
Whole-stream Metabolism - Field & Laboratory Analysis

Dr. Jennifer L Tank - Laboratory Standard Operating Procedure

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SUMMARY

The following protocol describes the methods for (1) collecting whole-stream metabolism data using equipment deployed in the field and (2) analyzing these data using the day-time regression model (Kosinski 1984) to estimate gross primary production (GPP), ecosystem respiration (ER), and reaeration (k). The model was developed by Drs. Mike Grace and Mike Harper at Monash University, Melbourne, Australia, and can be run using the Model Maker computer program (Cherwell Scientific, Ltd., Oxford, UK).

EQUIPMENT AND SUPPLIES

1 x Hydrolab mini-Sonde*	1 x 1-L Nalgene bottle
1 x Hydrolab Surveyor 4a*	8 x Duracell AA batteries
1 x Suveyor-Sonde connecting cable*	1 x PVC protective mini-Sonde case
1 x weight to cover & protect probe	1 x 2-ft metal cable & screw clamp
1 x Odyssey light meter*	2 x re-bar
1 x 5-gallon bucket	Electronic distance measurers (EDMs)
2 lbs NaCl (table salt)	1 x 50-m tape measure
1 x Marsh McBirney Flow-mate*	1 x wading rod
Computer with Model Maker software	

NOTES

* Several companies supply probes that may be programmed and deployed in the field to log the data necessary for calculating whole-stream metabolism. The suggested in-stream and light meters have been used by the Tank Laboratory, but may be substituted.

* In order to estimate GPP, ER, and k with Model Maker, you must record

- (1) dissolved oxygen (% saturation, but record mg L^{-1} , too)
- (2) temperature
- (3) light
- (4) average stream depth.

The first three data sets can be recorded using the deployed loggers. To estimate average stream depth, measure discharge (Q), average width (w), and average velocity (v). Then calculate average stream depth (d) as:

$$Q = w * d * v, \text{ therefore: } d = Q / w * v$$

To measure average velocity, conduct a slug release of salt. The time for the peak to pass a downstream conductivity meter a known distance from the salt release is the average velocity.

$$v = \text{distance} / \text{time}$$

To measure average velocity from a constant injection of salt or rhodamine, the time required for the stream to reach half the plateau conductivity is the time it takes the average solute to travel the length of the reach. Divide the reach length divided by the time to “½ height” to get the average velocity.

$$v = \text{distance} / \text{time}_{1/2 \text{ peak}}$$

FIELD METHODS

Deploying the Sonde

1. Choose a location to deploy the Sonde in the middle of the channel that has steady flow. **Do not** deploy the Sonde downstream of turbulent flow, which will be a section of high gas exchange with the atmosphere and will likely disrupt your data. **Do not** deploy the Sonde in a pool or very slow-moving water habitat.
2. Connect the Sonde to the Surveyor or a laptop to initiate probe calibration and create log files.
3. Equilibrate the Sonde with the temperature of the stream water. Allow the Sonde to be submersed for at least 5 minutes such that the probes and body of the Sonde are roughly the same temperature as the stream.
4. Calibrate the dissolved oxygen probe.
 - a. Fill the 1-L Nalgene bottle half-full with stream water. Shake the bottle consistently for 60 seconds to create 100% air-saturated water.
 - b. After 60 seconds, quickly dump the water in the calibration cup covering the probes out and pour the 100% air-saturated water into the calibration cup. Leave ~2 to 5 cm of space at the top and gently place the screw-top of the calibration cup back on top, but upside-down. The air space is important to ensure 100% air-saturated water and the cap prevents additional diffusion of oxygen into the atmosphere. If you screw the cap back on, additional pressure may disrupt the calibration process. Also, **do not** hold the Sonde by the calibration cup or allow direct sunlight into the calibration cup. Both these actions may warm the water during the calibration cup and result in a poor calibration.
 - c. Wait 3 minutes. To calibrate a Hydrolab mini-Sonde, choose the following options:
CALIBRATE → SONDE → LDO% → Enter 100.0 → CALIBRATE → Enter B. Pressure → Enter
 - d. A “Calibration Successful” message should appear immediately. Scroll back to the Main Menu. Repeat steps (b) and (c) at least twice. Continue repeating these processes until the LDO% reads 100.0 ± 0.2 after 3 minutes without entering the new calibration.

5. Program the Sonde to record (1) time and date, (2) dissolved oxygen as mg L^{-1} and percent saturation, (3) temperature as $^{\circ}\text{C}$, and (4) conductivity every **10 minutes** for at **least 36 hours**. The logging interval and length of deployment may vary. You can program the logger to record every 30 minutes and will not need to change the batteries as frequently.
 - a. First, check that the sonde as 3 or fewer files stored on the memory: FILES → SONDE → STATUS. The Sonde can store a maximum of 5 files, however, it is best to only have 3 or fewer when recording more data. **Before deleting any files, ensure they are downloaded and backed up.** Once the files are deleted from the Sonde, they can not be retrieved!
 - b. To program a new file, FILES → SONDE → CREATE → TIME-TRIG
 - c. Enter a unique log file name (typically SITE.DATE.SONDE).
 - d. Enter a START and STOP time (format: MMDDYYYYHHMMSS).
 - e. Set LOGGING INTERVAL to 10 minutes (format: HHMMSS).
 - f. Set SENSOR and CIRCLTR (circulator) warm-up to 30 seconds (format: HHMMSS).
 - g. Set AUDIO to on or off.
 - h. Choose to record at least the following parameters: (1) Date/Time, (2) Temp(C), (3) Cond (us/s), (4) LDO (% sat), and (5) LDO (mg/L).
6. Detach the Sonde and Surveyor. Place the Sonde in the protective PVC tubing, if desired, and deploy the instrument in the stream. We recommend securing the Sonde to the re-bar with a cable and screw clamp to assure the Sonde is not lost, should discharge increase during the deployment.

Additional Field Measurements

1. Measure the wetted width of the stream at 20 transects to estimate the average stream width.
2. Measure stream discharge.
3. Measure the average velocity of the stream using a slug of a conservative solute (e.g., salt, rhodamine-wt dye). If using the same Sonde to measure the change in conductivity or rhodamine-wt concentration, conduct the slug release prior to deployment or when you return to retrieve the Sonde. Release the slug and known distance upstream of the Sonde and record the time from the release to the peak of the slug at the downstream location.

MODELING METABOLISM DATA

Running the Model

1. Arrange your data in Microsoft Excel to include a column of time (seconds; 86400 for a 24-hr period), dissolved oxygen (percent saturation), light data (units do not matter; the model uses change in light over time), and temperature (degrees Celsius). In addition, the model will require the average temperature and initial DO.
2. The minimum amount of data required for the model is 24 hours, which will provide a measure of daily metabolism. Model Maker allows longer time periods up to multiple days, but will only provide one integrative measure of metabolism over the entire period. We recommend running multiple days only when discharge and light conditions are relatively stable (e.g., do not run consecutive days if one is overcast and the other full sun).
3. Open an existing Model Maker file to input the new data. Immediately **save** the re-written file with a new name so to not save over a previously run file.

- a. On the **Main** tab, double click on **Av_temp** and enter the average temperature for your run where it reads “Equation: =”. Then double click **Cinit** and enter the initial dissolved oxygen percent saturation.
 - b. On the **Page 1** tab under **Model Data**, enter the time step (in seconds) and dissolved oxygen (in % saturation). If the previous file had a longer period for integration, be sure to delete the additional lines from the bottom of the columns.
 - c. On the **Page 1** tab under **Lookup Tables, Temp & I data**, enter the time step, light data, and temperature data.
 - d. On the **Parameters** tab, enter an initial set of parameters by double-clicking each parameter letter and entering a value into the box. An initial parameter set may be:
 - A (scaling constant) = 0.001 (constraint range: 0 to 1)
 - K1 (reaeration) = 0.001 (range: 0 to 0.005)
 - p (light saturation coefficient) = 0.2 (range: 0 to 1)
 - R (respiration coefficient) = 0.0005 (range: 0 to 0.005)
 - e. If necessary, the constraint ranges may be adjusted at the **Constraints** tab in the **Parameter Definition** box, which can be opened by double clicking the specific parameter while under at the **Parameters** page.
4. To run the model, choose **Model → Optimize**. Press **OK** and the model will begin running. A timer on the lower right will indicate the elapsed time between initiating the run. The optimization may require anywhere from 10 seconds to 1 hour. If the model runs longer than 1 hour, we would recommend stopping the run and changing the initial parameters.
 5. The model will end with one of several messages. If the model indicates “the model run was completed successfully” or “the machine accuracy has been reached”, then the model was successful and you can continue to Step 5. If the model indicates “R violated its constraint range” or “a single curvature matrix was encountered”, you will need to return to Step 2d and choose different initial parameters. Only change one at a time by an order of magnitude until the model runs successfully.
 6. After the successful model run, update the new parameters. On the **Parameter Results** tab, you will see the new estimated parameters. Right click the letter (e.g., A, K1, p, & R) and choose **Update Parameter**. This action will replace the initial value in the model under the **Parameters** tab with the new estimated parameter. Do this for all 4 parameters. Also, copy these 4 parameters into your Excel file for later calculations.
 7. Integrate the model with the new parameters by choosing **Model → Integrate** and enter the appropriate length of time (in seconds) that you will be integrating over.
 8. Once the model has been integrated, you can view how the model fits to your data under the **DO percent** tab or check the model fit as the r^2 value under the **Optimization Statistics** tab. Record the r^2 value into an Excel file to compare this parameter set against later runs.
 9. Copy the data in the **photosynth** column under **Photosynthesis rate** into your Excel file. The sum of these values is the measure of GPP in $\text{mg O}_2 \text{ L}^{-1} \text{ day}^{-1}$.
 10. **Repeat Steps 2d to 8 at least 5 additional times**, manipulating the parameters one-at-a-time or together. Choose extreme, but realistic values (i.e., change R an order of magnitude or more above and below the first estimate). Continue to record the new parameter value you are entering prior to optimization and the (1) new parameters, (2) r^2 value, and (3) photosynthesis rate produced by the model.

11. Choose the data with the highest r^2 value from your multiple runs. If the r^2 or parameter values were changing significantly from one iteration to the next, you may want to run additional iterations. Also, **always** check that the data are **realistic**. At times, the model parameters from a lower r^2 value may provide more realistic estimates of GPP, ER, and k .

Calculating Metabolism Parameters

1. The GPP value, as $\text{mg O}_2 \text{ L}^{-1} \text{ day}^{-1}$, is the sum of the **photosynth** column multiplied by the scaled-time step (86.4), as noted in Step 8 above.
2. To calculate ER values expressed as $\text{mg O}_2 \text{ L}^{-1} \text{ day}^{-1}$ from $\text{mg O}_2 \text{ L}^{-1} \text{ s}^{-1}$ multiply the R parameter value by the time step (in seconds). For example, ER for a 24-hour data set is: $\text{ER} = \text{R} * 86400$
3. To calculate k as day^{-1} from s^{-1} , multiply the K1 value by the time step (in seconds): $k = \text{K1} * 86400$
4. To calculate GPP or ER values as $\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ from $\text{mg O}_2 \text{ L}^{-1} \text{ day}^{-1}$, multiply the values from Steps 1 and 2 by the average stream depth in meters.

Trouble-shooting

Important Notes

- * Generally good agreement between measured and modeled k values, when k is low (i.e., ~10 to 30 day^{-1}), but model does not predict k well when the value is high (i.e., ~100+ day^{-1}).
- * The model can be run for multiple days at one time. The values you will obtain will be integrated over that entire time period. If you will be running data for a period > 24 hours, we suggest also running daily measurements to compare the parameter estimates.
- * No metabolism model works well with rapidly changing discharge. Therefore, some data may not be able to be integrated.
- * Each parameter has its own error, which you can record if you choose. The A and p parameters should have similar error because they are correlated. Also, if the p value has a small value for error, photo-inhibition may be occurring, but this is only likely for streams with little to no canopy cover.
- * The R parameter has a maximum constraint of 0.005, which can be adjusted if your stream may have high ER. The p parameter has a constraint between 0 and 1, but cannot be changed because it is an exponent in the model.
- * To keep any of the parameters constant during the optimization, uncheck the **Optimize** box under the **Parameters** tab. For instance, if you measured k using a SF_6 or propane release, you can enter a known value and instruct the model to estimate the other parameters.

APPENDIX

Typical values for GPP, ER, and k for different lotic ecosystems (compiled by Dr. Mike Grace)

Stream Type	Typical Discharge (ML d ⁻¹)	Typical Range for K _{O2} (d ⁻¹)	Typical Range for GPP (mg O ₂ L ⁻¹ d ⁻¹)	Higher GPP values (mg O ₂ L ⁻¹ d ⁻¹)	Typical Range for ER (mg O ₂ L ⁻¹ d ⁻¹)	Higher ER values (mg O ₂ L ⁻¹ d ⁻¹)
Rivers	20 – 2000	0.2 – 20	0.1 – 20	Up to 80	-1 to -20	Up to - 40
Creeks	0.1 – 40	5 – 250	0.2 – 50	Up to 280	-8 to -100	Up to - 200

If data analysis produces parameters outside the typical ranges (and especially if the “higher limits” are exceeded), then the following points need to be considered to determine whether these parameters are correct or an artifact arising from problems with the data analysis:

1. K_{O2} - if the stream is large and slow flowing (especially if also deep), then low values of K_{O2} are expected. Values higher than 20 d⁻¹ would only occur if there water is very turbulent (lots of air entrainment)
2. K_{O2} - values < 5 d⁻¹ in small streams would be highly unusual unless stream flow was slow and nearly laminar with no visible turbulence, riffle zones, cascades etc.
3. K_{O2} - values > 100 d⁻¹ have frequently been reported in small, turbulent streams. The issue in these circumstances is the impact that any uncertainty in K_{O2} will have on the estimates of GPP and CR.
4. GPP - values are usually < 15-20 mg O₂ L⁻¹ d⁻¹ in larger streams and < 50 mg O₂ L⁻¹ d⁻¹ in smaller streams. Values larger than this should be treated with suspicion unless there is significant visible evidence of primary producers in the study reach (e.g. phytoplankton, benthic algae, significant stands of macrophytes – submerged or emergent). It is strongly recommended that when very high GPP values are expected or recorded in the absence of macrophytes, then water samples (and if appropriate, sediment samples) be collected for chlorophyll-a analysis. Conversely, if large amounts of primary producers are observed on days with clear skies and GPP is < 2 mg O₂ L⁻¹ d⁻¹, then doubt is cast on this GPP value. Further investigation would be required as other factors might be responsible for the low GPP.
5. ER - values are generally confined within a relatively narrow range of -1 to -40 mg O₂ L⁻¹ d⁻¹. Although GPP can often be negligible due to severe nutrient and/or light limitation, ER values smaller than -1 mg O₂ L⁻¹ d⁻¹ are considered unlikely and would be an immediate cause for investigating the data analysis. Values in excess of - 50 mg O₂ L⁻¹ d⁻¹ have been recorded but tend to be limited to streams with highly visible large stores of organic matter (e.g. decaying vegetation, large standing stocks of benthic algae). Again a visual check of the study reach (which should be done anyway!) will determine whether a ‘high’ ER value is sensible. If a high ER value is obtained without any large and obvious source of organic matter, then two aspects should be investigated before discarding the data (but after checking the data analysis has been performed correctly): a) is there a large source of dissolved organic carbon in the stream (perhaps from a pollution event or continuous discharge of effluent); or b) there is significant hyporheic flow in the study reach with a large rate of hyporheic respiration. This possibility should be checked by salt addition. As noted in that section, if groundwater inflow accounts for more than 5%, then the study reach may be considered unsuitable as a monitoring site.