Region 4
U.S. Environmental Protection Agency
Science and Ecosystem Support Division
Athens, Georgia

OPERATING PROCEDURE

Title: **Water Column Oxygen Metabolism**

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Revision History

The top row of this table shows the most recent changes to this controlled document. For previous revision history information, archived versions of this document are maintained by the SESD Document Control Coordinator on the SESD local area network (LAN).

History	Effective Date
SESDPROC-504-R3, *Water Column Oxygen Metabolism*, replaces SESDPROC-504-R2. **General:** Corrected any typographical, grammatical, and/or editorial errors. **Cover Page:** Changed the Ecological Assessment Branch Chief from Bill Cosgrove to John Deatrick. Changed the FQM from Liza Montalvo to Bobby Lewis. **Revision History:** Changes were made to reflect the current practice of only including the most recent changes in the revision history. **Section 1.2:** Added the following statement – "Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use."	December 4, 2013
SESDPROC-504-R2, *Water Column Oxygen Metabolism*, replaces SESDPROC-504-R1.	February 12, 2010
SESDPROC-504-R1, Water Column Oxygen Metabolism, replaces SESDPROC-504-R0.	November 1, 2007
SESDPROC-504-R0, Water Column Oxygen Metabolism, Original Issue	February 05, 2007

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1 General Information

1.1 Purpose

The purpose of this procedure is to assess the gross primary production (GPP), net primary production (NPP), and respiration (R) associated with phytoplankton communities in the water column.

1.2 Scope/Application

This document describes both general and specific methods to be used by field investigators when determining the water column oxygen metabolism in the field. On the occasion that Science and Ecosystem Division (SESD) field investigators determine any of the procedures described in this section to be inappropriate, inadequate, or impractical and another procedure to be appropriate for assessing the water column oxygen metabolism, the variant procedure will be documented in the field log book (in accordance with SESD Operating Procedure for Logbooks (SESDPROC-010)), along with a description of the circumstances requiring its use. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

1.3 Documentation/Verification

This procedure was prepared by persons deemed technically competent by SESD management, based on their knowledge, skills and abilities and has been tested in practice and reviewed in print by a subject matter expert. The official copy of this procedure resides on the SESD Local Area Network (LAN). The Document control Coordinator (DCC) is responsible for ensuring the most recent version of the procedure is placed on the LAN and for maintaining records of review conducted prior to its issuance.

1.4 References

American Public Health Association (APHA), American Waterworks Association (AWWA), and the Water Environment Federation (WEF). 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. Washington, D.C.

Belfort Instrument Company. 1981. Instruction Book for Pyranograph, Cat. No. 5-3850. Book No. 11900. Baltimore, Maryland.

LI-COR, Inc. 1990. LI-COR Radiation Sensors: Instruction Manual, Underwater Type SA: LI-192SA Underwater Quantum Sensor, LI-193SA Spherical Quantum Sensor. Publication number 8609-57. Lincoln, Nebraska. (Original published 1986).

LI-COR, Inc. 1991. LI-COR Radiation Sensors: Instruction Manual, Terrestrial Type SA: LI-190SA Quantum Sensor, LI-200SA Pyranometer Sensor, LI-210SA Photometric Sensor, LI-191SA Line Quantum Sensor. Publication number 8609-56. (Original published 1986).
LI-COR, Incorporated. 1998. Model LI-1400 Datalogger Instruction Manual. Publication number 984-06626. Lincoln, Nebraska.

SESD Operating Procedure for Logbooks, SESDPROC-010, Most Recent Version.

SESD Operating Procedure for Field Sampling Quality Control, SESDPROC-011, Most Recent Version.

SESD Operating Procedure for Measurement of Dissolved Oxygen, SESDPROC-106, Most Recent Version.

SESD Operating Procedure for Equipment Inventory and Management, SESDPROC-108, Most Recent Version.

United States Environmental Protection Agency (USEPA). 2001. Region 4 Ecological Assessment Branch Standard Operating Procedures and Quality Assurance Manual Science and Ecosystem Support Division, Region 4, Athens, Georgia.

USEPA. 2007. Safety, Health and Environmental Management Program Procedures and Policy Manual. Science and Ecosystem Support Division, Region 4, Athens, Georgia.

1.5 General Precautions

1.5.1 Safety

Proper safety precautions must be observed when measuring water column oxygen metabolism. Refer to the SESD Safety, Health and Environmental Management Program Procedures and Policy Manual (USEPA 2007) and any pertinent site-specific Health and Safety Plans (HASP) for guidelines on safety precautions. These guidelines, however, should only be used to complement the judgment of an experienced professional. When using this procedure, one may be exposed to toxic chemicals. If so, minimize exposure to potential health hazards through the use of protective clothing, eye wear and gloves. Address chemicals that pose specific toxicity or safety concerns and follow any other relevant requirements, as appropriate.

1.5.2 Procedural Precautions

Documentation of field sampling is done in a bound logbook. All documentation in the field logbook will follow SESD Operating Procedure for Logbooks (SESDPROC-010).

2 Sampling Procedure and Considerations

2.1 Sampling Procedure

1. Deploy a device to measure solar radiation in a location representative of the study area.

2. Once on station, obtain the percent light transmission profiles through the water column, recording the depth at which the measurement is made and the corresponding light levels of both the deck and sea cells. Utilize appropriate increments of depth based upon professional judgment. For example, half- foot increments may be used until the photic zone has been identified. Once the photic zone has been identified, use of whole-foot increments of depth may be appropriate.

3. Select bottle deployment depths based upon light transmission percentages that will permit integration of the water column/euphotic zone. As an example, in very clear waters, bottles would be deployed at percent transmission values such as 90, 50, 10, and 1% (Figure 1). Such excellent percent transmission values are rarely encountered so onsite selection of appropriate depths based on percentages that fully integrate the water column must be made using professional judgment.

   At each selected depth, fill bottles for initial dissolved oxygen (DO) determination, and bottles for light and dark deployment. [Note: If the Winkler titration technique will not be used for DO determination, initial DO measurements may be made *in situ* at each depth if appropriate for the method being used.] All measurements of DO should be conducted in accordance with SESD Operating Procedure for Measurement of Dissolved Oxygen (SESDPROC-106).

4. If the total depth at the station is greater than the depth of the photic zone (defined as the zone with 1% or greater ambient light), then additional dark bottles should be filled within one foot of the bottom. In this case, light bottles do not need to be deployed on the bottom. Refer to Figure 1 for a schematic of the bottle deployment.

5. Deploy light and dark bottles at the depths from which they were filled, recording the time at which deployment was initiated. This start time should take into account time at which bottles were filled. Allow deployed bottles to incubate at depth for approximately one to four hours, depending on location conditions.

6. After incubation is complete, retrieve bottles and immediately measure the oxygen concentration of each bottle following the SESD Operating Procedure for Dissolved Oxygen Measurement (SESDPROC-106). [Note: If measuring the oxygen concentration by the Winkler technique, “fix” the initial bottles with the manganous sulfate and alkali iodide azide solutions (Winkler reagents “1” and “2”). Set the bottles aside for processing later via the Winkler titration method. The bottles should

be placed in the dark to prevent photodegradation of the iodine which results in erroneous DO measurement. Once fixed, the bottles can sit for several hours before addition of sulfuric acid (Winkler reagent "3") and titration. Add the sulfuric acid, "reagent 3," immediately before beginning titration].

2.2 Suggested Equipment

The following table is a suggested equipment list. Refer to the manufacturers' instructions for operation and maintenance of the pyrheliometers, light meters, and photometers. Refer to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al*. 1998) for procedural information related to the azide modification of the Winkler titration method. Modifications or revisions may be necessary and are appropriate based upon the judgment of the investigator:

Table 1: Suggested Equipment

Item	Use/Notes
Pyranograph (pyrheliometer or pyranometer)	To record solar energy during incubation period and over the course of the study day.
Light meter/photometer	To determine the percent transmission of light through the water column.
Winkler titration kits / instrument capable of measuring oxygen in BOD bottles	To determine the dissolved oxygen concentration of samples contained in BOD bottles via the Winkler titration method.
25-ml burette precise to 0.05 mg/l	For titrating the dark bottles under supersaturated conditions in order to better estimate small changes in dissolved oxygen.
Floats with chains graduated at 0.5 ft. intervals	For bottle deployment.
Light and dark BOD* bottles	Should be equipped to be deployed in pairs on the floatation device if employing the Winkler titration method for oxygen measurement.
Plastic caps for light and dark bottles	To ensure that the stoppers will stay in the bottles. Black plastic caps for dark bottles keep the light from penetrating the bottles and triggering photosynthesis. Aluminum foil may be substituted for the black plastic caps.
4-liter horizontal water sampler / submersible 12-volt pump (include 12-volt battery and hose marked at 1-ft. intervals)	For filling bottles.
Rubber bands	For securing paired bottles together to prevent breakage during rough sea conditions that may be encountered during incubations periods.
Cooler filled with ice	For chilling bottles in the case of supersaturation.
Weights	Large (to anchor the entire bottle apparatus) and small (to ensure that the pump and deployed bottle chains remain vertical).

* Notes: BOD = biological oxygen demand; ml = milliliter; mg/l = milligrams per liter; ft. = foot.

2.3 Special Considerations

The following considerations may not be necessary in all cases, but may increase the accuracy of water column metabolism measurements made with the light bottle/dark bottle method under certain circumstances.

2.3.1 Pyrheliometer/Pyranometer Deployment

The pyrheliometers/pyranometers do not need to be deployed at each station. A central location that is easily accessible over the course of the study is ideal. Do not deploy the pyrheliometers/pyranometers in a location such that they will be shaded during the day or lit at night. Open fields or high locations (such as on a roof or post) are often appropriate.

2.3.2 Winkler Reagents

It is recommended that sufficient reagents be available for assessing one and one half the actual number of stations expected, allowing for extreme oxygen concentrations due to the supersaturation of dissolved oxygen in the light bottles.

If supersaturated conditions are anticipated, it is recommended that approximately 20 (milliliters) ml of sodium thiosulfate be estimated per bottle.

2.3.3 Pump Operation and Bottle Filling

If in a fast-flowing waterbody, it may be necessary to attach additional weight to the pump in order for the pump to pull from the correct depth. Once the pump is positioned at each appropriate depth, allow enough time for water from that depth to clear the pump. Pinch the hose in order to stop the flow and insert it into the bottom of the bottle. In order to avoid adding additional dissolved oxygen to the sample, continue to pinch the hose, but lessen the pressure to allow enough flow to escape to slowly fill the bottles. Allow each bottle to overflow to twice its volume. Minimize each bottle's exposure to light and examine each bottle prior to deployment to ensure that no air bubbles are present within. These precautions will help prevent erroneous changes in DO. Filling all of the bottles should usually take no more than 15 to 20 minutes.

2.3.4 Deployment

Light and dark bottle measurements should occur generally during the middle of the day when solar intensity is usually greatest. The duration of the incubation periods should be adjusted based on the productivity of the waterbody. In extremely productive systems, the incubation period may be only one to two hours. Ideal incubation times are usually between 0900-1500 hours. The location

selected for the deployment of the bottles should be mid-channel in an area representative of the station. The deployment location should not become shaded as the angle of the sun's incidence changes during the incubation period.

It may be necessary to attach additional weight to keep the deployed bottle chains vertical in lotic environments.

Additional light and dark bottles may be deployed to monitor the progress of DO production and respiration. These additional bottles may be retrieved prior to the scheduled retrieval time to evaluate the progress of production or respiration. Based on the DO concentrations in the extra bottles, retrieval times of the remaining bottles may be adjusted using professional judgement.

2.3.5 Re-deployment and Other Dark Bottle Considerations

If no discernable difference in dissolved oxygen concentration is observed in the dark bottles, it may be necessary to re-deploy the bottles. If appropriate, pull the light bottles in order to avoid super-saturation. Be sure to note the separate incubation times for each set of bottles in the field book. Additionally, if using the Winkler method for oxygen concentration determination, a finer scale burette may be used to more precisely measure a small change in oxygen.

2.3.6 Supersaturation

If air bubbles are observed in the light bottles following deployment, supersaturated conditions may exist. Prior to beginning to measure oxygen concentration, place the bottles on ice in order to lower the temperature and allow the oxygen to go back into solution. The cool temperatures and darkness of the cooler should simultaneously stop or at least lower the metabolic activity within the bottles as to minimally affect the final dissolved oxygen concentration.

3 General Considerations

3.1 Records

Information generated or obtained by SESD field investigators will be organized and accounted for in accordance with the SESD Operating Procedure for Control of Records (SESDPROC-002) . Field notes will be recorded in a bound field notebook. Figure 2 shows a suggested format for field notes for water column metabolism. All measurements shall be traceable to the field investigator(s) making the measurements and the equipment utilized.

3.2 Oxygen Measurement Methods

Measurements of oxygen may be conducted using methods other than the Winkler technique, if determined to be appropriate.

Figure 1: *In Situ* Bottle Schematic

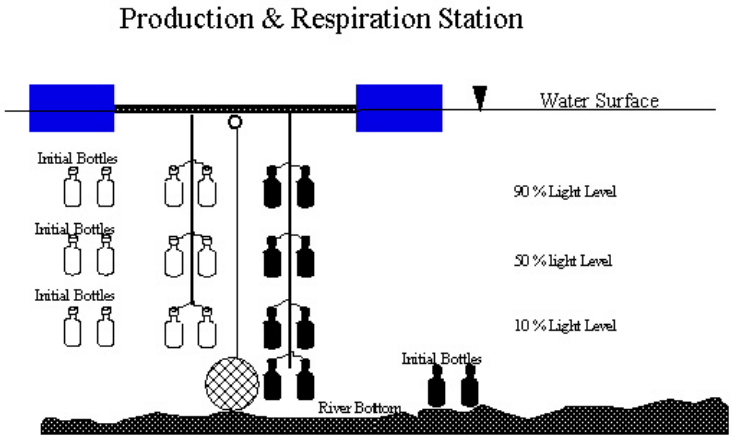

Figure 2: Suggested Logbook Format

Project Name **EPA Project ID** **Page 1 of X**

Light Profile Time: _____

Light Sensor ID:_____ Light Meter ID: _____

Depth (Feet)	Deck Cell (mmol s-1 m^{-2}) ($watts/m^2$)	Sea Cell (mmol s-1 m^{-2}) ($watts/m^2$)	% Light Transmission	Bottle Deployment Depth
Deck				
Surface				
Subsurface (~0.1)				
0.5				
1				
2				
3				
4				
5				

Bottle filling method: _____

P&R Bottle Deployment Time_____ Retrieval Time _____

		Initial			Light Bottles			Dark Bottles		
Depth (feet)	% light Trans-mission	Time (Meter)	Bottle #	DO (mg/L)	Time (Meter)	Bottle #	DO (mg/L)	Time (Meter)	Bottle #	DO (mg/L)

LDO Probe ID: _____ LDO Meter ID:_____

DO analyst: _____

Notes:_____