Probing whole-stream metabolism: influence of spatial heterogeneity on rate estimates

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SUMMARY

1. Whole-stream metabolism has been estimated by measuring in-stream oxygen (O_2) concentrations since the method was introduced over 50 years ago. However, the influence of measurement location and estimation method on metabolism rates is understudied. We examined how the placement of O_2 probes (i.e. depth, separation from the thalweg), differences in methodology (1-station, 2-station, area-weighted) and reach lengths influenced estimated rates of whole-stream metabolism in a tallgrass prairie watershed.

2. Metabolism estimates made in the thalweg differed from estimates made in backwaters due to disconnection in flow, and estimates made in deep pools differed from surface estimates due to thermal stratification (temporary flow disconnection). The 1-station respiration estimates differed from short 2-station reach-scale estimates (*c.* 20 m) but were more similar to larger 2-station reach-scale estimates (*c.* 100 m). In contrast, the 1-station gross primary production was most similar to the short 2-station reaches occurring immediately upstream and became less similar at longer 2-station reach lengths. The different estimation methodologies (1-station, 2-station, area-weighted) accounting for the longest reach scale did not result in different metabolism rates.

3. The temporary phenomena of thermal stratification of stream pools during a warm day, which disconnected pool bottoms from the surface waters, likely affected not only the pool estimates but also estimates made in the downstream thalweg (i.e. an O_2 deficit accrued from respiration during the day in the bottom of the pool abruptly moved downstream during mixing).

4. Oxygen probe placement mattered and affected rate estimates according to habitat type and reach length (i.e. scale) due to the influence of small-scale heterogeneity on community respiration. Selection of reach length can be critical for studies depending on whether local heterogeneity is of interest or should be averaged.

5. We conclude that the intuitive use of thalwegs and reaches that are at least 10 times the stream width are likely appropriate for whole-stream metabolism estimates, although the exact reach length necessary and potential stream-specific characteristics, such as stratified pools, need to be carefully considered in probe placement. We encourage other studies to report the placement characteristics of O_2 probes in streams as well as consider the potential confounding factor of local habitat heterogeneity.

Keywords: habitat heterogeneity, scale, tallgrass prairie, whole-stream metabolism

Introduction

Ecosystems are driven by the sum of the energy produced within the system through photosynthesis (autochthonous carbon) and the subsidies from adjacent ecosystems (allochthonous carbon; Dodds, 2007). Gross primary production (GPP) is the amount of carbon (C) fixed through photosynthesis and community respiration (CR) is the amount of C respired by autotrophs and heterotrophs. The net flux of C in aquatic ecosystems is

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referred to as net ecosystem production (NEP), which is calculated by subtracting CR from GPP; the NEP has been used to assess changes in ecosystem trophic status and could potentially be used to assess system stress (e.g. Dodds, 2007; Battin et al., 2008). Estimates of GPP, CR and NEP are collectively termed ecosystem metabolism or, in lotic systems, whole-stream metabolism.

In aquatic ecosystems, dissolved oxygen (O_2) flux is oftentimes used as a surrogate for C flux because it is stoichiometrically linked to the processes of oxygenic photosynthesis and aerobic respiration (Dodds, 2007). Photosynthesis, respiration and reaeration (i.e. the flux of O_2 gained or lost from the atmosphere through physical processes) are the driving factors influencing O_2 concentrations in streams. Data on daily trends in O_2 have long been used to estimate whole-stream metabolism (e.g. Odum, 1956; Hoellein, Bruesewitz & Richardson, 2013) but critical information regarding O_2 sampling location is sparse, despite our knowledge that variables, such as width, depth, flow and canopy cover influence metabolism rates (e.g. Demars et al., 2011; Dodds et al., 2013).

Stream heterogeneity, and subsequently O_2 probe placement, could influence metabolism estimates and offer different types of information about stream ecosystems because physical heterogeneity can translate to metabolic heterogeneity (Cardinale et al., 2002; Demars et al., 2011; Demars, Thompson & Manson, 2015). However, it is not always clear how metabolic activity varies across habitat types (e.g. backwaters and thalwegs and differing depths within pools) or at which scales heterogeneity is evident (e.g. among stream habitat patches or reaches). For example, backwater and hyporheic habitats can have different flow properties and rates of microbial activity compared to the thalweg, and these differences could impact metabolism estimates (Mulholland et al., 1997; Naegeli & Uehlinger, 1997; Behn, Kennedy & Hall, 2010). Resazurin–resorufin tracer measures in streams documented metabolic hot-spots and spatial heterogeneity due to bed materials, large woody debris and transient storage (e.g. González-Pinzón et al., 2014). In our system (and likely many others), stream pools can temporarily stratify under high temperature and this too could influence O_2 dynamics as the assumption of a well-mixed stream is not supported.

In most cases, O_2 probes are placed in the thalweg of the stream where turbulent mixing and maximum flow occurs (Bott, 2006), so the metabolic characteristics of a stream reach can be averaged. Bott (2006) advised that variation in lateral and vertical O_2 dynamics be examined when placing O_2 probes, but the potential for actual influences of this variation on rate estimates has not been quantified. Dye studies are not commonly employed to determine the actual thalweg location in a stream. The habitats where O_2 probes are placed are rarely well described in the literature, which could be an important detail that influences metabolism estimates and thus reduces comparability of rates across studies.

The commonly used reach-scale methods for estimating metabolism in lotic systems are the open channel 1- and 2-station methods (Odum, 1956; Bott, 2006; Holtgrieve et al., 2010), but less is known about how probe placement influences these two methods or whether these methods are comparable in the light of heterogeneity of stream metabolism. An inter-biome comparison of metabolism found that 1- and 2-station methods provided similar results for both GPP and CR when comparing a total of 72 urban, agricultural and reference streams across multiple biomes (Bernot et al., 2010b) and 1- and 2-station methods provided similar rates of GPP but not CR in New Zealand streams (Young & Huryn, 1999).

The 1-station method requires less equipment but estimates could be influenced by unspecified portions of the stream (e.g. the thalweg may influence measurements from a greater distance upstream than do backwaters, and low O_2 groundwater inputs can inflate CR estimates; Hall & Tank, 2005). The 2-station method has a major benefit of explicitly accounting for in-stream changes in O_2 of a defined area between two O_2 probes (assuming the two stations are within the 'footprint' of the lower sensor; Demars et al., 2015). Both methods are probably influenced by specific habitats with different properties (e.g. velocity, width, depth, retention structures, etc.) of the selected reach due to the fact that these methods tend to assume homogeneity of reaches (Demars et al., 2011, 2015). To reduce heterogeneity in oxygen dynamics and improve metabolic estimates, it has been suggested to average the diel O_2 swings from two or more probes within a reach (Demars et al., 2011, 2015). Measurable stream properties are incorporated as average model variables into both 1- and 2-station metabolism modelling (e.g. average depth, width and velocity), and thus rate estimates depend upon exactly where variables are measured.

Reach length is another important consideration when estimating metabolism through diel O_2 measurements, both with respect to how long of a reach should be used for 2-station (upstream–downstream) characterisation and how far upstream 1-station methods integrate O_2 patterns (Demars et al., 2015). If a reach is too short, it may be impossible to measure differences in O_2 between

two stations (Reichert, Uehlinger & Acuña, 2009; Riley & Dodds, 2013) as metabolism will not have enough time to exert influence on O_2 dynamics. On the other hand, if a reach is too long, reaeration effects may diminish the ability to detect O_2 dynamics produced by metabolism (Grace & Imberger, 2006; Demars *et al.*, 2015). Most 1-station estimates do not explicitly consider how far upstream the 1-station estimates actually integrate (but see Hoellein *et al.*, 2009; Hondzo *et al.*, 2013), and such estimates must assume homogeneity for the stream reach. We are not aware of specific empirical research studying the scales of such stream reach integration effects between 1- and 2-station methods of estimating metabolism or of any study that tested probe placement in conjunction with reach length and methodology.

In this study, we investigated how lateral, vertical and longitudinal placement of O_2 probes affected metabolism estimates and addressed the following predictions by placing an array of O_2 probes simultaneously within stream reaches: (1) *Lateral* placement of probes in a stream channel would influence metabolism estimation. Specifically, we predicted backwaters would have distinctive metabolic characteristics compared to those of the nearest thalweg. (2) *Vertical* placement of probes in stream pools could be important for metabolism estimation because diurnal stratification, which we had observed previously during summer in this stream, would constrain water mixing. (3) *Longitudinal* placement of probes, such as the distance between probes, would alter metabolism estimates because of spatial heterogeneity in biological activity. Specifically, we predicted that: (3a) the 1-station method would be most similar to the 2-station method in the reach immediately above the point of measurement and become more dissimilar with increased distance. (3b) Area-weighting of several 2-station contiguous metabolism estimates of subreaches with different habitat integrations would yield more representative, and likely different, estimates because the area-weighted approach would better integrate stream heterogeneity, which would be differentially captured by both 1-station and long 2-station reach approaches.

Methods

Study area

Data for this study were collected at three sites along Kings Creek, a headwater prairie stream located on the Konza Prairie Biological Station (KPBS), a long-term ecological research site. The KPBS is a 35 km² tallgrass prairie preserve in the Flint Hills near Manhattan, KS, U.S.A. (39°5′55.65″N, 96°36′19.91″W). The stream reaches were surrounded almost entirely by gallery forest with some open patches of native prairie grasses such as *Andropogon gerardii* (big bluestem). Dominant tree species in the gallery forests included *Quercus macrocarpa* (bur oak), *Quercus muehlenbergii* (chinquapin oak) and *Celtis occidentalis* (hackberry). Kings Creek is an intermittent stream subjected to repeated floods and drought (Dodds *et al.*, 2004). This stream has received considerable research on metabolic rates (e.g. Dodds *et al.*, 1996; Mulholland *et al.*, 2001; Bernot *et al.*, 2010b), but only modest attention has been paid to the major factors influencing metabolic heterogeneity (e.g. Riley & Dodds, 2013).

The three study sites differed in size, canopy cover and management practice within the Kings Creek watershed (Table 1). All data collected from sites 1 and 2 were previously analysed to address a separate research question (Riley & Dodds, 2013) and were reanalysed for this study to address how estimation methodology (1- and 2-station) influences rate estimates across reach scales (short, medium and long reaches; previously only analysed for 2-station of the short reach scale). Site 1 (named 'K2A' watershed on KPBS) was a total of 91 m in length and subdivided into three heterogeneous subreaches (i.e. encompassing mixed stream habitats): 1a, 1b and 1c (Table 1). Dissolved oxygen was measured for 48 h each during the summers of 2007 and 2009 at this site. Site 2 (named 'N4D' watershed on KPBS) was 155 m in length

Table 1 Characteristics of the study sites. Range refers to the subreaches as well as measurements across years for sites 1 and 2 (except for subreach length) with the exception of discharge, which was determined for the overall site only and range thus reflects only the years.

	Bison grazed	Burn regime	Total reach length (m)	Number of subreaches	Range in subreach length (m)	Range in average subreach width (m)	Range in average subreach depth (m)	Range in site discharge (m³ min⁻¹)	Median site nutrient concentrations (DIN:SRP µg L⁻¹)
Site 1	No	2 year	91	3	27–35.5	2.9–4.2	0.05–0.29	0.43–1.1	N/A
Site 2	Yes	4 year	155	4	22.5–63.5	0.9–1.7	0.05–0.10	0.09–0.53	328:15
Site 3	No	1–2 years	137	5	20–45.5	1.8–4.4	0.1–0.5	0.38	431:14

and subdivided into four heterogeneous subreaches: 2a, 2b, 2c and 2d with O_2 measured for 48 h each during the summers of 2006, 2007 and 2009. Site 3 (named 'AL' watershed on KPBS) was 137 m in length and contained five subreaches: 3a, 3b, 3c, 3d and 3e. At site 3, we intentionally placed O_2 probes in transitional areas between riffles and pools. Thus, subreaches 3a, 3c and 3e corresponded with riffles, while 3b and 3d corresponded with pools. At site 3, we measured O_2 for 25 h during the summer of 2012. For all sites, subreach 'a' corresponded with the most upstream subreach, while subsequent letters corresponded to downstream subreaches. Additionally, at site 3, we placed two probes in slower moving backwaters (referred to as backwaters 1 and 2) to compare metabolism in slower moving water to the nearest thalweg probe location (Fig. 1). Backwater 1 was a depositional site created by channel widening that slowed flow while backwater 2 was partially separated from the main flow by a land bar projecting into the stream and thus creating a physical barrier to the main channel. We placed a set of two probes within two pools (i.e. subreaches 3b and 3d) at depths of 15 cm below the water surface and 15 cm above the benthic zone in the deepest location of each pool to determine if there were differences in O_2 concentration due to vertical probe placement (see Table S1 in Supporting Information for characteristics of the two pool sites).

Field methodology presented below is more detailed for site 3 (see Riley & Dodds, 2013 for field methodology and more specifics on sites 1 and 2). The main methodological differences are noted where applicable in the methods.

Habitat characterisation

We selected subreaches based primarily on travel times and the 20 m minimum reach lengths recommended by Riley & Dodds (2013) for this stream to measure significant differences in O_2 concentrations. We also selected reaches based on habitat heterogeneity, lack of major tributaries or groundwater inputs, constrictions between habitats and average depth. We measured stream widths using a minimum of 10 measurements per subreach (Bott, 2006).

Travel times and discharge

We used a conservative tracer (rhodamine WT) pumped into the stream at a continuous rate of 8–12 mL min^{-1} using a FMI pump (model QBG; Fluid Metering, Inc.,
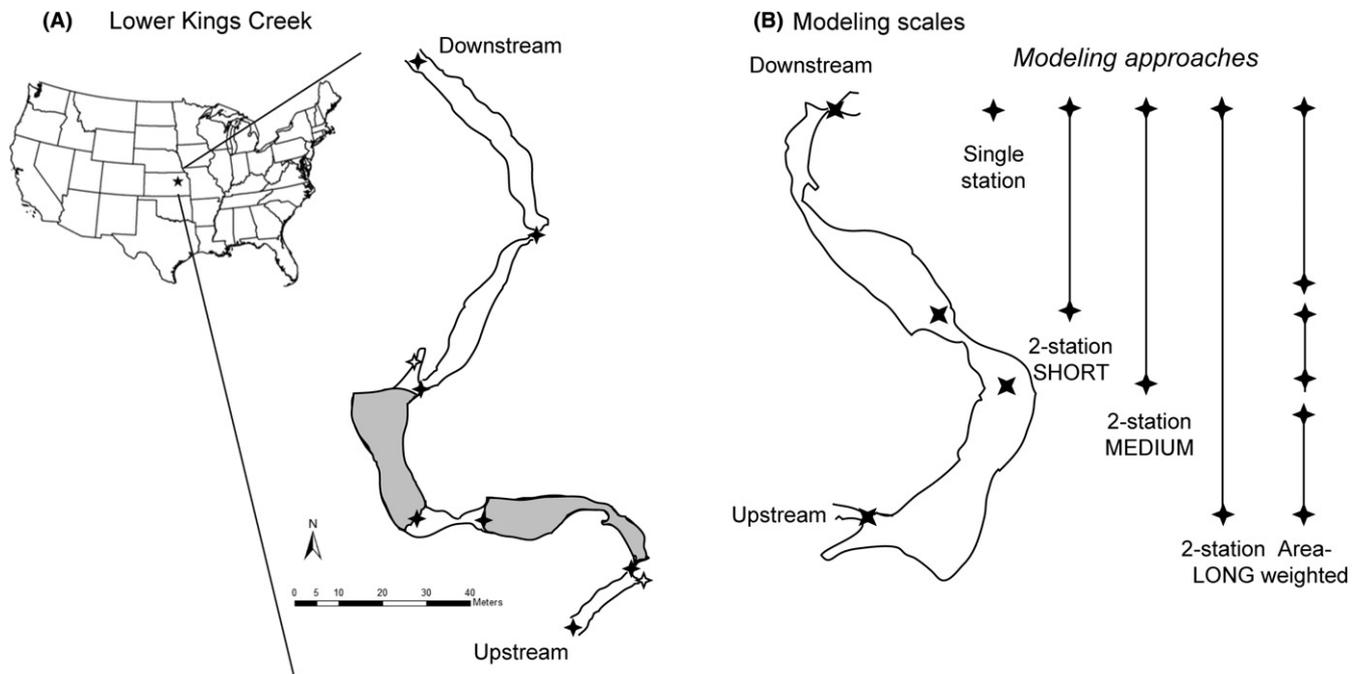

Fig. 1 (A) Konza Prairie Biological Station and locations of where O_2 probes were placed at site 3. Areas in white represent riffles and areas shaded in grey represent pools. Black stars indicate locations where O_2 probes were placed in the thalweg (the deepest area of the channel) and white stars represent locations where probes were placed in backwaters (where water flow was slower in side channels). (B) A conceptual model of how we categorised short, medium and long reaches and how we calculated metabolism using the area-weighted method.

Syosset) to determine travel times and discharge at each site. We measured rhodamine fluorescence with an Aquafluor fluorometer (model 8000-010; Turner Designs, Sunnyvale) to determine plateau and measured travel times as the time it took in-stream concentration of the conservative tracer to reach half of the ultimate plateau concentration and converted these measurements to velocity using reach length (Mulholland *et al.*, 2009). Discharge was calculated from proportional dilution of tracer stock and pump rate. We calculated average depth using average velocity, discharge and width. In backwaters, we used average depth as measured upstream from the nearest thalweg probe to calculate areal metabolic rates expressed in per unit area.

Dissolved oxygen & light

Oxygen probes were placed in each reach based on the 2-station upstream–downstream approach for an entire reach as well as all the subreaches (Bott, 2006). Sites 1 and 2 corresponded to the reanalysed data from Riley & Dodds (2013) and measurement methods are described therein. Site 3 used data collected solely for this manuscript. For site 3 measurements, we calibrated YSI ProODO meters (Yellow Springs Instruments, Yellow Springs) in the laboratory for O_2 using the air-saturated water approach and a standardised barometric pressure. We placed all probes in a bucket of air-saturated water for 30 min prior to and immediately after deploying the probes to check calibration and drift. After calibration, we deployed the first O_2 probe in the thalweg at the upstream station of each reach and subsequent downstream probes at each subreach boundary, with the most downstream probe being placed at the end of the preselected reach (Fig. 1). We affixed all probes to a steel bar hammered vertically into the substrate at ~50% of water depth. Data were logged continuously every 10 min for 25 h (48 h sites 1 and 2) to capture a minimum of one full diel O_2 swing. Calibration of all O_2 probes was maintained throughout the deployment.

Light intensity was measured at the same intervals as O_2 (i.e. 10 min) with Odyssey Photosynthetic Irradiance Recording Systems (Dataflow Systems PTY LTD, Christchurch) attached to the top of each steel bar. The values were converted to photosynthetically active radiation (PAR) based on calibration coefficients obtained from a calibration run of the loggers against a calibrated LiCOR from the National Ecological Observatory Network.

Estimating metabolism rates

We used the Bayesian Single-Station Estimation model (BASE) v2.1 published by Grace *et al.* (2015) as corrected for light estimation (Song *et al.*, 2016) for the 1-station models that provided parameter estimates and measures of variation and uncertainty. Each Bayesian model was run with 20 000 iterations with 10 000 burn-in for three parameters (GPP, CR and K). In addition, we used the modelling approach from Riley & Dodds (2013) to model 2-station metabolism because the BASE model is for 1-station metabolism only (see Table S2 in Supporting Information for differences in CR and GPP estimates between 1-station BASE and 1-station Dodds *et al.*, 2013 modelling approaches). The basic approach for all of these models adjusted rates of CR and GPP (and reaeration if not measured) based on the measured physical characteristics of the stream (i.e. temperature and the reach averages of discharge, velocity and channel width) and the environment (i.e. PAR and atmospheric pressure) to predict diel trends in O_2. Of course, physical attributes changed with, and were adapted for, increasing reach length as more heterogeneity was included, which would also be reflected in the metabolism estimates. Since we were interested in the effect of reach length, such heterogeneity was critical to our research question.

At sites 1 and 2, we used reaeration estimates published in Riley & Dodds (2013) for all subreaches, which measured reaeration using inert gases (i.e. propane, acetylene or SF_6) and conservative tracers (i.e. rhodamine WT or Br^-). At site 3, and for the combined reaches at sites 1 and 2 (see below), we modelled reaeration (Riley & Dodds, 2013). The model used the 'Solver' option in Microsoft Excel (version 2013; Microsoft Corporation, Redmond) to find estimates of CR and GPP that minimised the sum of squared error between the observed and modelled O_2 (see Data S1 in Supporting Information for the specific equations used in the Riley & Dodds (2013) 2-station modelling approach).

All comparisons for lateral and vertical influences of probe placement on metabolism estimates were conducted using data from site 3. We modelled metabolism using the 1-station method at site 3 for the two backwaters, as well as the nearest thalweg locations and at the surface and bottom locations in the pools. We used physical characteristics upstream of each nearest thalweg probe to estimate metabolism because our objective was to determine how biased whole-stream estimates would be if probes were placed in locations other than the thalweg.

Metabolism was modelled using the 1-station method for the most downstream probes for each sampling date and site to test for longitudinal placement effects. Additionally, metabolism was modelled for each sampling date and site using the 2-station method for the entire reach, each subreach, as well as reaches that represented an increased distance from the lowermost O_2 probe (e.g. downstream probe at subreach 1c and upstream probe of subreach 1b for the reach 1c+1b), so that we could compare metabolism estimates for increased reach length and, thus, integrated spatial heterogeneity (Fig. 1). We refer to 'medium' and 'long' reaches as the combination of two or three subreaches respectively (Fig. 1B). Thus, we modelled metabolism for six 'combined' subreaches (medium reaches: 1c+1b, 2d+2c, 3c+3b and long reaches: 1a+1b+1c, 2d+2c+2b, and 3c+3b+3a). Medium and long reaches ranged in size from 42 to 100 m and 73 to 133 m in length respectively (Table 1), with long reaches always longer than medium reaches within a site.

We also used an area-weighted approach (Fig. 1B) based on total reach surface area to estimate metabolism rates for the entire stream reach to compare these rates to the 1-station rates of the most downstream probe and the 2-station rates of the entire reach at each site. This approach weighted the metabolism estimate of each subreach by the relative aerial proportion of the subreach within the total reach area. In this method, metabolism of the entire reach was calculated as a function of area-weighted metabolism estimates from all subreaches.

Data analyses

Due to low replication ($n = 2$) for lateral and vertical probe placement, we chose not to compare the differences statistically. For longitudinal placement, we used the mean squared error (MSE) to find the differences between 1- and 2-station rates of CR and GPP for all reach sizes (i.e. short, medium and long) using eqn 1.

$$\text{MSE} = \sqrt{\frac{(1\text{-station rate} - 2\text{-station rate})^2}{\text{number of reaches}}} \quad (1)$$

The MSE allowed us to compare how closely the 1-station rate matched with respect to different 2-station reach lengths. Specifically, the MSE comprises both the variance and bias, and thus provides precision (small variance) and its accuracy (small bias). We compared MSE between the 1-station rates and the most downstream short subreach followed by the medium and long subreaches ($n = 6$ site-year comparisons for short,

medium and long reaches against 1-station). We assumed that a MSE < 1.5 and the points in close proximity to the 1:1 line was indicative of high congruence between the two methods.

We determined differences among the area-weighted, 1- and 2-station approaches by finding the differences in rates of CR and GPP at each site on all sampling dates. For area-weighted estimates at sites 1 and 2, we used as fine a resolution of subreaches as possible (i.e. three subreaches at site 1 and four subreaches at site 2), but we divided site 3 into an upper and lower section (i.e. upper section represented by 3a, 3b and 3c and the lower section represented by 3d and 3e) to best incorporate the heterogeneity at that site. Since we had a total of seven sampling site-year combinations (site 1: 2007 and 2009, site 2: 2006, 2007 and 2009, two sections of site 3 in 2012), we had a total of seven sets of measurements for which we calculated the differences between the methods (i.e. area-weighted – 1-station, area-weighted – 2-station, 1-station – 2-station). We then bootstrapped these differences 5000 times in R version 3.0.2 (R Foundation for Statistical Computing, 2013) using the package 'boot' (Canty & Ripley, 2014) to attain a mean difference and 95% confidence intervals for estimates using the various methods.

Results

Measurements taken in the more slowly moving backwaters differed from the nearby thalwegs, supporting prediction 1 (Table 2, Fig. 2). Differences in CR were more pronounced than those of GPP which were similar between the backwater and thalweg, but the directionality (higher/lower) of the CR differences varied between the two backwaters studied (Table 2).

Metabolism differed between probes placed at the surface and bottom of pools, supporting prediction 2 (Table 2, Fig. 3). The pools stratified by temperature and

Table 2 One-station community respiration (CR) and gross primary production (GPP) rates to test for differences in lateral and vertical probe placement in Kings Creek.

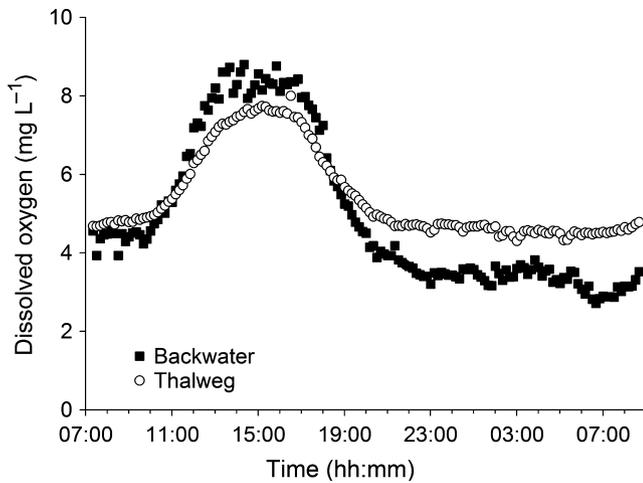|	CR (g O_2 m^{-2} d^{-1})	GPP (g O_2 m^{-2} d^{-1})
Backwater 1	−3.4	1.7
Thalweg 1	−11.9	3.3
Backwater 2	−2.7	1.4
Thalweg 2	−3.5	1.6
Pool 3b surface	−4.5	2.3
Pool 3b bottom	−9.0	1.4
Pool 3d surface	−1.8	1.1
Pool 3d bottom	−10.7	2.2

Fig. 2 O_2 concentrations in backwater 1 (closed squares) and the nearest thalweg (open circles).
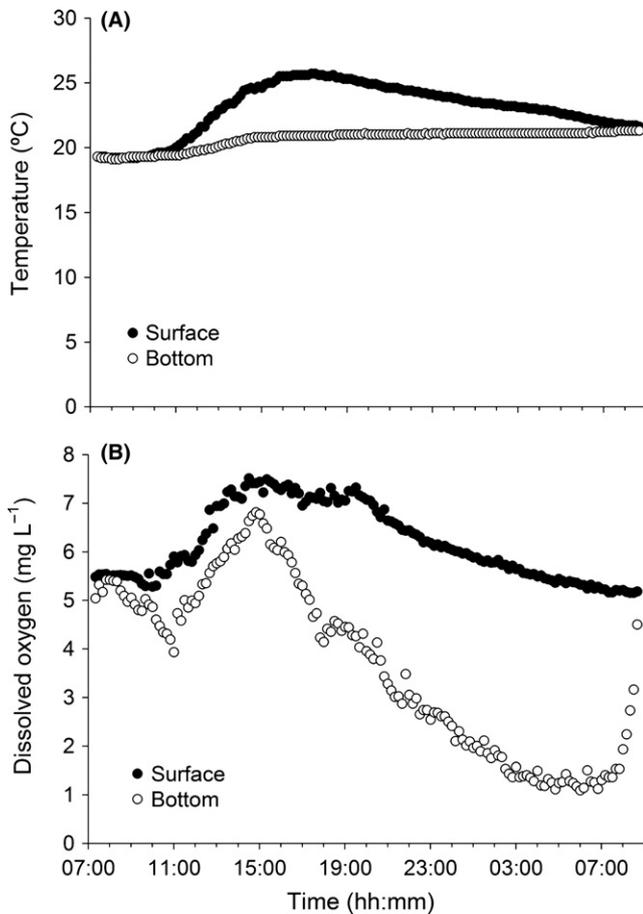

Fig. 3 Changes in temperature (A) and O_2 (B) between measurements made 15 cm below the surface (closed circles) and 15 cm above the bottom (open circles) of pool 3d. Pool 3b displayed similar patterns.

the surface water was a maximum of 4.8 and 3.1 °C warmer than the bottom water of pools 3d and 3b respectively (Fig. 3A). Stratification also altered O_2

Table 3 The mean squared error (MSE) between 1- and 2-station methods for the three tested reach lengths. A lower MSE suggested improved congruence between methodologies. CR, community respiration; GPP, gross primary production.

Site-years at each reach scale ($n = 6$)	MSE	
	CR	GPP
Short (20–64 m)	2.1	0.9
Medium (42–100 m)	1.1	1.6
Long (73–133 m)	1.5	1.4

patterns (Fig. 3B), and thus the modelled metabolism rates differed at the surface and bottom of the pools. Within the pools, rates of CR ranged from −1.8 to −10.7 g O_2 m^{-2} d^{-1}, whereas GPP rates were less variable and ranged from 1.1 to 2.3 g O_2 m^{-2} d^{-1} (Table 2). The estimated CR rates were 2–6× greater at the bottom of pools than at the surface, while GPP rates were similar between the surface and the bottom of each pool.

The 1-station estimates of modelled reaeration compared with measured water velocity yielded estimates of ~95 m for the 50% O_2 sensor 'footprint' using the equation of Demars *et al.* (2015) (see Data S1), indicating that the 2-station segments we chose (Table 1) were within the distance of assumed homogeneity. The longest 2-station estimate (Table 3) was ~30% longer than the calculated 'footprint' (e.g. 133 m).

Longitudinal placement of O_2 probes influenced metabolism estimates, but not as we specifically predicted, and thereby partially supported prediction 3. The 1-station rates were most similar to 2-station rates for the short reaches closest to the single station for GPP, but not for CR (Fig. 4). The 1- and 2-station CR estimates deviated most from each other for shortest reaches (20–64 m), but were more concordant with what we called medium (42–100 m) and long reaches (73–133 m) as indicated by a reduction in MSE as more reach area was integrated in the 2-station metabolism estimate (Table 3, Fig. 4A). The estimated GPP rates of the 1-station models were most similar to the 2-station short reaches, as indicated by the lowest MSE (Table 3, Fig. 4B). The MSE increased when comparing 1-station GPP estimates to 2-station medium and long reaches meaning the 1-station estimates were most reflective of GPP occurring immediately upstream of this O_2 probe in the short reaches. Therefore, in this stream ecosystem, longitudinal placement influenced the rate estimations of both CR and GPP, but in opposite ways.

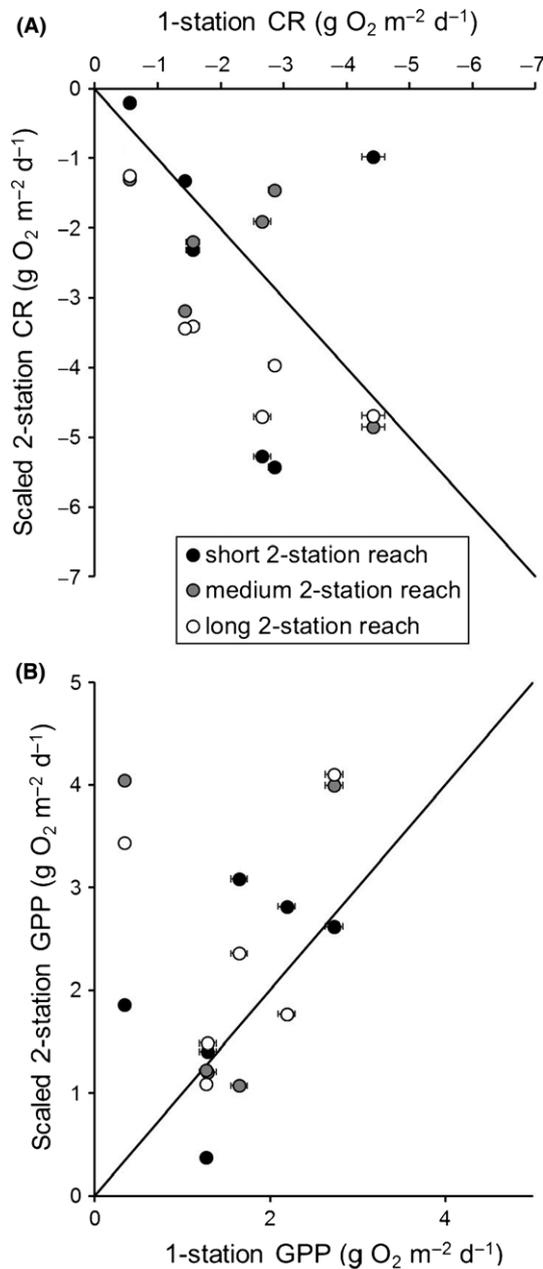Our data did not support prediction 3b, that the use of area-weighted subreaches would yield more

Fig. 4 One-station community respiration (CR) (A) and gross primary production (GPP) (B) rates compared to 2-station rates for short (black circles), medium (grey circles) and long (white circles) reaches. The black lines indicate 1:1 lines; thus, points occurring near the line indicate agreement between 1-station and 2-station rates. Error bars for 1-station estimates represent ± 1SD. For MSE, see Table 3.

representative, and likely different, estimates from those of the 1- and 2-station methods. The 1-station, 2-station and area-weighted modelling approaches all provided similar metabolism estimates as the 95% confidence intervals of the differences spanned zero (Fig. 5). However, 95% confidence intervals were large, potentially due to the low replication, especially considering the
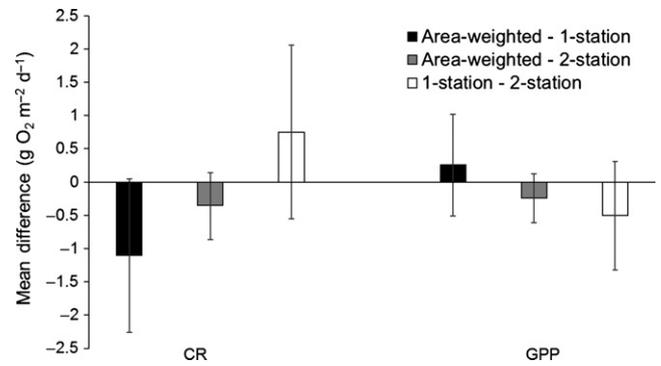

Fig. 5 Means and 95% confidence intervals for differences in community respiration (CR) and gross primary production (GPP) between methods after bootstrapping 5000 times. Black indicates differences between area-weighted and 1-station rates. Grey indicates differences between area-weighted and 2-station rates. White indicates differences between 1-station and 2-station rates.

high variance observed in 1-station versus 2-station CR across reaches. Overall, vertical, lateral and longitudinal placement and methodology affected CR estimates at small spatial scales, whereas GPP was more influenced at larger scales.

Discussion

Scaling of metabolic rates in streams over multiple adjacent reaches has been largely unexplored and we predicted that habitat heterogeneity and, subsequently, placement of O_2 probes would influence the estimation of measured rates and extrapolation to other scales. This study demonstrated that both CR and GPP estimates varied depending upon lateral, vertical and longitudinal placement of probes. The longer reaches provided similar metabolic estimates to those weighted by surface area, suggesting they all captured and integrated small-scale heterogeneity. Our data indicated that spatial heterogeneity can be linked to metabolic heterogeneity. Up-scaling should be used with caution for short reach length measurements of GPP because these measurements tended to be more influenced by small-scale heterogeneity than longer reaches. Our data do not indicate that our longest reach lengths were beyond the limit of detecting differences in metabolism and where reaeration, rather than in-stream photosynthesis, becomes the dominating factor influencing O_2 dynamics.

Influence of lateral and vertical probe placement

Lateral placement of O_2 probes resulted in different estimates of CR, but not GPP, in backwaters compared to

those of the thalweg. The two backwaters varied both in direction and magnitude for CR, suggesting that 'backwater' was either a poor descriptor of CR or that CR rates are highly variable among backwaters and thus not captured well with our low sample size ($n = 2$). The differences between the backwaters may be due to hyporheic exchange or low water exchange between habitats as well as organic matter availability (Gantzer, Rittmann & Herricks, 1991; Mulholland *et al.*, 1997). Our finding that GPP was similar among backwaters and the thalweg agree with the results comparing GPP in backwaters to the main channel of the Colorado River (Behn *et al.*, 2010). Other studies have shown GPP to be highly variable in many contexts (Demars *et al.*, 2011), especially across biomes (e.g. Bernot *et al.*, 2010b).

Similar to backwaters, we found the deep-water habitat of the pools to be temporarily disconnected from the main stream flow through stratification. The effects of such stratification can greatly influence the estimate of aquatic metabolism in lakes (Coloso, Cole & Pace, 2011) but has not yet been studied in streams. While we did not determine the exact degree of mixing between the surface and bottom of the pools, we do know that exchange of water (eddy diffusion) was inhibited because the surface and bottom waters had distinct temporal patterns of both temperature and O_2 (Fig. 3). Dye tracer releases (data not shown) indicated that the surface water of the pool slid quickly across deeper waters and did not display vertical dispersion, further indicating isolation of the bottom waters. If water velocity is changing throughout the day due to this stratification, this could also impact model variables required by some models to estimate metabolism. If a pool is suspected to stratify, one could make several velocity measurements throughout the day and use the average velocity during the period of O_2 measurement. This short-term stratification led to a situation where the O_2 was similar at the surface and bottom of the pool when the temperatures were similar, but as temperatures diverged, the O_2 dropped more rapidly at the bottom of the pool. It is unlikely the rapid drop in O_2 in the bottom of the pool was due to high groundwater influence because groundwater tends to have substantially lower temperatures and is about 70% saturated with O_2 in this area (Edler & Dodds, 1996). When temperatures converged (*c.* 7:00 hours) at the time of destratification, there was a rapid increase in O_2 in the bottom of the pool, which could be incorrectly interpreted as a burst of GPP in the pool (Fig. 3B). The injection of low O_2 from the deep pool water could also influence apparent metabolic patterns detected in the downstream riffle (burst of CR),

although the mass effect on pool surface water concentrations was not as apparent (Fig. 3B).

Stratification could lead to anomalous interpretations of diurnal metabolic rates, as well as affect organisms and other ecological processes, and feedback to influence metabolism. The differences in estimates found between surface and bottom of the pools may thus be influenced by both biological and physical constraints. The stream-pool stratification led to hypoxic conditions for a ~12-h period before mixing, with O_2 concentrations dropping below 2 mg O_2 L^{-1}. Low O_2 concentrations can reduce fungal diversity, fungal biomass, fungal sporulation and leaf decomposition rates (Medeiros, Pascoal & Graça, 2009), which can shift dominant biogeochemical processes and affect CR rates (Gulis & Suberkropp, 2003; Dodds & Whiles, 2010). Mobile animals may be forced into the upper, oxygenated pool where they are more prone to predation, whereas plants and microbes must simply adjust to transient low O_2 concentrations. Fishes may congregate in the shallow pools and increase metabolic activity (Martin *et al.*, 2016), at least until oxygen is depleted. Trophic complexity can increase N uptake (Bernot, Martin & Bernot, 2010a) and thus metabolism rates. Stratification could thus lead to metabolism estimates affected by heterogeneity where oxic processes become less dominant, and destratification and O_2 mixing could influence metabolic rate estimates made downstream of the individual pool. We are not aware of any studies that have measured metabolism in backwater or pool habitats of small streams. These habitats could function similarly to transient storage zones or other zones of high influence which have been found using resazurin–resorufin tracer approaches (González-Pinzón *et al.*, 2014). Stratified pools are temporary transient storage zones, as they are isolated for only part of the day. In any case, stratified pools, almost certainly exert some influence on stream metabolism estimates and the overall influence of these habitats warrants further investigation.

Influence of longitudinal probe placement, estimation methodology and metabolism scaling

Spatial heterogeneity is a key determinant of stream metabolic rates, and longitudinal small-scale heterogeneity was integrated and accounted for in longer reaches (maximum of 155 m long reaches). The 1-station method best integrated CR in medium 2-station reaches and somewhat comparably to the long 2-station stream reaches, suggesting that heterogeneity across pools and riffles could be successfully integrated into a single

metabolism model, but this trend was not fully clear. These findings do not completely contradict Demars *et al.* (2011, 2015), who suggested averaging the results of several O_2 profiles to obtain more reliable metabolism estimates. Most of our reaches were short enough that the assumption of homogeneity was reasonable and when several O_2 probes are not available, the 1-station method may provide reasonable results.

Spatial variability in metabolism can occur at all scales of study: patch scales (Cardinale *et al.*, 2002), reach scales (Reichert *et al.*, 2009; this study) and stream networks (Vannote *et al.*, 1980; Gawne *et al.*, 2007). In our study, the 1-station method best matched variability of shorter reach lengths (20–64 m) for GPP, but was more representative of CR at longer reach scales. If a research question is not specifically aimed towards what we are calling reach-scale variability, our data indicate that the integration by 1- and 2-station methods are appropriate reflections of variability at small reaches as no difference was found among them and the area-weighted method. Thus, the general methods applied to date have been confirmed by our study regarding integration of heterogeneity by longer reaches and use of well-mixed probe locations in the thalweg. However, such findings and our general assumptions about probe placements need to be confirmed in other streams.

Ecological scaling implications

The issue of scale has been a dominant theme and persistent issue in ecology (Levin, 1992), and in stream ecology in particular (e.g. Frissell *et al.*, 1986; McGuire *et al.*, 2014). Streams are complex and nested hierarchical constructs with multiple system levels (e.g. patch versus reach versus watershed scales; Frissell *et al.*, 1986; Hawkins *et al.*, 1993; Montgomery & Buffington, 1997), and each system level is applicable for metabolism studies. However, differences among system levels/scales are inevitable because driving processes are likely to vary with the scale considered. For example, light drives reach-scale GPP across diverse biomes (e.g. Bernot *et al.*, 2010b), and light is expected to vary with position in the watershed and physical orientation of features that could shade the stream. We found GPP to be similar at various patch and reach scales, suggesting that at the scale of ~ 10–100 of metres in our watershed, light might not be variable enough to drive heterogeneity. However, the CR rates did not match well when up-scaling from short reach scales to longer reach scales potentially due to differences in physical and chemical properties of the subreaches. Thus, GPP and CR appeared to scale differently in our study. We documented metabolic heterogeneity of what might be considered meso-scale heterogeneity in our streams, where variation occurred across pool-riffle complexes. The variation at the largest scale (whole watersheds) has yet to be well characterised, yet strides have been made describing physical variation at that scale (Rüegg *et al.*, 2016).

With finer grain sampling, future research could examine cross-section variability in O_2 by deploying several O_2 probes and coupling them with fine-scale flow measurement. Algae and substrate can display spatial heterogeneity at the millimetre-scale (Dodds, 1991; Wilson & Dodds, 2009), which can translate into differences in metabolism and nutrient uptake at the micro-scale (Hoellein *et al.*, 2009; Koopmans & Berg, 2015; Lupon *et al.*, 2016). Eddy-covariance methods are currently being developed for use in streams (Koopmans & Berg, 2015) that could be used to investigate the centimetre to metre scale of heterogeneity more thoroughly.

Having the ability to predict metabolism at various scales could help to determine stream trophic status and possibly reference conditions for degraded streams (e.g. Dodds, 2007; Atkinson *et al.*, 2008). Cross-biome comparisons of metabolism found that GPP across North American biomes was more variable than CR rates primarily due to large differences in light availability across sites (Mulholland *et al.*, 2001; Bernot *et al.*, 2010b), which differs from our smaller scale findings within a single, small watershed where variation in light is much smaller. Phosphorus concentrations and channel hydraulic conditions were the driving factors controlling CR across biomes (Mulholland *et al.*, 2001) which may have been contributing factors in our streams as well (e.g. more slowly flowing backwaters). We found that GPP was relatively constant throughout our sites and that CR was a more variable process, but our measurements were made at a single time of year, so canopy cover was relatively constant. Metabolism rates tend to vary throughout the year in many streams (Hill, Mulholland & Marzolf, 2001; Hill & Dimick, 2002; Roberts, Mulholland & Hill, 2007), suggesting that our findings may only apply to the season of study. More detailed measurements made at sites 1 and 2 did in fact exhibit distinct seasonal patterns (Riley & Dodds, 2012). In a specific region and season, the prediction of GPP across stream sites with similar canopy cover and nutrient concentrations may be feasible (i.e. at the meso-scale) as seen with our sites, but such an option may not be available for CR as its estimates may be more driven by factors varying at smaller spatial scales such as organic matter availability.

Conclusions

Mixing patterns of water in streams should be considered when planning placement of O_2 probes and selecting the metabolism estimation methodology. Shorter reaches using the 2-station method will best capture stream metabolic heterogeneity, particularly if they are within the 'footprint' of the O_2 sensors (Demars *et al.*, 2015), while longer reaches may be more representative if extrapolation across several pool-riffle complexes is desired. We confirmed that measurements of O_2 made in the thalweg tend to average metabolism due to constant mixing, which avoids small-scale variation and provides more accurate site-to-site comparisons. Our work leaves open the possibility that important heterogeneity is missed using the thalweg alone if sub-habitat is a characteristic of interest. For example, pool stratification could lead to changes in O_2 dynamics as the waters in the pool are mixed into surface waters when stratification breaks or as water exchange with backwaters varies at different flows. Future research could examine what ecological variables (e.g. light, velocity, geomorphology) are driving metabolic heterogeneity at various spatial scales to determine what information is retained, integrated and lost when up-scaling.

References

Atkinson B.L., Grace M.R., Hart B.T. & Vanderkruk K.E.N. (2008) Sediment instability affects the rate and location of primary production and respiration in a sand-bed stream. *Journal of the North American Benthological Society*, **27**, 581–592.

Battin T.J., Kaplan L.A., Findlay S., Hopkinson C.S., Marti E., Packman A.I. *et al.* (2008) Biophysical controls on organic carbon fluxes in fluvial networks. *Nature Geoscience*, **1**, 95–100.

Behn K.E., Kennedy T.A. & Hall R.O. (2010) *Basal Resources in Backwaters of the Colorado River below Glen Canyon Dam – Effects of Discharge Regimes and Comparison with Mainstem Depositional Environments*. U.S. Geological Survey Open-File Report 2010-1075. Available at: http://pubs.usgs.gov/of/2010/1075/.

Bernot M.J., Martin E.C. & Bernot R.J. (2010a) The influence of trophic complexity on preferential uptake of dissolved inorganic and organic nitrogen: a laboratory microcosm experiment. *Journal of the North American Benthological Society*, **29**, 1199–1211.

Bernot M.J., Sobota D.J., Hall R.O., Mulholland P.J., Dodds W.K., Webster J.R. *et al.* (2010b) Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology*, **55**, 1874–1890.

Bott T.L. (2006) Primary productivity and community respiration. In: *Methods in Stream Ecology*, 2nd edn (Eds F.R. Hauer & G.A. Lamberti), pp. 663–690. Academic Press, San Diego.

Canty A. & Ripley B. (2014) *boot: Bootstrap R (S-Plus) Functions*. R package version 1.3-11.

Cardinale B.J., Palmer M.A., Swan C.M., Brooks S. & Poff N.L. (2002) The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. *Ecology*, **83**, 412–422.

Coloso J.J., Cole J.J. & Pace M.L. (2011) Short-term variation in thermal stratification complicates estimation of lake metabolism. *Aquatic Sciences*, **73**, 305–315.

Demars B.O.L., Manson J.R., Olafsson J.S., Gislason G.M., Gudmundsdottir R., Woodward G. *et al.* (2011) Temperature and the metabolic balance of streams. *Freshwater Biology*, **56**, 1106–1121.

Demars B.O.L., Thompson J. & Manson J.R. (2015) Stream metabolism and the open diel oxygen method: principles, practice, and perspectives. *Limnology and Oceanography-Methods*, **13**, 356–374.

Dodds W.K. (1991) Micro-environmental characteristics of filamentous algal communities in flowing freshwaters. *Freshwater Biology*, **25**, 199–209.

Dodds W.K. (2007) Trophic state, eutrophication, and nutrient criteria in streams. *Trends in Ecology & Evolution*, **22**, 669–676.

Dodds W.K., Gido K., Whiles M.R., Fritz K.M. & Matthews W.J. (2004) Life on the edge: the ecology of Great Plains prairie streams. *BioScience*, **54**, 205.

Dodds W.K., Hutson R.E., Eichem A.C., Evans M.A., Gudder D.A., Fritz K.M. *et al.* (1996) The relationship of floods, drying, flow and light to primary production and producer biomass in a prairie stream. *Hydrobiologia*, **333**, 151–159.

Dodds W.K., Veach A.M., Ruffing C.M., Larson D.M., Fischer J.L. & Costigan K.H. (2013) Abiotic controls and temporal variability of river metabolism: multiyear analyses of Mississippi and Chattahoochee River data. *Freshwater Science*, **32**, 1073–1087.

Dodds W.K. & Whiles M.R. (2010) *Freshwater Ecology: Concepts and Environmental Applications of Limnology*, 2nd edn. Academic Press, Burlington.

Edler C. & Dodds W.K. (1996) The ecology of a subterranean isopod, *Caecidotea tridentata*. *Freshwater Biology*, **35**, 249–259.

Frissell C.A., Liss W.J., Warren C.E. & Hurley M.D. (1986) A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmental Management*, **10**, 199–214.

Gantzer C.J., Rittmann B.E. & Herricks E.E. (1991) Effect of long-term water velocity changes on streambed biofilm activity. *Water Research*, **25**, 15–20.

Gawne B., Merrick C., Williams D.G., Rees G., Oliver R., Bowen P.M. et al. (2007) Patterns of primary and heterotrophic productivity in an arid lowland river. *River Research and Applications*, **23**, 1070–1087.

González-Pinzón R., Haggerty R. & Argerich A. (2014) Quantifying spatial differences in metabolism in headwater streams. *Freshwater Science*, **33**, 798–811.

Grace M.R., Giling D.P., Hladyz S., Caron V., Thompson R.M. & Mac Nally R. (2015) Fast processing of diel oxygen curves: estimating stream metabolism with BASE (BAyesian Single-station Estimation). *Limnology and Oceanography: Methods*, **13**, 103–114.

Grace M.R. & Imberger S.J. (2006) *Stream Metabolism: Performing & Interpreting Measurements*. Water Studies Centre Monash University, Murray Darling Basin Commission and New South Wales Department of Environment and Climate Change. Available at http://www.sci.monash.edu.au/wsc/docs/tech-manual-v3.pdf.

Gulis V. & Suberkropp K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology*, **48**, 123–134.

Hall R.O. & Tank J.L. (2005) Correcting whole-stream estimates of metabolism for groundwater input. *Limnology and Oceanography: Methods*, **3**, 222–229.

Hawkins C.P., Kershner J.L., Bisson P.A., Bryant M.D., Decker L.M., Gregory S.V. et al. (1993) A hierarchical approach to classifying stream habitat features. *Fisheries*, **18**, 3–12.

Hill W.R. & Dimick S.M. (2002) Effects of riparian leaf dynamics on periphyton photosynthesis and light utilisation efficiency. *Freshwater Biology*, **47**, 1245–1256.

Hill W.R., Mulholland P.J. & Marzolf E.R. (2001) Stream ecosystem responses to forest leaf emergence in spring. *Ecology*, **82**, 2306–2319.

Hoellein T.J., Bruesewitz D.A. & Richardson D.C. (2013) Revisiting Odum (1956): a synthesis of aquatic ecosystem metabolism. *Limnology and Oceanography*, **58**, 2089–2100.

Hoellein T.J., Tank J.L., Rosi-Marshall E.J. & Entrekin S.A. (2009) Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale. *Journal of the North American Benthological Society*, **28**, 305–318.

Holtgrieve G.W., Schindler D.E., Branch T.A. & A'mar Z.T. (2010) Simultaneous quantification of aquatic ecosystem metabolism and reaeration using a Bayesian statistical model of oxygen dynamics. *Limnology and Oceanography*, **55**, 1047–1063.

Hondzo M., Voller V.R., Morris M., Foufoula-Georgiou E., Finlay J., Ganti V. et al. (2013) Estimating and scaling stream ecosystem metabolism along channels with heterogeneous substrate. *Ecohydrology*, **6**, 679–688.

Koopmans D.J. & Berg P. (2015) Stream oxygen flux and metabolism determined with the open water and aquatic eddy covariance techniques. *Limnology and Oceanography*, **60**, 1344–1355.

Levin S.A. (1992) The problem of pattern and scale in ecology. *Ecology*, **73**, 1943–1967.

Lupon A., Martí E., Sabater F. & Bernal S. (2016) Green light: gross primary production influences seasonal stream N export by controlling fine-scale N dynamics. *Ecology*, **97**, 133–144.

Martin E.C., Gido K.B., Bello N., Dodds W.K. & Veach A. (2016) Increasing fish taxonomic and functional richness affects ecosystem properties of small headwater prairie streams. *Freshwater Biology*, **61**, 887–898.

McGuire K.J., Torgersen C.E., Likens G.E., Buso D.C., Lowe W.H. & Bailey S.W. (2014) Network analysis reveals multiscale controls on streamwater chemistry. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 7030–7035.

Medeiros A.O., Pascoal C. & Graça M.A.S. (2009) Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biology*, **54**, 142–149.

Montgomery D.R. & Buffington J.M. (1997) Channel-reach morphology in mountain drainage basins. *Bulletin of the Geological Society of America*, **109**, 596–611.

Mulholland P.J., Fellows C.S., Tank J.L., Grimm N.B., Webster J.R., Hamilton S.K. et al. (2001) Inter-biome comparison of factors controlling stream metabolism. *Freshwater Biology*, **46**, 1503–1517.

Mulholland P.J., Hall R.O., Sobota D.J., Dodds W.K., Findlay S.E.G., Grimm N.B. et al. (2009) Nitrate removal in stream ecosystems measured by 15N addition experiments: denitrification. *Limnology and Oceanography*, **54**, 666–680.

Mulholland P.J., Marzolf E.R., Webster J.R., Hart D.R. & Hendricks S.P. (1997) Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. *Limnology and Oceanography*, **42**, 443–451.

Naegeli M.W. & Uehlinger U. (1997) Contribution of the hyporheic zone to ecosystem metabolism in a prealpine gravel-bed river. *Journal of the North American Benthological Society*, **16**, 794–804.

Odum H.T. (1956) Primary production in flowing waters. *Limnology and Oceanography*, **1**, 102–117.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/.

Reichert P., Uehlinger U. & Acuña V. (2009) Estimating stream metabolism from oxygen concentrations: effect of spatial heterogeneity. *Journal of Geophysical Research: Biogeosciences*, **114**, 1–15.

Riley A.J. & Dodds W.K. (2012) The expansion of woody riparian vegetation, and subsequent stream restoration, influences the metabolism of prairie streams. *Freshwater Biology*, **57**, 1138–1150.

Riley A.J. & Dodds W.K. (2013) Whole-stream metabolism: strategies for measuring and modeling diel trends of dissolved oxygen. *Freshwater Science*, **32**, 56–69.

Roberts B.J., Mulholland P.J. & Hill W.R. (2007) Multiple scales of temporal variability in ecosystem metabolism rates: results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems*, **10**, 588–606.

Rüegg J., Dodds W.K., Daniels M., Sheehan K., Baker C., Bowden W. *et al.* (2016) Baseflow physical characteristics differ at multiple spatial scales in stream networks across diverse biomes. *Landscape Ecology*, **31**, 119–136.

Song C., Dodds W.K., Trentman M.T., Rüegg J. & Ballantyne F. (2016) Methods of approximation influence aquatic ecosystem metabolism estimates. *Limnology and Oceanography: Methods*, **14**, 557–569.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.

Wilson K.C. & Dodds W.K. (2009) Centimeter-scale stream substratum heterogeneity and metabolic rates. *Hydrobiologia*, **623**, 53–62.

Young R.G. & Huryn A.D. (1999) Effects of land use on stream metabolism and organic matter turnover. *Ecological Applications*, **9**, 1359–1376.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of pools and differences in temperature between the surface and the bottom.

Table S2. The metabolic parameter estimates (GPP, gross primary production; CR, community respiration) using two modelling approaches: BASE (Grace *et al.*, 2015) and Dodds *et al.*, (2013) (=Dodds, 2013).

Data S1. Equations used in the Riley and Dodds (2013) modelling approach and the equation from Demars *et al.*, (2015) describing how to calculate the 50% 'footprint' of an O$_2$ probe.

(Manuscript accepted 13 December 2016)