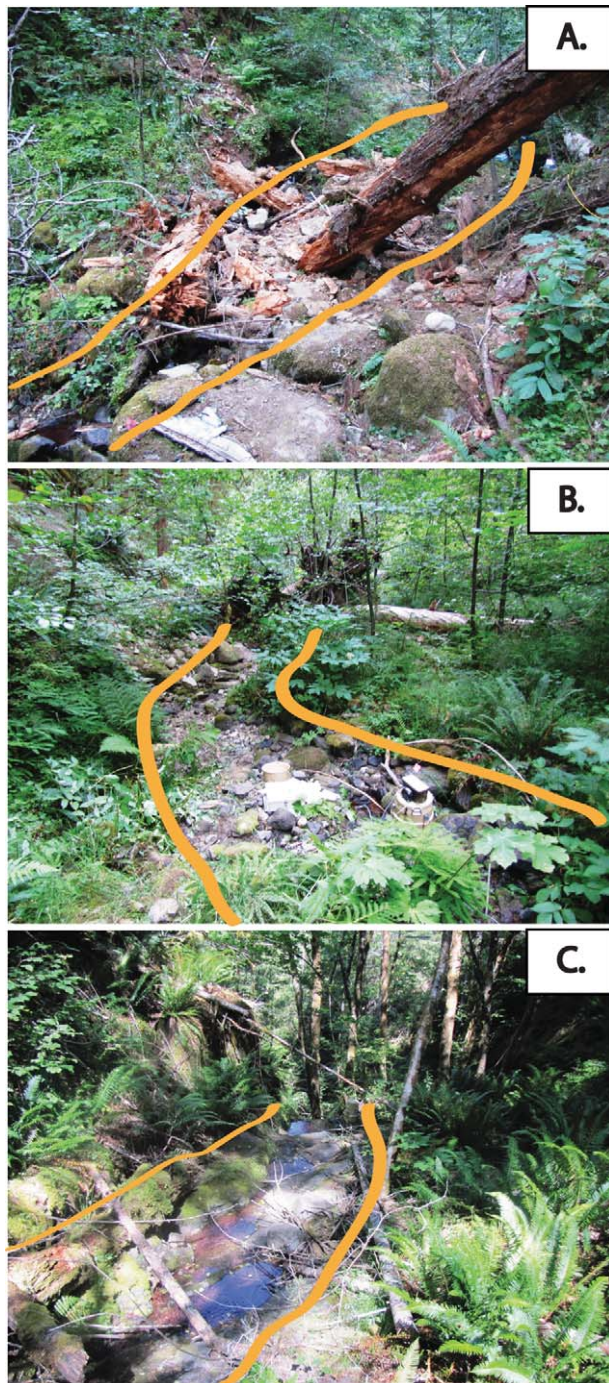


Figure 2. A.—WS03 subreach SC–SD, characterized by step–pool and pool–riffle bedforms, with stream water flowing through large woody debris, which increases retention times, microbial colonization, and hyporheic exchange. B.—WS03 subreach SE–SF, characterized by almost complete subsurface (hyporheic) flow with stream water reemerging only at the end of the reach. C.—WS01 subreaches SD–SE and SE–SF, characterized by sequences of pools and riffles formed over a bedrock outcrop. Added lines help visualize the stream path.

DISCUSSION

The Raz–Rru system can be used to quantify spatial differences in metabolism because Raz is a tracer that undergoes an irreversible transformation to Rru in the presence of aerobic respiration (González-Pinzón et al. 2012, Stanaway et al. 2012). Once lateral and vertical mixing is reached in a stream, a 1-D model describing the transport and reactivity of Raz can be used to estimate in-stream metabolism (Haggerty et al. 2009, Argerich et al. 2011, González-Pinzón and Haggerty 2013). We developed a parsimonious method to estimate respiration rates (λ_{resp}) in streams with the Raz–Rru system, and used it to investigate the relationship between metabolism and hydrodynamics at the subreach scale.

Our proposed method accounts for the interaction between hydrology and biological reactivity to define λ_{resp} and explicitly estimates the uncertainty in the estimates, which are bounded by $\lambda_{0,\text{sz}}^{\text{Raz}}$ and an easy-to-measure empirical constant, $K_{\text{DO}}^{\text{Raz}}$ (Eqs 1, 2). Once λ_{resp} is estimated, we use an O_2 mass balance to estimate rate coefficients and fluxes of respiration, reaeration, and primary production. The main difference between our method and traditional methods is the choice of the target process to estimate independently to close the O_2 mass balance. The Raz–Rru system targets independent estimation of λ_{resp} , whereas traditional methods estimate reaeration rate coefficients (λ_{reae}). We argue that respiration should be the target process for estimating in-stream metabolism in headwater streams because it primarily drives atmosphere–stream O_2 mass transfer, which occurs as a system response to equilibrate the imbalance produced by respiration. Furthermore, estimating λ_{reae} can be highly uncertain and method dependent, as has been discussed extensively in the literature (Kilpatrick et al. 1989, Genereux and Hemond 1992, Melching and Flores 1999, Jha et al. 2004, Aristegi et al. 2009).

λ_{resp} estimated with the Raz–Rru system was more heterogeneous in WS03 than in WS01, which is consistent with the more heterogeneous hydrodynamic conditions observed during field sampling and reported in more detail by Kasahara and Wondzell (2003) and Ward et al. (2012). Spatial variability in λ_{resp} , quantified with the CV, was higher in WS03 than in WS01, i.e., $\text{CV}_{\text{ws03}} \lambda_{\text{resp}} = 61\%$ and $\text{CV}_{\text{ws01}} \lambda_{\text{resp}} = 33\%$. Higher λ_{reae} corresponded with subreaches that had intensive respiration activity, i.e., respiration drove atmosphere–stream mass transfer, which resulted in net small changes in DO concentrations along the stream reaches (Figs 4A–D, 5A–D). Furthermore, λ_{pp} was low, particularly in subreaches without hyporheic exchange (WS01, SE–SF) and with almost nonexistent main-channel flow (WS03, SE–SF). Previous investigators of stream metabolism in the H. J. Andrews Experimental Forest site (and elsewhere) quantified metabolic fluxes either at the reach scale or at the point scale and, therefore, missed the spatial heterogeneity observed in our study (Ashkenas et al. 2004, Argerich et al. 2011, Sobota et al. 2012, and AA

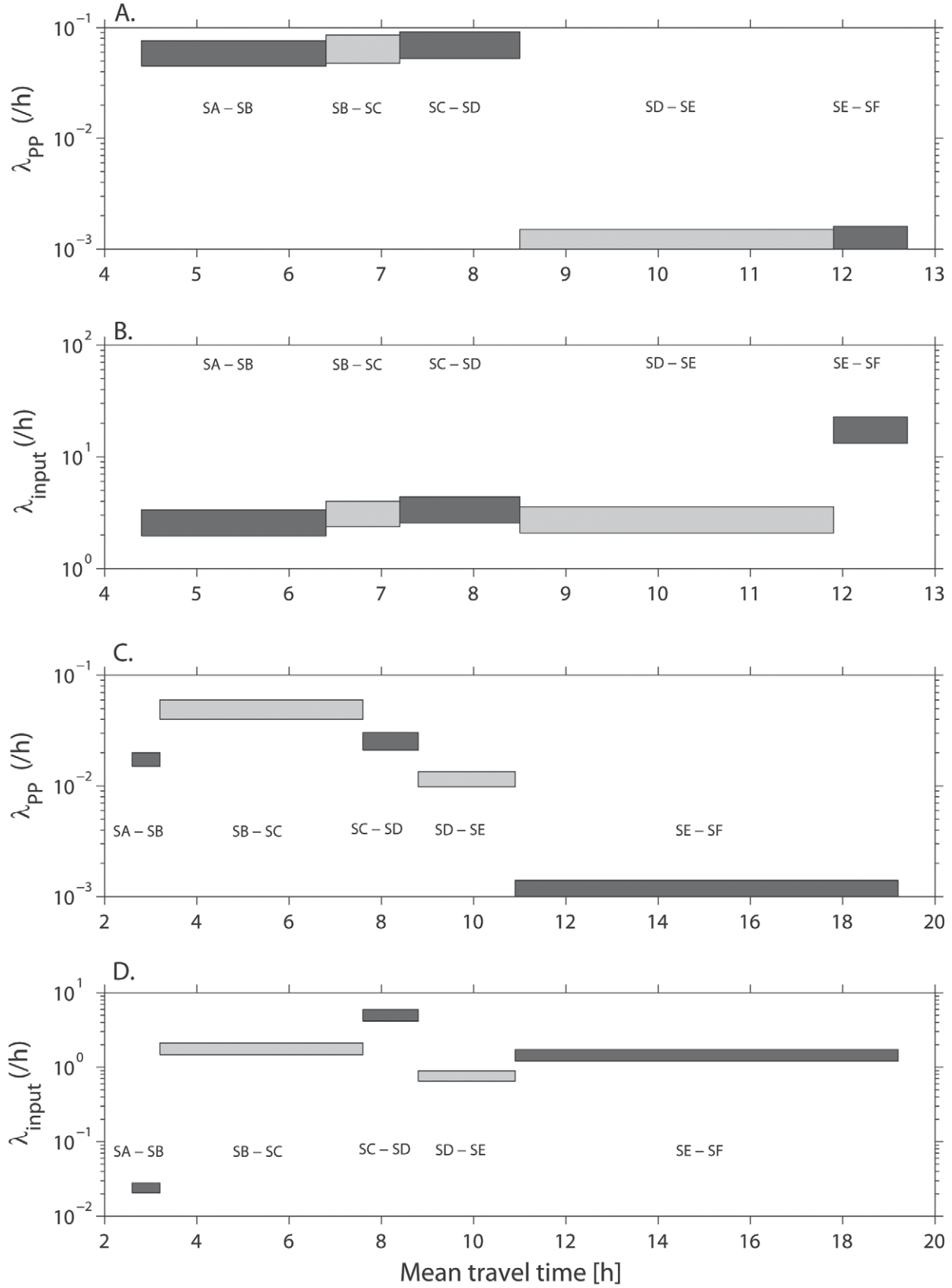


Figure 3. Gross primary production (λ_{PP}) (A, C) and net input O₂ ($\lambda_{input} = \lambda_{PP} + \lambda_{reac}$, where λ_{reac} is the reaeration rate coefficient) (B, D) rate coefficients estimated from the resazurin–resorufin (Raz-Rru) system for different subreaches in WS01 (A, B) and WS03 (C, D). Rate coefficients were computed using daytime and nighttime O₂ histories. Lower and upper bounds (indicated by the height of each bar) describe the uncertainty in the estimates as (derived after estimating λ_{resp} from Eq. 2).

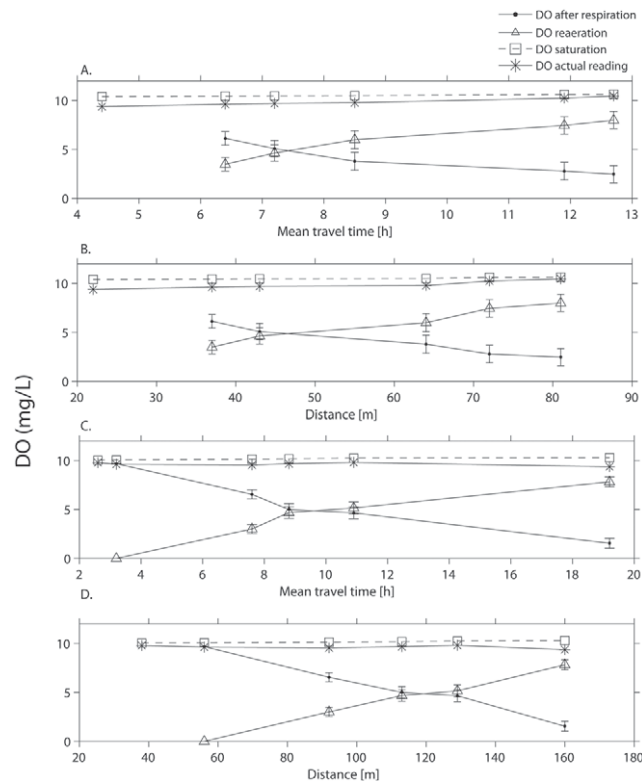


Figure 4. Longitudinal profile for dissolved O_2 concentrations over travel time (A, C) and distance (B, D) in WS01 (A, B) and WS03 (C, D) in nighttime conditions. Markers indicate the locations of stations SA–SF. Concentrations were estimated with Eq. 11. Lower and upper bounds describe the uncertainty in the estimates (as derived after estimating λ_{resp} from Eq. 2).

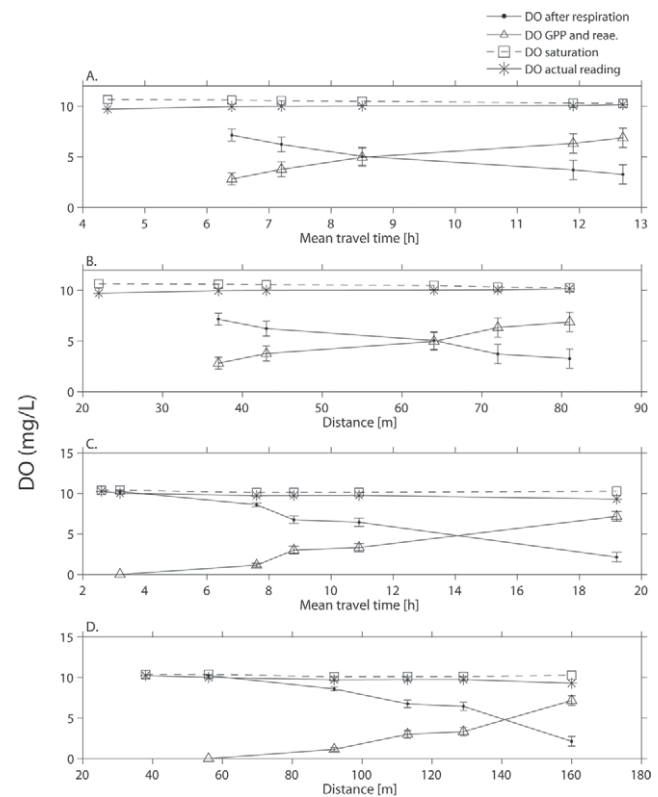


Figure 5. Longitudinal profile for dissolved O_2 concentrations over travel time (A, C) and distance (B, D) in WS01 (A, B) and WS03 (C, D) in daytime conditions. Markers indicate the locations of stations SA–SF. Concentrations were estimated with Eq. 11. Lower and upper bounds describe the uncertainty in the estimates (as derived after estimating λ_{resp} from Eq. 2).

and S. L. Johnson [Oregon State University], unpublished data). Coupling hydrodynamic and metabolic processes in streams with highly heterogeneous geomorphology is expensive and highly uncertain. Thus, the Raz-Rru system might be an improved technique in applications designed to understand stream dynamics at different spatial scales because of the simplicity and thoroughness offered by in-stream tracers.

We compared λ_{reae} (Fig. 6A, B) and NM (Fig. 7A, B) estimated with the Raz-Rru system and traditional approaches (i.e., propane evasion and nighttime regression methods) in subreaches with available information. For these comparisons, we plotted the magnitudes of each quantity and their confidence bounds as: 1) Raz-Rru system: upper and lower limits describing the uncertainty in estimating λ_{reae} from the transport equations (Eq. 2); 2) propane evasion: upper and lower limits for λ_{reae} estimated from field samples ($n = 10$) collected at each site; and 3) nighttime regression: upper and lower limits for λ_{reae} , representing 10% error bars from estimated slope values. For the last 2 methods, other metabolic rate coefficients are based on estimates of reaeration.

The confidence bounds shown in Figs 6A, B and 7A, B do not provide information regarding method accuracy because the intervals have different information content, i.e., uncertainty in coupled transport and reactive processes to infer rate coefficients (Raz-Rru system), sampling precision (propane evasion), and data fitting (nighttime regression). Moreover, a standard reference method to estimate stream metabolism does not exist (see Introduction). Instead, a combination of different techniques can be applied to stream ecosystems, primarily as a function of stream geomorphology.

The Raz-Rru system yielded higher λ_{reae} (Fig. 6A, B) and lower estimates of stream NM (Fig. 7A, B) than the other methods. These results were a consequence of the higher λ_{resp} estimated with Raz, which resulted in more negative estimates of NM (i.e., a more heterotrophic ecosystem). These results are supported by the dense forest present along the stream reaches in the H. J. Andrews Experimental Forest. The magnitude of stream NM estimated with the Raz-Rru system in all the subreaches that we studied in WS01 (-8.47 to -0.52 g $O_2/m^2/d$) and WS03 (-3.74 to

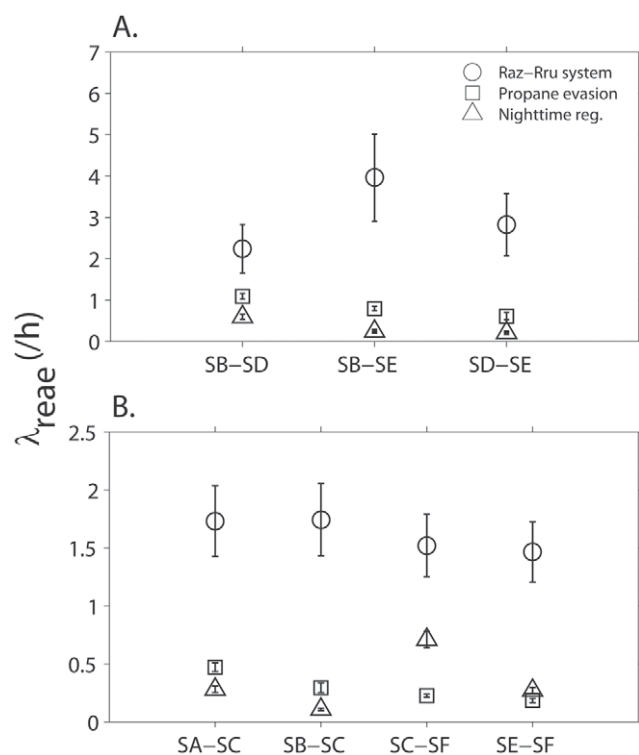


Figure 6. Comparison of reaeration rate coefficients (λ_{ree}) estimated with the resazurin–resorufin (Raz-Rru) system, the propane evasion, and nighttime regression techniques for sites in WS01 (A) and WS03 (B) with comparable information. Lower and upper bounds describe confidence bounds, as explained in the text.

–0.35 g O₂/m²/d) are similar to values reported by other authors for the same streams in summer: -3.67 ± 0.90 g O₂/m²/d in WS01 (mean $\pm$ SE; $n = 3$ summers) (AA and S. L. Johnson, unpublished data) and -0.34 g O₂/m²/d at night in WS03 (Argerich et al. 2011). Our estimates of stream metabolism are similar to those estimated in Mack Creek, a stream surrounded by old-growth forest in the H. J. Andrews Experimental Forest: -9.10 g O₂/m²/d (Ashkenas et al. 2004) and -4.57 g O₂/m²/d (Sobota et al. 2012).

The Raz-Rru system offers theoretical and logistic advantages for estimating stream metabolism at different scales of interest, but metabolic rate coefficients estimated with this method might be affected by uncertainties caused by Raz sorption and Raz nontargeted reactivity. Laboratory experiments suggest that the transformation rate coefficient of Raz to Rru is at least 1 order of magnitude greater than the nontargeted reaction rate coefficient of Raz (Haggerty et al. 2008, Stanaway et al. 2012, Lemke et al. 2013a). The effects of nontargeted transformations of Raz can be assumed to be effectively negligible because the reaction rate coefficient of Raz in the storage zone accounts for the sum of the transformation of Raz to Rru (Eq. 1) and, implicitly, for the nontargeted decay and sorption rate coefficients (i.e., $\lambda_{SZ}^{Raz} =$

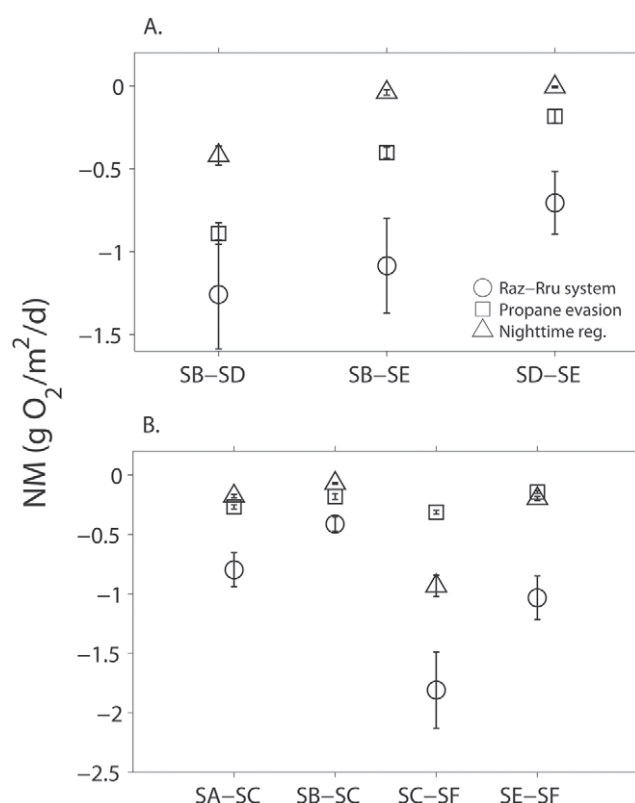


Figure 7. Comparison of net rates of metabolism (NM) estimated with the resazurin–resorufin (Raz-Rru) system, the propane evasion, and nighttime regression techniques for sites in WS01 (A) and WS03 (B) with comparable information. Lower and upper bounds describe confidence bounds, as explained in the text.

$\lambda_{SZ}^{Raz \text{ to Rru}} + \lambda_{SZ}^{nontarget} + \lambda_{SZ}^{Raz \text{ sorption}}$ for Eqs 1 and 2 by González-Pinzón and Haggerty 2013). This assumption is supported by results presented by Haggerty et al. (2009), who showed that photodecay, the nontargeted transformation of Raz and Rru thought to be of most importance (Haggerty et al. 2008), affected Rru but not Raz during a 20-h injection in a less-shaded stream in Catalonia (northeastern Spain). Thus, neglecting sorption of Raz would generate the highest error in estimates of stream metabolism with respect to conventional approaches.

If irreversible sorption within the sampling time scale were large relative to respiration, then it would be a significant source of error. Reversible sorption within the sampling timescale would not be a source of error because Raz is not lost, i.e., Raz would be recovered within the 0th temporal moment analysis. Previous field experiments done in Catalonia (fig. 4 by Haggerty et al. 2009), Germany (fig. 2 by Lemke et al. 2013b), and at the H. J. Andrews Experimental Forest (fig. 3 by Argerich et al. 2011) showed that Raz breakthrough curves are characterized by a much sharper fall than that of the concurrent conservative tracer in-

jected. These results suggest that either: 1) the sorption effects of Raz are small compared to its fast transformation in natural stream sediments, or 2) Raz experiences irreversible sorption within the sampling time scale. More experiments are needed to resolve this duality. However, the only experiments available to date where Raz has been injected into sterile sediments (0.73 and 0.13% organic C content) yielded Raz breakthrough curves with longer tails than that of fluorescein (conservative tracer) (fig. 3 by Lemke et al. 2013a), i.e., the opposite behavior than has been observed in experiments with natural sediments. This result suggests that the microbially mediated transformation of Raz in natural sediments is more important than sorption.

The methods we propose to estimate λ_{resp} and, subsequently, all stream metabolism rate coefficients and fluxes, were developed with consideration of the extent of sorption in the apparent transformation rate coefficient of Raz and of experiments suggesting that the transformation of Raz is primarily the result of aerobic metabolic activity (Haggerty et al. 2008, 2009, González-Pinzón et al. 2012, Stanaway et al. 2012). Furthermore, the methods we propose are based solely on the irreversible transformation of Raz because sorption and photodecay of Rru appear to be more significant under common field conditions (Haggerty et al. 2009, Argerich et al. 2011, Lemke et al. 2013a, b).

Conclusions

The use of tracers to overcome uncertainties associated with the characterization of geomorphic and biochemical in-stream properties has become widespread (e.g., Newbold et al. 1983, Mulholland et al. 2008, Leibundgut et al. 2009). Tracers allow hydrologists and stream ecologists to integrate biogeochemical processes within suitable theoretical and logistical frameworks. We proposed the use of the Raz-Rru tracer system as an alternative technique to quantify metabolism in headwater streams and presented a simple modeling framework to estimate rates and fluxes of community respiration, primary production, and reaeration. Because Raz is a tracer, it can help to bridge the gap between point-scale and reach-scale estimates of metabolism, i.e., it can be used to estimate within reach-scale metabolism and provide insight into the distribution and frequency of hotspots of metabolic activity. Results from our field experiments in 2 headwater streams demonstrated that the Raz-Rru system was capable of detecting spatial variations of in-stream metabolic activity. We found higher respiration rate coefficients in subreaches with extensive hyporheic flow and flow through large woody debris and higher reaeration rate coefficients in subreaches with intensive respiration and higher flow velocities. The differences between metabolic rate coefficients in hotspot subreaches and adjacent subreaches with average metabolic activity were as large as 2 orders of magnitude. The relationships between Raz

reactivity (a proxy for respiration) and (otherwise) difficult-to-quantify geomorphic and hydrodynamic conditions defining metabolic hotspots were self-consistent. Therefore, our methods can be used to inform stream-management and water-quality programs regarding in-stream metabolism and metabolic hotspots operating at different spatial scales.

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