This document is Chapter 9 of the Volunteer Estuary Monitoring Manual, A Methods Manual, Second Edition, EPA-842-B-06-003. The full document be downloaded from: http://www.epa.gov/owow/estuaries/monitor/

Voluntary Estuary Monitoring Manual

Chapter 9: Dissolved Oxygen and Biochemical Oxygen Demand

March 2006

Chapter 9

Oxygen



Dissolved oxygen concentrations indicate how well aerated the water is, and vary according to a number of factors, including season, time of day, temperature, and salinity. Biochemical oxygen demand measures the amount of oxygen consumed in the water by chemical and biological processes.

Photos (l to r) K. Register, R. Ohrel, R. Ohrel

Overview

Nearly all aquatic life needs oxygen to survive. Because of its importance to estuarine ecosystems, oxygen is commonly measured by volunteer monitoring programs. When monitoring oxygen, volunteers usually measure dissolved oxygen and biochemical oxygen demand.

Dissolved oxygen concentrations indicate how well aerated the water is, and vary according to a number of factors, including season, time of day, temperature, and salinity. Biochemical oxygen demand measures the amount of oxygen consumed in the water by chemical and biological processes.

This chapter discusses the role of dissolved oxygen and biochemical oxygen demand in the estuarine environment. It provides steps for measuring these water quality variables. Finally, a case study is provided.

Why Monitor Oxygen?

Of all the parameters that characterize an estuary, the level of oxygen in the water is one of the best indicators of the estuary's health. An estuary with little or no oxygen cannot support healthy levels of animal or plant life.

Unlike many of the problems plaguing estuaries, the consequences of a rapid decline in oxygen set in quickly and animals must move to areas with higher levels of oxygen or perish. This immediate impact makes measuring the level of oxygen an important means of assessing water quality. ■

DISSOLVED OXYGEN (DO)

Oxygen enters estuarine waters from the atmosphere and through aquatic plant photosynthesis. Currents and wind-generated waves boost the amount of oxygen in the water by putting more water in contact with the atmosphere.

Dissolved Oxygen in the Estuarine Ecosystem

DO is one of the most important factors controlling the presence or absence of estuarine species. It is crucial for most animals and plants except for a small minority that can survive under conditions with little or no oxygen. Animals and plants require oxygen for respiration—a process critical for basic metabolic processes.

In addition to its use in respiration, oxygen is needed to aid in decomposition. An integral part of an estuary's ecological cycle is the breakdown of organic matter. Like animal and plant respiration, this process consumes oxygen. Decomposition of large quantities of organic matter by bacteria can severely deplete the water of oxygen and make it uninhabitable for many species.

An overload of nutrients from wastewater treatment plants or runoff from various land uses also adds to the problem. Nutrients fuel the overgrowth of phytoplankton, known as a bloom. The phytoplankton ultimately die, fall to the bottom, decompose, and use up oxygen in the deep waters of the estuary. Although nutrients from human activities are a major cause of depleted oxygen, low oxygen conditions may also naturally occur in estuaries relatively unaffected by humans. Generally, however, the severity of low DO and the length of time that low oxygen conditions persist in these areas are less extreme.

DO and nutrients can be connected in another way. When oxygen is low, nutrients bound to bottom sediments can be released into the water column, thereby permitting more plankton growth and eventually more oxygen depletion. Other pollutants may also be released from sediments under low oxygen conditions, potentially causing problems for the estuarine ecosystem.

Oxygen availability to aquatic organisms is complicated by the fact that its solubility in water is generally poor. Salt water absorbs even less oxygen than fresh water (e.g., seawater at 10°C can hold a maximum dissolved oxygen concentration of 9.0 mg/l, while fresh water at the same temperature can hold 11.3 mg/l). Warm water also holds less oxygen than cold water (e.g., seawater can hold a dissolved oxygen concentration of 9.0 mg/l at 10°C, but that concentration drops to 7.3 mg/l when the temperature increases to 20°C). Therefore, warm estuarine water can contain very little dissolved oxygen, and this can have severe consequences for aquatic organisms.

Levels of Dissolved Oxygen

Although we may think of water as homogeneous and unchanging, its chemical constitution

does, in fact, vary over time. Oxygen levels, in particular, may change sharply in a matter of hours. DO concentrations are affected by physical, chemical, and biological factors (Figure 9-1), making it difficult to assess the significance of any single DO value.

At the surface of an estuary, the water at midday is often close to oxygen saturation due both to mixing with air and the production of oxygen by plant photosynthesis (an activity driven by sunlight). As night falls, photosynthesis ceases and plants consume available oxygen, forcing DO levels at the surface to decline. Cloudy weather may also cause surface water DO levels to drop since reduced sunlight slows photosynthesis.

DO levels in an estuary can fluctuate greatly with depth, especially during certain times of the year. Temperature differences between the surface and deeper parts of the estuary may be quite distinct during the warmer months. Vertical **stratification** in estuarine waters (warmer, fresher water over colder, saltier water) during the late spring to summer period is quite effective in blocking the transfer of oxygen between the upper and lower layers (see Figure 9-1). In a well-stratified estuary, very little oxygen may reach lower depths and the deep water may remain at a fairly constant low level of DO. Changing seasons or storms, however, can cause the stratification to disintegrate, allowing oxygen-rich surface water to mix with the oxygen-poor deep water. This period of mixing is known as an **overturn**.

When DO declines below threshold levels, which vary depending upon the species, mobile animals must move to waters with higher DO; immobile species often perish. Most animals and plants can grow and reproduce unimpaired when DO levels exceed 5 mg/l. When levels drop to 3-5 mg/l, however, living organisms often become stressed. If levels fall below 3 mg/l, a condition known as **hypoxia**, many species will move elsewhere and immobile species may die. A second condition, known as **anoxia**, occurs when the water becomes totally depleted of oxygen (below 0.5 mg/l) and results in the death of any organism that requires oxygen for survival. Figure 9-2 summarizes DO thresholds in estuarine waters. ■

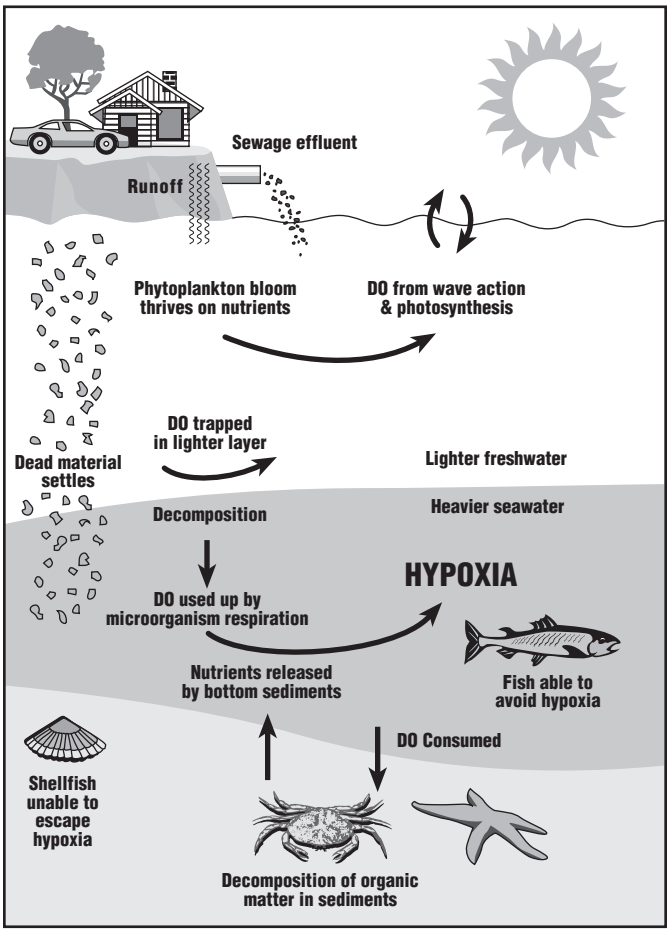

Figure 9-1. Physical, chemical, and biological processes that affect dissolved oxygen concentrations in estuaries. *(Redrawn from USEPA, 1998.)*

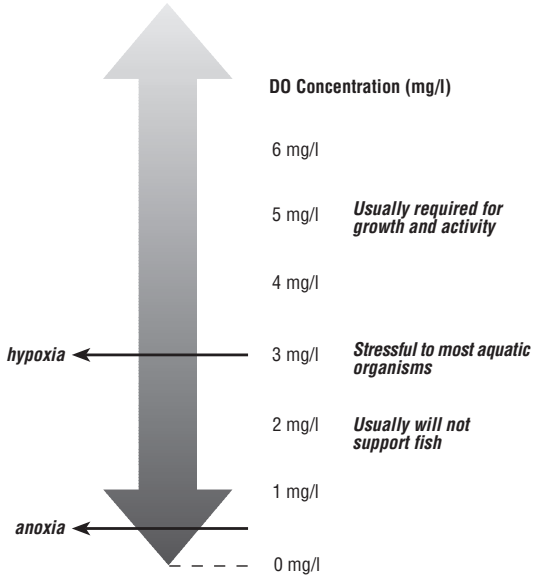

Figure 9-2. Dissolved oxygen in the water. A minimum DO concentration of 5 mg/l is usually necessary to fully support aquatic life.

Sampling Considerations

Chapter 6 summarized several factors that should be considered when determining monitoring sites, where to monitor in the water column, and when to monitor. In addition to the considerations in Chapter 6, a few additional ones specific to oxygen monitoring are presented here.

When to Sample

In estuarine systems, sampling for DO throughout the year is preferable to establish a clear picture of water quality. If year-round sampling is not possible, taking samples from the beginning of spring well into autumn will provide a program with the most significant data. Warm weather conditions bring on hypoxia and anoxia, which pose serious problems for the estuary's plants and animals. Because these conditions are rare during winter, cold weather data can serve as a baseline of information.

Sampling once a week is generally sufficient to capture the variability of DO in the estuary. Since DO may fluctuate throughout the day, **volunteers should sample at about the same time of day** each week. This way, they are less likely to record data that largely capture daily fluctuations. Some programs suggest that volunteers sample in the morning near dawn as well as mid-afternoon to capture the daily high and low DO values.

In some areas, especially large tidal swings can work to weaken the stratification in the estuary. Tidal effects, then, could be a consideration when collecting and analyzing DO data.

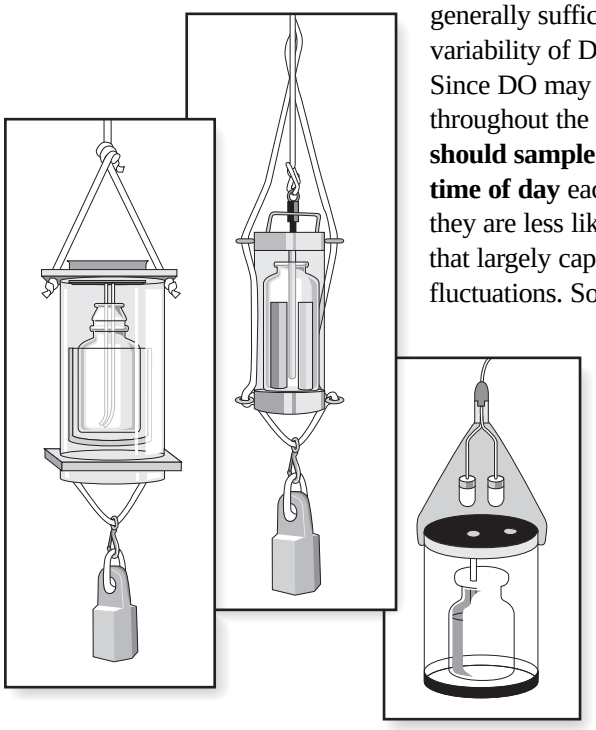

Figure 9-3. Dissolved oxygen samplers. Many of these instruments may also be used to collect samples for the analysis of other water quality variables.

Where to Sample

As mentioned previously, estuary stratification can have an impact on DO levels at different depths. Stratification is especially evident during the summer months, when warm fresh water overlies colder, saltier water. Very little mixing occurs between the layers, forming a boundary to mixing.

Because DO levels vary with depth—especially during the summer—volunteer groups may wish to collect samples at different depths. Van Dorn and Kemmerer samplers (see Chapter 7) are commonly used to collect these kinds of samples. In addition, there are several water samplers designed primarily for collecting DO samples at different depths (Figure 9-3). Appendix C provides a list of equipment suppliers.

Choosing a Sampling Method

Citizen programs may elect to use either a DO electronic meter or one of the several available DO test kits (Table 9-1). If the volunteer group wants its data to be used by state or federal agencies, it is wise to confer with the appropriate agency beforehand to determine an acceptable monitoring method.

Meters

The electronic meter measures DO based on the rate of molecular oxygen diffusion across a membrane. The results from a DO meter are extremely accurate, providing the unit is well-maintained, calibrated, and the membrane is handled in accordance with the manufacturer's instructions before each use. To properly calibrate some DO meters, knowledge of the sampling site's salinity is necessary.

The DO probe may be placed directly into

Table 9-1. Summary of dissolved oxygen monitoring methods. Depending on the method used, DO measurements may be made in the field or in a laboratory *(USEPA, 1997)*.

Method	Location of Measurement	Comments
Meter	Field	• The meter must be properly calibrated, accounting for salinity. • The meter is fragile; handle it carefully.
Test kit (Winkler titration)	Field or Lab	• If measured in lab, the sample is fixed in the field and titrated in the lab. • Lab measurement must take place within 8 hours of sample collection.

the estuary for a reading or into a water sample drawn out by bucket for a surface measurement. Depending on the length of its cable, a meter may allow monitors to get DO readings directly from various depths. Some meters allow volunteers to take both DO and temperature readings simultaneously.

Though easy to use, a reliable DO meter will likely cost more than $1,000. It also uses batteries, which last a long time but must be disposed of properly. To offset upfront and maintenance costs, monitoring groups might consider sharing equipment (Stancioff, 1996).

Because of the expense, a volunteer program might be able to afford only one meter. Consequently, only one team of monitors can measure DO and they will have to do it at all sites. Dissolved oxygen meters may be useful for programs in which many measurements are needed at only a few sites, volunteers sample at several sites by boat, or volunteers plan on running DO profiles (many measurements taken at different depths at one site).

Test Kits

If volunteers are sampling at several widely scattered sites, one of the many DO kits on the market may be more cost-effective. These kits rely on the Winkler titration method or one of its modifications. The modifications reduce the effect of materials in the water, such as organic matter, which may cause inaccurate results.

The kits are inexpensive, generally ranging from $30 to $200, depending on the method of titration they use. While inexpensive upfront, the kits require reagent refills as the reagents are used up or degrade over time. Reagents cannot be reused. Unused reagents and waste generated during the performance of tests must be disposed of properly (see Chapter 7). Volunteers must also take appropriate safety precautions when using the reagents, which can be harmful if used improperly.

Kits provide good results if monitors adhere strictly to established sampling protocols. Aerating the water sample, allowing it to sit in sunlight or unfixed (see box, page 9-6, for an explanation of fixing), and titrating too hastily can all introduce error into DO results.

For convenience, the volunteer monitors may keep their kits at home and take them to the sampling site each week. The program manager must provide the monitors with fresh chemicals as needed. Periodically, the manager should check the kit to make sure that each volunteer is properly maintaining and storing the kit's components. At the start of the monitoring program, and periodically thereafter if possible, the program manager should directly compare kit measurements to those from a standard Winkler titration conducted in a laboratory. ■

Titration

Titration is an analytical procedure used to measure the quantity of a substance in a water sample by generating a known chemical reaction. In the process, a reagent is incrementally added to a measured volume of the sample until reaching an obvious **endpoint**, such as a distinct change in color (Figure 9-4).

The volunteer on the left is titrating a water sample, while the other volunteer is "fixing" another sample (photo by K. Register).

Volunteers can use titration to assess the quantity of dissolved oxygen at a sampling site. This procedure, known as the Winkler titration, uses iodine as a substitute for the oxygen dissolved in a "fixed" sample of water. A **fixed sample** is one in which the water has been chemically rendered stable or unalterable, meaning that atmospheric oxygen will no longer affect the test result. Iodine stains the sample yellow-brown. Then, a chemical called sodium thiosulfate reacts with the free iodine in the water to form another chemical, sodium iodide. When the reaction is complete, the sample turns clear. This color change is called the endpoint.

Since the color change is often swift and can occur between one drop of reagent and the next, a starch indicator should be added to the solution to exaggerate the color change. The starch keeps the sample blue until all the free iodine is gone, at which time the sample immediately turns colorless. The amount of sodium thiosulfate used to turn the sample clear translates directly into the amount of dissolved oxygen present in the original water sample.

Figure 9-4. Titration of a reagent into a water sample.

Reminder!

To ensure consistently high quality data, appropriate quality control measures are necessary. See "Quality Control and Assessment" in Chapter 5 for details.

Not all quality control procedures are appropriate for all water quality analyses. Blanks and standards are not usually used for Winkler DO titrations, due to problems with contamination by oxygen from the air. To check the accuracy of the procedure, one has at least two options:

- *Create an oxygen-saturated sample by shaking and pouring water back and forth through the air, then titrate the sample and compare the results to published tables of oxygen solubility versus temperature (salinity must be known to determine oxygen solubility).*
- *Use a standard solution of potassium bi-iodate to check the accuracy of the titrant (standard solutions can be ordered from chemical supply companies—see Appendix C). The amount of titrant required to make the sample colorless should equal the amount of potassium bi-iodate added to the sample, ±0.1 ml.*

(Excerpted and adapted from Mattson, 1992.)

How to Monitor Dissolved Oxygen

General procedures for collecting and analyzing dissolved oxygen samples are presented in this section for guidance only; they do not apply to all sampling methods. **Monitors should consult with the instructions that come with their sampling and analyzing instruments. Those who are interested in submitting data to water quality agencies should also consult with the agencies to determine acceptable equipment, methods, quality control measures, and data quality objectives (see Chapter 5).**

Before proceeding to the monitoring site and collecting samples, volunteers should review the topics addressed in Chapter 7. It is critical to confirm the monitoring site, date, and time; have the necessary monitoring equipment and personal gear; and understand all safety considerations. Once at the monitoring site, volunteers should record general site observations, as discussed in Chapter 7.

STEP 1: Check equipment.

In addition to the standard sampling equipment and apparel listed in Chapter 7, the volunteer should bring the following items to the site for each sampling session:

If using the Winkler method

- large clean bucket with rope (if taking surface sample or if unable to collect sample directly in DO bottles);
- Kemmerer, Van Dorn, DO sampler, or homemade sampler (if taking a full DO profile);
- fully stocked dissolved oxygen kit with instructions;
- extra DO bottles;
- equipment for measuring temperature and salinity (necessary to calculate percent saturation—see page 9-12); and
- enough reagents for the number of sites to be tested.

If using a meter and probe

- calibrated DO meter and probe with operating manual (the meter must be calibrated according to the manufacturer's instructions);
- extra membranes and electrolyte solution for the probe;
- extra batteries for the meter;
- extra O-rings for the membrane;
- extension pole; and
- equipment for measuring temperature and salinity (necessary to calculate percent saturation—see page 9-12), if temperature and salinity cannot be measured by the meter.

STEP 2: Collect the sample.

This task is necessary if the volunteer is using a DO kit or if a sample is being drawn for a DO meter (rather than placing the DO probe directly in the estuary). Chapter 7 reviews general information about collecting a water sample.

Although the task of collecting a bottle of water seems relatively easy, volunteers must follow strict guidelines to prevent contamination of the sample. The citizen monitor must take care during collection of the water; jostling or swirling the sample can result in aeration and cause erroneous data. Using a bucket to collect the sample increases the risk of introducing oxygen to the sample. It is preferable to use a standard DO sampling bottle rather than a simple bucket since a washed and capped bottle is less likely to become contaminated than an open container.

Reminder!

The water sample must be collected in such a way that you can cap the bottle while it is still submerged. That means you must be able to reach into the water with both arms and the water must be deeper than the sample bottle.

If using a bucket

- Rinse the sample bucket with estuary water twice before sampling. Rinse and empty the bucket away from the collection area.
- Drop the bucket over the side of the dock, pier, or boat and allow water from just under the surface to gently fill the container until it is about two-thirds full. There should be no air bubbles in the bucket.
- Lift the bucket carefully to the working platform.
- If using a DO kit, rinse two DO bottles twice each with estuary water before filling them from the sample bucket. Then, submerge each capped bottle in the bucket, remove the lid, and slowly fill. Avoid agitating the water in the bucket to minimize the introduction of oxygen to the sample.
- While the bottle is still under water, tap its side to loosen any air bubbles before capping and lifting the bottle from the bucket.
- Check the sample for bubbles by turning the bottle upside-down and tapping. If you see any bubbles, repeat the filling steps.

Figure 9-5. Taking a water sample for DO analysis. Point the bottle against the tide or current and fill gradually. Cap the bottle under water when full, ensuring that there are no air bubbles in the bottle *(USEPA, 1997).*

If collecting samples directly in bottles

- Rinse two DO bottles twice each with estuary water away from the collection area before filling them with the sample.
- Make sure you are positioned downcurrent of the bottle.
- Submerge each capped bottle in the water, facing into the current.
- Remove the lid, and slowly fill (Figure 9-5). Avoid agitating the water to minimize the introduction of oxygen to the sample.
- While the bottle is still under water, tap its side to loosen any air bubbles before capping and lifting the bottle from the water.
- Check the sample for bubbles by turning the bottle upside-down and tapping. If you see any bubbles, repeat the filling steps.

If collecting samples from other samplers

- Follow the manufacturer's instructions.
- Make sure that no air bubbles are introduced into the sample.
- The sampler should have a mechanism for allowing the DO bottle to fill from the bottom to the top.

If using a test kit, take the water temperature by setting the thermometer in the bucket and allow it to stabilize while preparing for the DO test. Most meters will have a thermometer included. The bucket of water used for measuring DO can also be used for many of the other water quality tests.

Temperature and salinity should also be measured to calibrate a DO meter or if the volunteer group wishes to calculate percent saturation (see box, page 9-12).

STEP 3: *Measure DO.*

Many citizen monitoring programs use the "azide modification" of the Winkler titration to measure DO. This test removes interference due to nitrites—a common problem in estuarine waters.

If using the Winkler method

Gloves should be worn when doing this test.

Part One: "Fix" the sample immediately

- Proceed with the DO test for both sample bottles by carefully following the manufacturer's instructions. Allow some of the sample to overflow during these steps; this overflow assures that no atmospheric oxygen enters the bottled contents. After the sample is fixed, exposure to air will not affect the oxygen content of the sample. Be careful not to introduce air into the sample while adding the reagents. Simply drop the reagents into the test sample, cap carefully, and mix gently.
- Once the sample has been fixed in this manner, it is not necessary to perform the titration procedure immediately. Thus, several samples can be collected and "fixed" in the field, then carried back to a testing station or laboratory where the titration procedure is to be performed. The titration portion of the test should be carried out within 8 hours. In the meantime, keep the sample refrigerated and in the dark.

Part Two: Titrating the sample

- Continue with the titration of both samples, again following specific instructions included with the kit or provided by the program manager.
- Carefully measure the amount of fixed sample used in titration; this step is critical to the accuracy of the results. The bottom of the meniscus should rest on top of the white line on the titration test tube. (A **meniscus** is the curved upper surface of a liquid column that is concave when the containing walls are wetted by the liquid—see Figure 9-6.)
- Fill the syringe in the test kit, following instructions.
- Insert the syringe into the hole on top of the test tube and add 1 drop of sodium thiosulfate to the test tube; swirl the test tube to mix. Add another drop of the sodium thiosulfate and swirl the tube. Continue this titration process one drop at a time until the yellow-brown solution in the test tube turns a pale yellow. Then pull the syringe out of the hole (with the remaining sodium thiosulfate) and put it aside for a moment.

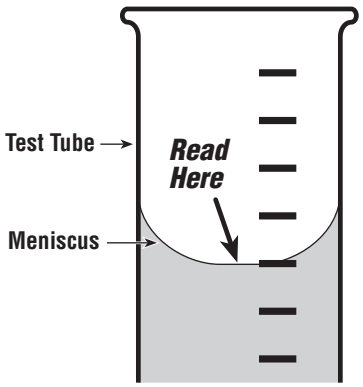

Figure 9-6. Measurements should be made at the bottom of the meniscus.

- Add starch solution to the test tube through the hole on top of the lid, according to directions. Swirl the tube to mix. The solution should turn from light yellow to dark blue.
- Now put the syringe back into the hole on the test tube. Continue the titration process with the remaining sodium thiosulfate, until the test tube solution turns from blue to clear. Do not add any more sodium thiosulfate than is necessary to produce the color change. Be sure to swirl the test tube after each drop.
- Using the scale on the side of the syringe, read the total number of units of sodium thiosulfate used in the experiment. Each milliliter of thiosulfate used is equivalent to 1 mg/l DO.
- Each volunteer should carry out all steps on two samples to minimize the possibility of error. The two samples can either be titrated from the one bottle of fixed sample solution or, for better quality assurance, from two water samples fixed in the field.

- If the discrepancy between the two DO concentrations is significant, the volunteer should run a third titration. The program's quality assurance project plan should define what difference is considered "significant." Some monitoring programs stipulate that a third sample must be analyzed if the DO concentrations of the first two samples differ by more than 0.6 mg/l.

NOTE: Samples with high levels of DO are brown, while low DO samples are generally pale yellow before the starch indicator is added. A few minutes after reaching the colorless endpoint, the sample may turn blue once again. This color reversion is not cause for concern—it is simply proof of a precise titration.

Helpful Hint

If volunteers are to collect and fix two water samples at each of their monitoring sites, be sure to provide each monitor with the appropriate number of DO bottles (e.g., 4 bottles for 2 monitoring sites, etc.). The bottles can be permanently marked with site location names. Volunteers will collect and fix the samples in the field, then titrate the samples within 8 hours. After the DO bottles have been emptied and cleaned, they are ready for the next monitoring session.

If using a meter and probe

- Make sure the unit is calibrated according to the manufacturer's instructions. Knowledge of salinity is needed to properly calibrate most meters (Green, 1998).
- After inserting the DO probe into the bucket or placing it over the side of the boat or pier, allow the probe to stabilize for at least 90 seconds before taking a reading.
- With some meters, you should manually stir the probe without disturbing the water to get an accurate measurement.

STEP 4: *Clean up and submit data.*

If using the Winkler titration method, make sure to thoroughly rinse all glassware in the kit and tightly screw on the caps to the reagent bottles. Check to ensure that each bottle contains sufficient reagents for the next DO analysis. Properly dispose of wastes generated during the performance of tests (see Chapter 7).

If using a laboratory to analyze the samples, deliver the fixed samples and field data sheets to the lab as soon as possible, as the sample analysis must be done within 8 hours.

Make sure that the data sheet is complete and accurate. Volunteers should make a copy of the completed data sheet before forwarding it to the project manager in case the original data sheet becomes lost. ■

Case Study: Dissolved Oxygen Monitoring in New Jersey

In New Jersey, the Alliance for a Living Ocean coordinates the Barnegat Bay Watch Monitoring Program. Dissolved oxygen testing is one of the more complicated monitoring activities undertaken by program volunteers.

The volunteer monitors use a modified Winkler titration test kit that is user-friendly and has a good degree of accuracy. With each test kit, the monitors receive a test procedure sheet and a monitor's testing manual. Often, monitors tape a simplified version of the test procedures to the inside of their test kits.

The program provides several tips to minimize any confusion about the test procedure:

- Because the test kit uses five reagents, monitors are encouraged to label the reagent bottles as #1, #2, etc.
- It is suggested that the bottles be arranged in numeric order in the test kit. This simplifies looking for the next reagent.
- Solutions 1, 2, 3, and 5 are each added 8 drops at a time. (Solution #4 is added one drop at a time.) The monitors can mark these reagent bottles with the words "8 drops." When the monitors' hands are wet or the wind is blowing, it is much easier to read the label on a bottle than an instruction sheet.

Many monitors conduct tests from their boats in the Barnegat Bay. These monitors are encouraged to "fix" the water sample by adding the first three reagents, and then return to land. Once on shore, volunteers can resume the test, which includes filling a titration tube to exactly 20 ml and titrating Solution #4 one drop at a time. In this manner, inaccuracies caused by a rocking boat are avoided.

Monitors are reminded to remove all air bubbles from the water sample by tapping the sample bottle while it is submerged. Monitors also double-check for air bubbles in the sample and the titration plunger before beginning a test. Air bubbles in the plunger are avoided by depressing the plunger before drawing up the titration solution. These practices greatly reduce data error.

The program suggests that volunteers perform the dissolved oxygen test several times at home or in the laboratory before going out in the field. Through practice, they can become familiar with the order of reagents and what the water sample should look like at each step.

For More Information:

Alliance for a Living Ocean
P.O. Box 95
Ship Bottom, NJ 08008
Phone: 609-492-0222
Fax: 609-492-6216
E-mail: livingocean@worldnet.att.net

DO Saturation and Percent Saturation

DO saturation, or potential DO level, refers to the highest DO concentration possible under the environmental limits of temperature, salinity (or chlorinity), and atmospheric pressure. As salinity or chlorinity increases, the amount of oxygen that water can hold decreases substantially. For example, at 20°C, 100% DO saturation for fresh water (for which salinity and chlorinity are zero) is 9.09 mg/l. At the same temperature, 100% saturation for water with 36 parts per thousand (ppt) salinity is 7.34 mg/l.

Table 9-2 summarizes DO saturation levels for different salinities and temperatures at sea level. Tables showing saturation levels in waters of various chlorinity can be found in APHA (1998).

Percent saturation is the amount of oxygen in the water relative to the water's potential DO saturation. It is calculated as follows:

$$\text{Percent saturation} = \frac{\text{measured DO}}{\text{DO saturation}} \times 100$$

(Excerpted and adapted from Green, 1998.)

Temperature °C	Oxygen Saturation Concentration (mg/l)				
	Salinity: 0 ppt	9 ppt	18 ppt	27 ppt	36 ppt
0.0	14.6	13.7	12.9	12.1	11.4
1.0	14.2	13.4	12.5	11.8	11.1
2.0	13.8	13.0	12.2	11.5	10.8
3.0	13.5	12.7	11.9	11.2	10.5
4.0	13.1	12.3	11.6	10.9	10.3
5.0	12.8	12.0	11.3	10.6	10.0
6.0	12.4	11.7	11.0	10.4	9.8
7.0	12.1	11.4	10.8	10.2	9.6
8.0	11.8	11.2	10.5	9.9	9.4
9.0	11.6	10.9	10.3	9.7	9.2
10.0	11.3	10.6	10.0	9.5	9.0
11.0	11.0	10.4	9.8	9.3	8.8
12.0	10.8	10.2	9.6	9.1	8.6
13.0	10.5	10.0	9.4	8.9	8.4
14.0	10.3	9.7	9.2	8.7	8.2
15.0	10.1	9.5	9.0	8.5	8.1
16.0	9.9	9.3	8.8	8.4	7.9
17.0	9.7	9.2	8.7	8.2	7.8
18.0	9.5	9.0	8.5	8.0	7.6
19.0	9.3	8.8	8.3	7.9	7.5
20.0	9.1	8.6	8.2	7.7	7.3
21.0	8.9	8.4	8.0	7.6	7.2
22.0	8.7	8.3	7.9	7.5	7.1
23.0	8.6	8.1	7.7	7.3	7.0
24.0	8.4	8.0	7.6	7.2	6.8
25.0	8.3	7.8	7.4	7.1	6.7
26.0	8.1	7.7	7.3	7.0	6.6
27.0	8.0	7.6	7.2	6.8	6.5
28.0	7.8	7.4	7.1	6.7	6.4
29.0	7.7	7.3	7.0	6.6	6.3
30.0	7.6	7.2	6.8	6.5	6.2
31.0	7.4	7.1	6.7	6.4	6.1
32.0	7.3	7.0	6.6	6.3	6.0
33.0	7.2	6.8	6.5	6.2	5.9
34.0	7.1	6.7	6.4	6.1	5.8
35.0	7.0	6.6	6.3	6.0	5.7

Table 9-2. Dissolved oxygen saturation concentrations (mg/l) in waters of various salinity (ppt) and temperature (°C) at sea level *(adapted from Campbell and Wildberger, 1992, and APHA, 1998).* Readers are referred to APHA (1998) for DO saturation concentrations using chlorinity instead of salinity (salinity = 1.80655 x chlorinity).

Common Questions About DO Testing

Should I pour off any of the water in my sample bottle before I add the reagents?

No. Pouring off some of the water allows space for an air bubble to be trapped when the bottle is capped. When you shake the bottle, this oxygen mixes with the sample and causes erroneously high results. It's OK for some liquid to overflow as you add the fixing reagents. (If you are concerned about spillage, put the bottle on a paper towel.)

How should I hold the dropper bottles to dispense the reagents?

Hold the dropper bottles completely upside down (i.e., vertical). This ensures a uniform drop size.

What is meant by saying that the sample is "fixed"?

After the first three reagents are added, the sample is fixed; this means that contact with atmospheric oxygen will no longer affect the test result because all the dissolved oxygen in the sample has reacted with the added reagents. The final titration actually measures iodine instead of oxygen. Fixed samples may be stored up to 8 hours, if kept refrigerated and in the dark.

What if I spill some of the acid as I am fixing the sample?

As part of the fixing process, acid crystals or liquid are added to the sample. The addition of the acid will dissolve the flocculate. You can spill a few acid crystals and not have to start over—but you should be sure to clean up the spill (see Chapter 7). If a few grains of acid do not go into the solution and all the flocculate is dissolved, you may continue the titration.

Sometimes after I add the acid, some brown "dots" remain. Is this OK?

The brown particles should be dissolved before you continue the test. Try shaking the sample bottle again. If this doesn't work, add one more drop of acid. You may occasionally find that organic material or sediment in the sample will not dissolve. This will not affect the test results.

What if my sample is colorless after it's fixed?

This means there is no dissolved oxygen in the sample. If this happens, you might want to test a sample that you know contains oxygen to make sure that your kit is functioning properly. One way to do this is to intentionally introduce an air bubble into the water sample, shake well, then fix the sample. You should see a yellow color.

When filling the syringe with the thiosulfate reagent, how far back should I pull the barrel?

The point of the black neoprene tip should be set right at zero. This is extremely important.

What if my syringe runs out of the sodium thiosulfate titrant?

In colder water, the amount of DO may be above 10 mg/l, so you will have to refill the syringe. For accurate results, fill to 0 mark and add the amount titrated from second syringe-full to the 10 from the first syringe-full.

How much starch solution should I add?

When and how much starch solution is added is not critical to the test. The important thing is that the sample turns blue.

(Excerpted and adapted from Green, 1997, and Ellett, 1993.)

BIOCHEMICAL OXYGEN DEMAND (BOD)

Biochemical oxygen demand measures the amount of oxygen that microorganisms consume while decomposing organic matter; it also measures the chemical oxidation of inorganic matter (i.e., the extraction of oxygen from water via chemical reaction). The rate of oxygen consumption in an estuary is affected by a number of variables, including temperature, the presence of certain kinds of microorganisms, and the type of organic and inorganic material in the water.

The Role of Biochemical Oxygen Demand in the Estuarine Ecosystem

BOD directly affects the amount of dissolved oxygen in estuaries. The greater the BOD, the more rapidly oxygen is depleted. This means less oxygen is available to aquatic organisms. The consequences of high BOD are the same as those for low dissolved oxygen: many aquatic organisms become stressed, suffocate, and die. Examples of BOD levels are provided in Table 9-3. Sampling locations with traditionally high BOD are often good candidates for more frequent DO sampling.

Table 9-3. Significant BOD Levels *(from Campbell and Wildberger, 1992).*

Type of Water	BOD (mg/l)
unpolluted, natural water	<5
raw sewage	150-300
wastewater treatment plant effluent	8-150*

**Allowable level for individual treatment plant specified in discharge permit*

Sources of BOD include leaves and woody debris; dead plants and animals; animal waste; effluents from pulp and paper mills, wastewater treatment plants, feedlots, and food-processing plants; failing septic systems; and urban stormwater runoff. Although some waters are naturally organic-rich, a high BOD often indicates polluted or eutrophic waters. ■

Sampling Considerations

BOD is affected by the same factors that affect DO. Chlorine can also affect BOD measurements by inhibiting or killing the microorganisms that decompose the organic and inorganic matter in a sample. In some water samples, chlorine will dissipate within 1-2 hours of being exposed to light. Such exposure often happens during sample handling or transport. However, if you are sampling in heavily chlorinated waters, such as those below the effluent discharge point from a wastewater treatment plant, it may be necessary to neutralize the chlorine with sodium thiosulfate (see APHA, 1998). ■

How to Measure Biochemical Oxygen Demand

The standard BOD test is a simple means of measuring the uptake of oxygen in a sample over a predetermined period of time. Citizens can easily collect the required water samples as they monitor the water for other variables. The BOD test does, however, demand a several-day period of water storage in the dark to obtain results. Test for BOD using the following steps:

- Collect two water samples from the same place in the water column (surface or at depth) using the water sampling protocol described earlier for DO. Each bottle should be labeled clearly so that the samples will not be confused. Make sure there is no contact between the sample water and the air.
- Immediately measure the first sample for DO using either a DO meter or DO kit. Record the time of sample collection and the water temperature. Place the second sample in a standard BOD bottle. The bottle should be black to prevent photosynthesis. You can wrap a clear bottle with black electrician's tape, aluminum foil, or black plastic if you do not have a black or brown glass bottle.
- Incubate the bottle of untested sample water at 20°C and in total darkness (to prevent photosynthesis). After 5 days of incubation, use the same method of testing to measure the quantity of DO in the second sample. Because of the 5-day incubation, the test should be conducted in a laboratory.
- The BOD is expressed in milligrams per liter of DO using the following equation:

BOD = DO (mg/l) of 1st bottle – DO of 2nd bottle

This represents the amount of oxygen consumed by microorganisms to break down the organic matter present in the sample bottle during the incubation period.

Sometimes by the end of the 5-day incubation period, the DO level is zero. This is especially true for monitoring sites with a lot of organic pollution (e.g., downstream of wastewater discharges). Since it is not known when the zero point was reached, it is not possible to tell what the BOD level is. In this case, it is necessary to collect another sample and dilute it by a factor that results in a final DO level of at least 2 mg/l. Special dilution water containing the nutrients necessary for bacterial growth should be used for the dilutions. Some supply houses carry premeasured nutrient "pillows" to simplify the process. APHA (1998) describes in detail how to dilute a sample and conduct the BOD analysis.

It takes some experimentation to determine the appropriate dilution factor for a particular sampling site. The final result is the difference in DO between the first measurement and the second after multiplying the second result by the dilution factor. ■

References and Further Reading

Portions of this chapter were excerpted and adapted from:

U.S. Environmental Protection Agency (USEPA). 1997. *Volunteer Stream Monitoring: A Methods Manual.* EPA 841-B-97-003. November. Office of Water, Washington, DC. 211 pp.

Other references:

American Public Health Association (APHA), American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed. L.S. Clesceri, A.E. Greenberg, A.D. Eaton (eds). Washington, DC.

Campbell, G., and S. Wildberger. 1992. *The Monitor's Handbook*. LaMotte Company, Chestertown, MD. 71 pp.

Ellett, K. 1993. *Chesapeake Bay Citizen Monitoring Program Manual*. Alliance for the Chesapeake Bay. Richmond, VA. 57 pp.

Green, L. 1997. "Common Questions About DO Testing." *The Volunteer Monitor* 9(1).

Green, L. 1998. "Let Us Go Down to the Sea—How Monitoring Changes from River to Estuary." *The Volunteer Monitor* 10(2): 1-3.

Mattson, M. 1992. "The Basics of Quality Control." *The Volunteer Monitor* 4(2): 6-8.

Stancioff, E. 1996. *Clean Water: A Guide to Water Quality Monitoring for Volunteer Monitors of Coastal Waters.* Maine/New Hampshire Sea Grant Marine Advisory Program and University of Maine Cooperative Extension. Orono, ME. 73 pp.

U.S. Environmental Protection Agency (USEPA). 1998. *Condition of the Mid-Atlantic Estuaries*. EPA 600-R-98-147. November. Office of Research and Development, Washington, DC. 50 pp.