

Dissolved Oxygen Protocol



Welcome

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Purpose

To measure the amount of oxygen dissolved in water

Overview

Students will use a dissolved oxygen kit or probe to measure the dissolved oxygen in the water at their hydrosphere study site. The exact procedure depends on the instructions in the dissolved oxygen kit or probe used.

Student Outcomes

Students will learn to:

- use a dissolved oxygen kit or probe;
- use technology in the classroom (DO probe);
- examine reasons for changes in the dissolved oxygen of a water body;
- communicate project results with other GLOBE schools;
- collaborate with other GLOBE schools (within your country or other countries); and
- share observations by submitting data to the GLOBE science database.

Science Concepts

Earth and Space Science

Earth materials are solid rocks, soils, water and the atmosphere.

Water is a solvent.

Each element moves among different reservoirs (biosphere, lithosphere, atmosphere, hydrosphere).

Physical Sciences

Objects have observable properties.

Life Sciences

Organisms can only survive in environments where their needs are met.

Earth has many different environments that support different combinations of organisms.

Organisms change the environment in which they live.

Humans can change natural environments.

All organisms must be able to obtain and use resources while living in a constantly changing environment.

Scientific Inquiry Abilities

Use a chemical test kit or probe to measure dissolved oxygen.

Identify answerable questions.

Design and conduct scientific investigations.

Use appropriate mathematics to analyze data.

Develop descriptions and explanations using evidence.

Recognize and analyze alternative explanations.

Communicate procedures and explanations.

Time

Kit: 20 minutes

Kit Quality Control Procedure: 20 minutes

Probe Setup: 20-30 minutes

Probe measurements: 10 minutes

Level

Middle and Secondary

Frequency

Weekly

Quality Control Procedure every 6 months

Probe calibration every time probe is used

Materials and Tools

[Hydrosphere Investigation Data Sheet](#)

[Dissolved Oxygen Protocol \(Test Kit\)](#)

[Field Guide](#)

[Dissolved Oxygen Protocol \(Probe\)](#)

[Field Guide](#)

Dissolved oxygen kit or probe

Latex gloves

Safety goggles

Waste bottle with cap

Distilled water

For dissolved oxygen kit Quality

Control Procedure:

- 100-mL graduated cylinder
- 250-mL polyethylene bottle with lid
- Clock or watch
- Thermometer
- [Solubility of Oxygen Table](#)
- [Correction for Elevation Table](#)
- [Hydrosphere Investigation Quality Control Procedure For Dissolved Oxygen Kits Data Sheet](#)



- [Quality Control Procedure for Dissolved Oxygen Kits Lab Guide](#)

For dissolved oxygen probe Quality Control Procedure:

- Zero oxygen solution (if appropriate for probe)
- Distilled water
- 250 mL polyethylene bottle with lid
- 100 ml beaker
- Thermometer
- Solubility of oxygen table
- Correction for elevation table
- Barometer (if available)

Preparation

Suggested activity: [Practicing Your Protocols: Dissolved Oxygen](#)

Find out what the elevation is at your school.

Dissolved oxygen probes must be calibrated before each use.

Prerequisites

Discussion of safety procedures when using chemical test kits

[Salinity Protocol](#) (where appropriate)

Dissolved Oxygen Protocol – Introduction

The GLOBE Dissolved Oxygen Protocol measures the amount of molecular oxygen (O_2) dissolved in water. It does not measure the amount of oxygen in the water molecule (H_2O). Students often confuse the oxygen that is part of the water molecule (the O in H_2O) with dissolved oxygen (O_2).

Just like animals that live on land, animals that live in water need molecular oxygen to breathe. However, there is much more oxygen available in the atmosphere for animal respiration than in water. Roughly, two out of ten air molecules are molecular oxygen. In water, however, there are only five or six oxygen molecules for every million water molecules. The amount of dissolved oxygen in the water determines what can live there. Some animals, like salmon or mayfly larvae, require higher oxygen levels than other animals like catfish or leeches.

We call the amount of dissolved oxygen the water will hold (under specific conditions) the solubility of dissolved oxygen. Factors affecting the solubility of dissolved oxygen include water temperature, atmospheric pressure, and salinity.

Cold water can dissolve more oxygen than warm water. For example, at $25^\circ C$, dissolved oxygen solubility is 8.3 mg/L, whereas at $4^\circ C$ the solubility is 13.1 mg/L. As temperature goes up, water releases some of its oxygen into the air. Water can hold less dissolved oxygen at higher elevations because there is

less pressure. Solubility of dissolved oxygen also decreases as salinity increases.

Dissolved oxygen can be added to water by plants during photosynthesis, through diffusion from the atmosphere, or by aeration. Aeration occurs when water is mixed with air. Such mixing occurs in waves, ripples, and waterfalls.

The amount of dissolved oxygen also is affected by what lives in the water. Just as photosynthesis by terrestrial plants adds oxygen to the air we breathe, photosynthesis by aquatic plants contributes dissolved oxygen to the water. Water may become supersaturated, meaning that the dissolved oxygen levels are greater than its solubility. The extra dissolved oxygen would then eventually be released back into the air or be removed through respiration.

The living biota of water systems makes up only a very small portion of the total organic matter of the system. Most organic matter in aquatic ecosystems is non-living and it is collectively referred to as detritus. The organic matter can be produced *in situ* or enter water bodies from the surrounding land (from both natural and human sources). The cycling of organic carbon between living and nonliving components is known as the carbon cycle. Organic matter is produced during photosynthesis and is consumed during respiration. During respiration, biota (fish, bacteria, etc.) consume dissolved oxygen.

Teacher Support

Supporting Protocols

Water Temperature: Oxygen solubility is dependent on temperature. It is therefore important to collect water temperature data along with dissolved oxygen data.

Salinity: Oxygen solubility is dependent on salinity. To find percent solubility, it is important to collect salinity data in water bodies where salinity is a factor. If your water body is less than 1 ppt (1000mg/L) you do not need to adjust for salinity.

Atmosphere Protocols: Atmosphere measurements such as cloud cover, precipitation, and air temperature may also be useful in interpreting dissolved oxygen data. Increased cloud cover, for instance, may result in a decrease in photosynthesis during the day.

Land Cover: It is also useful for measurements in the Hydrosphere Investigation to know about the land cover in your watershed. The land cover in a watershed can influence the amount of organic matter in the aquatic environment.

Advance Preparation

Dissolved Oxygen Kits

Students should do the quality control procedure as described in the *Quality Control Procedure for Dissolved Oxygen Kit Lab Guide* to test both the accuracy of their procedure and the precision of the kits. Doing the quality control will give students, teachers, and scientists confidence that the tests are being done properly.

Determine the elevation at the location (e.g., school) where the quality control procedure will be performed.

Dissolved Oxygen Probes

For measuring dissolved, you will hear references to either conductivity probes or meters. For clarification, probes are the instruments that measure voltage or resistance in a water sample. Meters are instruments that convert electrical (voltage or resistance) measurements to concentrations. In order to measure dissolved oxygen (or other types of measurements), both a probe

and meter are required. Sometimes the probe and meter are within one instrument and cannot be taken apart. Other instruments have probes that are separate from the meters and need to be connected to the meters in order to take the water measurements.

Dissolved oxygen probes must be calibrated before use. Check with the probe manufacturer to be sure the probe stores the most recent calibration. If it does, the dissolved oxygen probe will need to be calibrated 24 hours or less before taking measurements. If your probe does not keep the most recent calibration, you will need to calibrate the probe just before taking measurements taking care not to turn the probe or any associated software off.

Determine the elevation at your sampling site if you are not using a barometer.

Measurement Procedure

Dissolved Oxygen Kits

Dissolved oxygen test kits involve two parts – sample preservation (stabilization or fixing) and sample testing. Preservation involves the addition of a chemical to the sample that precipitates in the presence of dissolved oxygen, followed by the addition of a chemical that produces a colored solution. Testing involves adding drops of a titrant solution until the color disappears. The dissolved oxygen value is calculated from the volume of titrant added.

The amount of dissolved oxygen in the water can change rapidly after the sample has been collected. It is therefore important to do this test soon after the sample is collected. The water sample for the dissolved oxygen test should be ‘fixed’ at the water site (see instructions in your dissolved oxygen kit). After the sample is fixed, the sample may be taken back to the school to finish the test.

In following the instructions in the test kit, the following techniques should be followed.

Make sure there is no air in the bottle that contains the water you will test. To check for air bubbles in the sample bottle, turn the bottle upside down while it is capped and look for bubbles.



- Hold bottles and droppers vertically when adding drops of reagent to your water sample so that all of the drops of reagents are the same size.
- If students are asked to ‘mix’, they should cap the bottle and do a ‘windshield wiper motion’ to gently mix the chemicals.
- The precipitate is settled when there is a distinct line between the clear liquid at the top and the settled material at the bottom (fresh water). It takes a long time (greater than 15 minutes) for the precipitate to settle in salty and brackish water. Wait until there is a distinct line between clear liquid and settled material in the lower half of the bottle.
- Make sure you have no air bubbles in your titrator when you fill it.
- If your kit asks you to titrate to a “pale yellow”, hold a sheet of white paper behind the bottle and continue titration until the liquid is almost clear before adding the starch solution.

There is no elevation compensation required when measuring the actual amount of dissolved oxygen in a water sample from your Hydrosphere Study Site. This is only done on the quality control procedure.

Dissolved Oxygen Probes

Dissolved oxygen measured with an electronic probe **MUST** be measured at the site (or *in situ*). Samples cannot be taken back and analyzed in the lab.

Prepare your probe as instructed in the user’s manual. Many probes require a warm-up period before they will produce reliable results. Some probes need to remain on after calibration in order for them to retain their calibration. Be careful to follow the instructions that came with your probe.

When measuring dissolved oxygen in salt waters (conductivity greater than 1000 mg/L or salinity greater than 1 ppt), you will need to apply a salinity correction factor to the measurement taken by the probe. Saline water can hold less oxygen at the same temperature and pressure than can fresh water. Different probes have different procedures for this correction. Some have

the salinity correction before you measure DO and others afterward. Please refer to your manual for the procedure for your probe. As this correction can affect your measurement, it is necessary to measure salinity each time you measure DO and mark it down on your [*Hydrosphere Investigation Data Sheet*](#).

Quality Control Procedure

Dissolved Oxygen Kits

For the quality control procedure, students compare the measured dissolved oxygen in their standard solution with the saturated value from the table in order to determine if their kit and procedures are correct.

To make a saturated standard, students saturate distilled water by shaking a partially filled bottle of distilled water for 5 minutes. Since the solubility decreases with increasing temperature, increasing salinity, and decreasing air pressure, we control these variables in our dissolved oxygen standard by using distilled water, and correcting for the water temperature and elevation (an indirect measure of air pressure). You need to know the elevation (e.g., your school) where the procedure will be done. Table HY-DO-2 contains the correction values for various atmospheric pressures and elevations.

The shaken standard can be poured directly into the sample bottle until the bottle is completely filled. You will not add oxygen to the sample by pouring it since the water sample is already saturated with oxygen. After the sample bottle is filled, follow the instructions for the kit to measure the amount of dissolved oxygen.

Dissolved Oxygen Probes

Dissolved oxygen probes must be calibrated before use. Check with the probe manufacturer to be sure the probe stores the most recent calibration. If it does, the dissolved oxygen probe will need to be calibrated 24 hours or less before taking measurements. If your probe does not keep the most recent calibration, you will need to calibrate the probe just before taking measurements taking care not to turn the probe or any associated software off.

When you are in the field, check that the calibration has held by placing the probe in 100% saturated air with water. If the value is off by ± 0.2 mg/L then recalibrate in the field. Remember, different temperatures at the site might result in different total mg/L of oxygen at 100% saturation. This does not necessarily mean that your calibration is off. Check the calibration tables for the amount of oxygen present at 100% saturation at that temperature.

NEVER report Dissolved Oxygen data taken with an instrument that has not been calibrated before using.

Safety Precautions

- Students should wear gloves and goggles when handling chemicals and water that may contain potentially harmful substances such as bacteria or industrial waste.
- Local authorities should be consulted on the proper disposal of used chemicals.

Helpful Hints

Mark each item in the kit with a dot of paint or nail polish of the same color. Mark other kits with different colors to avoid having chemicals or titrators exchanged between kits.

When opening chemicals to use, write the date they were opened, and dispose of all chemicals appropriately when past their due date.

Managing Students

If there is not enough time to have students measure the dissolved oxygen of three different samples at the hydrosphere study site, have one or more students perform the whole measurement. Then have the other students use the same fixed sample for sample testing later in the classroom or lab.

Instrument Maintenance

Dissolved Oxygen Kits

1. Chemicals should be tightly capped immediately after they are used.
2. Rinse the sample bottle and titration tube with distilled water after use.
3. Discard chemicals from the dropper or titrator. They should not be put back into the original containers because they may be contaminated.
4. Do not rinse the titrator with distilled water as long as it has not been contaminated. Rinsing with distilled water often leaves a drop of water in the titrator that is difficult to remove.
5. Store the titrator with the plunger removed to avoid the rubber end sticking in the tube.

Dissolved Oxygen Probes

DO probes require meticulous care in order to maintain their accuracy and lifespan. Consult your manual for maintenance and storage instructions specific to your probe.

Questions for Further Investigation

How would a change in the amount of dissolved oxygen affect what lives in a water body?

How could warming or cooling of the atmosphere affect the amount of dissolved oxygen in your water?

How could changes in the land cover around your water site affect the amount of dissolved oxygen in your water?

Quality Control Procedure for Dissolved Oxygen Kits

Lab Guide

Task

Check the accuracy of your dissolved oxygen kit. Practice using your dissolved oxygen kit properly.

What You Need

- [Hydrosphere Investigation Quality Control Data Sheet](#)
- Distilled water
- 100-mL graduated cylinder
- 250-mL polyethylene bottle with lid
- Thermometer
- Waste bottle with cap for discarding used chemicals
- Dissolved oxygen test kit
- Latex gloves
- Goggles
- Pen or pencil
- Clock or watch

What To Do

1. Put on your gloves and protective goggles.
2. Rinse the 250-mL bottle twice with distilled water.
3. Pour 100 mL of distilled water into the 250-mL bottle.
4. Put the lid on the bottle. Shake the bottle vigorously for 5 minutes. This is the standard you will use to test your kit.
5. Uncap the bottle and take the temperature of the water (see *Water Temperature Protocol Field Guide*). Be sure the tip of the thermometer does not touch the bottom or sides of the bottle.
6. Record the temperature of the distilled water standard on the *Hydrosphere Investigation Quality Control Data Sheet*.
7. Pour the standard into the sample bottle in your dissolved oxygen kit. Fill the sample bottle completely to the top. Put the lid on the sample bottle. Turn the bottle upside down while it is capped. There should not be any air bubbles.

Note: It is not necessary to immerse the sample bottle in the water to collect your sample when you are doing the quality control procedure.

8. Follow the directions in your dissolved oxygen kit to measure the dissolved oxygen of your standard.
9. Record the amount of dissolved oxygen (mg/L) in your standard on your *Hydrosphere Investigation Quality Control Data Sheet*.

10. Look up the temperature you recorded earlier on the *Solubility of Oxygen Table*. See Table HY-DO-1.
11. Record the solubility for your water temperature.
12. Find the elevation closest to yours on the *Correction for Elevation/Pressure Table*. See Table HY-DO-2.
13. Record the correction value for your elevation.
14. Multiply the solubility of your standard times the correction value. This is the expected amount of dissolved oxygen in your standard.
15. Compare the amount of dissolved oxygen you measured with the kit to the expected amount for your standard.
16. If the measurement is within $\pm 1\text{mg/L}$, record the dissolved oxygen value on the *Hydrosphere Investigation Quality Control Procedure Data Sheet*. If the measurement is not within this range, repeat the entire quality control procedure.
17. If your measurements are still not in range, record the value you got and report to your teacher that the kit is not working properly.
18. Pour all used chemicals into the waste bottle. Clean your kit with distilled water.

Table HY-DO-1: Solubility of Oxygen in Fresh Water Exposed to Air at 1013.25 mB Pressure

Temp (°C)	Solubility (mg/L)	Temp (°C)	Solubility (mg/L)	Temp (°C)	Solubility (mg/L)
0	14.6	16	9.9	32	7.3
1	14.2	17	9.7	33	7.2
2	13.8	18	9.5	34	7.1
3	13.5	19	9.3	35	7.0
4	13.1	20	9.1	36	6.8
5	12.8	21	8.9	37	6.7
6	12.5	22	8.7	38	6.6
7	12.1	23	8.6	39	6.5
8	11.9	24	8.4	40	6.4
9	11.6	25	8.3	41	6.3
10	11.3	26	8.1	42	6.2
11	11.0	27	8.0	43	6.1
12	10.8	28	7.8	44	6.0
13	10.5	29	7.7	45	5.9
14	10.3	30	7.6	46	5.8
15	10.1	31	7.4	47	5.7

Table HY-DO-2: Correction Values For Various Atmospheric Pressures and Elevations

Pressure (millibars)	elev (m)	Correction value (%)	Pressure (millibars)	elev (m)	Correction value (%)
1023	-84	1.01	841	1544	0.83
1013	0	1.00	831	1643	0.82
1003	85	0.99	821	1743	0.81
993	170	0.98	811	1843	0.80
983	256	0.97	800	1945	0.79
973	343	0.96	790	2047	0.78
963	431	0.95	780	2151	0.77
952	519	0.94	770	2256	0.76
942	608	0.93	760	2362	0.75
932	698	0.92	750	2469	0.74
922	789	0.91	740	2577	0.73
912	880	0.90	730	2687	0.72
902	972	0.89	719	2797	0.71
892	1066	0.88	709	2909	0.70
882	1160	0.87	699	3023	0.69
871	1254	0.86	689	3137	0.68
861	1350	0.85	679	3253	0.67
851	1447	0.84	669	3371	0.66

HY-DO-3: Solubility of Oxygen in Salt Water at Sea Level (1013.25 mB) with Temperature and Salinity

Temperature (°C)	Salinity (ppt)												
	0	5	10	15	20	25	30	35	40	45	50	55	60
1	14.2	13.7	13.3	12.8	12.4	12.0	11.5	11.2	10.8	10.4	10.1	9.7	9.4
2	13.8	13.4	12.9	12.5	12.1	11.6	11.3	10.9	10.5	10.2	9.8	9.5	9.2
3	13.5	13.0	12.6	12.2	11.7	11.4	11.0	10.6	10.3	9.9	9.6	9.3	8.9
4	13.1	12.7	12.3	11.8	11.5	11.1	10.7	10.4	10.0	9.7	9.4	9.0	8.7
5	12.8	12.4	11.9	11.6	11.2	10.8	10.5	10.1	9.8	9.5	9.1	8.8	8.6
6	12.4	12.0	11.7	11.3	10.9	10.5	10.2	9.9	9.6	9.2	8.9	8.6	8.4
7	12.1	11.7	11.4	11.0	10.6	10.3	10.0	9.6	9.3	9.0	8.7	8.5	8.2
8	11.8	11.5	11.1	10.7	10.4	10.1	9.7	9.4	9.1	8.8	8.6	8.3	8.0
9	11.6	11.2	10.8	10.5	10.2	9.8	9.5	9.2	8.9	8.6	8.4	8.1	7.9
10	11.3	10.9	10.6	10.3	9.9	9.6	9.3	9.0	8.7	8.5	8.2	7.9	7.7
11	11.0	10.7	10.3	10.0	9.7	9.4	9.1	8.8	8.6	8.3	8.0	7.8	7.5
12	10.8	10.4	10.1	9.8	9.5	9.2	8.9	8.6	8.4	8.1	7.9	7.6	7.4
13	10.5	10.2	9.9	9.6	9.3	9.0	8.7	8.5	8.2	8.0	7.7	7.5	7.2
14	10.3	10.0	9.7	9.4	9.1	8.8	8.6	8.3	8.0	7.8	7.6	7.3	7.1
15	10.1	9.8	9.5	9.2	8.9	8.6	8.4	8.1	7.9	7.7	7.4	7.2	7.0
16	9.9	9.6	9.3	9.0	8.7	8.5	8.2	8.0	7.7	7.5	7.3	7.1	6.9
17	9.7	9.4	9.1	8.8	8.6	8.3	8.1	7.8	7.6	7.4	7.1	6.9	6.7
18	9.5	9.2	8.9	8.7	8.4	8.2	7.9	7.7	7.4	7.2	7.0	6.8	6.6
19	9.3	9.0	8.7	8.5	8.2	8.0	7.8	7.5	7.3	7.1	6.9	6.7	6.5
20	9.1	8.8	8.6	8.3	8.1	7.8	7.6	7.4	7.2	7.0	6.8	6.6	6.4
21	8.9	8.7	8.4	8.2	7.9	7.7	7.5	7.3	7.1	6.8	6.7	6.5	6.3
22	8.7	8.5	8.3	8.0	7.8	7.6	7.3	7.1	6.9	6.7	6.5	6.4	6.2
23	8.6	8.3	8.1	7.9	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.2	6.1
24	8.4	8.2	7.9	7.7	7.5	7.3	7.1	6.9	6.7	6.5	6.3	6.1	6.0
25	8.3	8.0	7.8	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.2	6.0	5.9
26	8.1	7.9	7.7	7.5	7.2	7.0	6.8	6.7	6.5	6.3	6.1	5.9	5.8
27	8.0	7.7	7.5	7.3	7.1	6.9	6.7	6.5	6.4	6.2	6.0	5.9	5.7
28	7.8	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.3	6.1	5.9	5.8	5.6
29	7.7	7.5	7.3	7.1	6.9	6.7	6.5	6.3	6.2	6.0	5.8	5.7	5.5
30	7.6	7.4	7.2	7.0	6.8	6.6	6.4	6.2	6.1	5.9	5.7	5.6	5.4
31	7.4	7.2	7.0	6.8	6.7	6.5	6.3	6.1	6.0	5.8	5.7	5.5	5.4
32	7.3	7.1	6.9	6.7	6.6	6.4	6.2	6.0	5.9	5.7	5.6	5.4	5.3
33	7.2	7.0	6.8	6.6	6.4	6.3	6.1	5.9	5.8	5.6	5.5	5.3	5.2
34	7.1	6.9	6.7	6.5	6.3	6.2	6.0	5.9	5.7	5.6	5.4	5.3	5.1
35	6.9	6.8	6.6	6.4	6.2	6.1	5.9	5.8	5.6	5.5	5.3	5.2	5.1
36	6.8	6.7	6.5	6.3	6.2	6.0	5.8	5.7	5.5	5.4	5.2	5.1	5.0
37	6.7	6.6	6.4	6.2	6.1	5.9	5.7	5.6	5.5	5.3	5.2	5.0	4.9
38	6.6	6.4	6.3	6.1	6.0	5.8	5.7	5.5	5.4	5.2	5.1	5.0	4.8
39	6.5	6.3	6.2	6.0	5.9	5.7	5.6	5.4	5.3	5.2	5.0	4.9	4.8
40	6.4	6.2	6.1	5.9	5.8	5.6	5.5	5.4	5.2	5.1	5.0	4.8	4.7



Frequently Asked Questions

1. Why does the amount of dissolved oxygen I measured not agree with the amount I calculated?

There are two reasons why these numbers may not match. First, you may not have followed the instructions on your kit exactly or you may have made small errors in the procedure you used. Here are some troubleshooting tips:

1. Make sure you do not have any air bubbles in your sample bottle or your titrator (for kits that use a titrator). To check for air bubbles in the sample bottle, turn the bottle upside down while it is capped and look for bubbles.
2. Measure accurately. If you are adding drops from a bottle, hold the bottle vertically so that all of the drops are the same size.
3. Allow all of the precipitate to settle. If you shake the bottle too hard before the precipitate settles, it may take 10 minutes or more for the settling to happen.
4. Record accurately. If your kit asks you to count drops, have two people count to insure accuracy. If your kit asks you to read a titrator, make sure to read the instructions for accurately reading the titrator that come with your kit.
5. If you are testing in salt waters make sure you refer to Table HY-DO-3 to determine the maximum amount of oxygen that waters with your salinity can hold. Salt waters can hold less oxygen when fully saturated than can freshwaters.



The second reason your measured value may not be the same as your calculated value is that there may be something wrong with the chemicals in your kit. In this case, you will need to get new chemicals.



Dissolved Oxygen Protocol (Test Kit)

Field Guide

Task

Measure the dissolved oxygen of your water sample using a test kit.

What You Need

- [Hydrosphere Investigation Data Sheet](#)
- Latex gloves
- Goggles
- Dissolved oxygen kit
- Distilled water
- Waste bottle with cap for used chemicals
- Pen or pencil

In the Field

1. Fill in the top of the *Hydrosphere Investigation Data Sheet*.
2. Put on the gloves and goggles.
3. Rinse the sample bottle and your hands with sample water three times.
4. Place the cap on the empty sample bottle.
5. Submerge the sample bottle in the sample water.
6. Remove the cap and let the bottle fill with water. Move the bottle gently or tap it to get rid of air bubbles.
7. Put the cap on the bottle while it is still under the water.
8. Remove the sample bottle from the water. Turn the bottle upside down to check for air bubbles. If you see air bubbles, discard this sample. Collect another sample.
9. Follow the directions in your Dissolved Oxygen Kit to test your water sample.
10. Record the dissolved oxygen in your water sample on the *Data Sheet* as *Observer 1*.
11. Have two other students repeat the measurement using a new water sample each time.
12. Record their data on the *Data Sheet* as *Observers 2* and *3*.
13. Calculate the average of the three measurements.
14. Each of the three measurements should be within 1 mg/L of the average. If one of the measurements is not within 1 mg/L of the average, find the average of the other two measurements. If both of these measurements are within 1 mg/L of the new average, record this average.
15. Discard all used chemicals into the waste container. Clean your dissolved oxygen kit with distilled water.

Dissolved Oxygen Protocol (Probe)

Field Guide

Task

Measure the dissolved oxygen of your water sample using a DO probe.

What You Need

- [Hydrosphere Investigation Data Sheet](#)
- Dissolved Oxygen Probe
- Zero Oxygen solution (if applicable for your probe)
- 250 mL polyethylene bottle with lid
- Latex gloves
- Distilled water
- Salinity correction tables (if appropriate)
- Barometer
- Pen or pencil

In the Lab or Field

Calibration (Performed within 24 hours before taking a measurement)

1. Warm up the probe as described in the probe manual.
2. Use the barometer to measure the atmospheric pressure at your site. If a barometer is not available, use your elevation to approximate the atmospheric pressure at your site.
3. Follow the probe manual instructions to enter calibration information for the probe.
4. Follow the probe manual instructions to measure the first calibration point (Zero oxygen point).
5. Rinse probe with distilled water and blot dry without touching membrane.
6. Follow the probe manual instructions to measure the second calibration point (100% oxygen).

In the Field

1. Warm up the probe as described in the probe manual.
2. Lower the tip of the probe into the water body that you are sampling and slowly move it back and forth. If you are measuring a stream or river and the water is moving past the probe, you can just hold the probe in place.
3. When reading has stabilized, record the dissolved oxygen in your water body on your *Hydrosphere Investigation Data Sheet*.
4. Repeat the readings two more times and record the dissolved oxygen under Observers 2 and 3.
5. Check to make sure that the three readings are within 0.2 mg/L of one another. If they are not, continue taking readings until the last three are within 0.2 mg/L of one another.
6. Apply the salinity correction (if appropriate).
7. Calculate the average of the three (adjusted if salinity correction applied) measurements.
8. Rinse the electrode with distilled water and blot dry. Cap electrode to protect membrane and turn off meter.

Frequently Asked Questions

1. Why do we have to do the measurements at the same time of day?

The amount of dissolved oxygen may change during the day as the water begins to warm up. More light penetrating the water causes more photosynthesis to occur. This can also increase the amount of dissolved oxygen. For this reason it is important to do your Hydrosphere measurements at the same time of day each week.

2. What will make my dissolved oxygen levels change over the year?

Besides seasonal differences in temperature, seasonal changes in the flow of your stream, changes in transparency, or changes in productivity (amount of growth of plants and animals in the water) will cause changes in dissolved oxygen levels.

3. Why does salt concentration affect oxygen saturation?



As the salt content increases in water, fewer oxygen molecules can be dissolved. Therefore, as salinity increases, saturated DO decreases in a water sample under the same temperature and pressure.

4. What is saturated DO?

Saturated DO refers to the maximum oxygen that water can hold at a particular temperature, pressure and salinity. When you calibrate your DO probe, the 100% saturation point is saturated Dissolved Oxygen or saturated DO.

5. Why do we need to measure salinity each time?

In arid and semi-arid areas, salinity or conductivity levels vary depending on whether it is a dry or rainy season. In estuaries, salinity can vary depending on the time of the tide or even in dry or wet years.



Dissolved Oxygen Protocol –Looking at the Data

Are the data reasonable?

The amount of dissolved oxygen you measure depends on your water site. Dissolved oxygen is added to water through aeration (water running or splashing), diffusion, and by photosynthesis of aquatic plants. It is used up by respiration. The maximum amount of dissolved oxygen your water can hold (saturated solution) depends on elevation (atmospheric pressure) at your site, water temperature, and salinity of your sample. Dissolved oxygen in natural waters may vary from 0.0 mg/L to around 16.0 mg/L. Distilled water at 0.0 C has a solubility of 14.6 mg/L at sea level. Warm, still waters might have dissolved oxygen levels of about 4 or 5 mg/L. Cold, running waters might have oxygen levels at 13 or 14 mg/L. Higher levels are possible due to photosynthesis by plants and lower levels are possible due to respiration.

Since dissolved oxygen levels are dependent on water temperature as well as other variables such as photosynthesis and respiration in the water, it is helpful to look for seasonal trends. Graph the dissolved oxygen and water temperature data over a year. Look for similarities in the seasonal patterns. In general, you should notice that as water temperature increases, the dissolved oxygen will tend to decrease (Figure HY-DO-1a).

Dissolved oxygen data should be collected at the same time of day each week since oxygen levels at a site will change throughout the day as the water warms up and photosynthesis increases during the afternoon. Data collected at different times of day make seasonal patterns much more difficult to interpret. In addition to finding seasonal patterns, graphing your data will help you to check for other potential errors, such as misplaced decimal points.

In Figure HY-DO-1b, the water temperature plot has been “turned off” (by clicking on the name of the plot in the legend); notice that two points of data are well below all others, a value of 3.0 in early February 1999 and less than 3.0 in late February/early March 2002. These values are extremely low and not a

normal value for this water body at this time of year. Based on the shape of the graph the expected value of dissolved oxygen would be much greater, perhaps around 11 to 13 mg/L. If you come across such values, contact the school and ask them to double check their *Data Sheets* and make sure that this is the value that is on the sheet.

After you have collected a few samples, you should know approximately what your value should be. If you get an unexpected measurement (higher or lower than you would expect based on the air temperature and values from previous weeks, do it again with a new water sample and clean sample bottles. If you get the same result, make a note in the metadata that you are aware of the unusual values for that date, and that they are indeed correct.

What do people look for in the data?

Most organisms will not exist at dissolved oxygen levels less than 3.0 mg/L. Some sensitive organisms will not live in oxygen levels less than 7.5 mg/L. Dissolved oxygen levels that drop at low levels (i.e., less than 5 mg/L) are a reason for concern. Excess nutrients (e.g., fertilizer, organic-rich waste water) added to the water body can cause an overgrowth of vegetation and algae, causing increased decay in the water. The bacteria that decompose the organic matter respire and use oxygen.

In addition to looking at the amount of dissolved oxygen in the water, it is also interesting to compare the amount of measured dissolved oxygen with a calculated value for saturation. This can tell us about the productivity of the water body. In a productive water body, plants will be producing oxygen through photosynthesis. Dissolved oxygen values will vary throughout the day, with maximum value occurring in the early afternoon and lowest levels occurring during the night (when respiration is not balanced by photosynthesis). At certain times of the day (typically early afternoon), some water bodies may actually have a dissolved oxygen measurement above the saturation level, indicating that more oxygen is being produced by photosynthesis that is being consumed by respiration. Water bodies that are highly turbid have low light penetration and low productivity. They are



typically characterized by low dissolved oxygen levels.

The GLOBE visualizations page on the Website displays values of saturated dissolved oxygen for your site that you can compare graphically with your actual measurements.

An Example of a Student Research Investigation

Forming a Hypothesis

A student interested in dissolved oxygen is looking at the time plot of dissolved oxygen at Reynolds Jr Sr High School SWS-02 site, called “Covered Bridge” (Figure HY-DO-2). She notices that the values of dissolved oxygen in late December 2000 through January 2001 were much lower than values in previous winters. During that time period the values ranged from 7 to 10 mg/L for about a month. During the previous three winters, dissolved oxygen consistently ranged from 11 to 15 mg/L. The low values are similar to those found during the warmer periods.

Knowing that the *saturated* dissolved oxygen levels are usually related to temperature, she hypothesizes that the *water temperature during this time period is higher than normal and the warmer water is responsible for the lower dissolved oxygen values.*

She contacts the school and learns that this water body is the Shenango River.

Collecting and Analyzing Data

She begins by plotting the monthly mean values of dissolved oxygen and temperature. See Figure HY-DO-3.

The unusually low January 2001 dissolved oxygen is even more apparent when looking at the monthly averages. However, there does not appear to be a corresponding increase in water temperature, which is about 3° C.

If temperature is normal, then the values of saturated dissolved oxygen should be high as well. This would mean that the *dissolved oxygen deficit*, which is the difference between the saturated and observed values, is unusually high for some reason.

The GLOBE visualizations page will calculate monthly averages for water temperature and measured dissolved oxygen, but not for saturated dissolved oxygen, so the student

Table HY-DO-3

Date	Water Temp (°C)	Dissolved oxygen (mg/L)	Saturated DO (mg/L)	DO use (mg/L)
1/2/1998	5	11.2	12.8	1.6
1/10/1998	5.5	10.5	12.6	2.1
1/17/1998	2	12.1	13.8	1.7
1/24/1998	1.5	12.6	14	1.4
1/31/1998	2	11.7	13.8	2.1
Average	3.2	11.6	13.4	1.8
<hr/>				
1/9/1999	0	12.3	14.6	2.3
1/16/1999	0	12.3	14.6	2.3
1/23/1999	1	10.8	14.2	3.4
1/30/1999	0.5	11.6	14.4	2.8
Average	0.4	11.8	14.5	2.7
<hr/>				
1/6/2000	3	13.6	13.5	-0.1
1/13/2000	1.2	13	14.1	1.1
1/20/2000	0	13	14.6	1.6
1/27/2000	0	13.3	14.6	1.3
Average	1.1	13.2	14.2	1.0
<hr/>				
1/5/2001	6	9.8	12.4	2.6
1/12/2001	1	9.8	14.2	4.4
1/19/2001	2	8.5	13.8	5.3
1/26/2001	1	7.4	14.2	6.8
Average	2.5	8.9	13.7	4.8

decides to calculate the monthly averages for saturated dissolved oxygen herself. She generates a plot with dissolved oxygen, saturated dissolved oxygen, and water temperatures, and then creates a data table. She transfers this information into a spreadsheet.

She extracts all the January values for each of the years (Table HY-DO-3). She then calculates the dissolved oxygen deficit (saturated dissolved oxygen – measured dissolved oxygen). Then for each year, she calculates the average for each of the four terms.

The average dissolved oxygen in 2001 was 8.9 mg/L. In 1998-2000, it was 11.6, 11.8 and 13.2, respectively.

However, the water temperature was about the same for all four Januarys: 3.2°, 0.4°, 1.1° and 2.5° C. The temperature was actually



warmer in January of 1998 than 2001, and the measured DO was higher. Therefore, the decrease in dissolved oxygen does not seem to be related to temperature.



The average dissolved oxygen deficit ranged from 1.0 to 2.7 mg/L the first three years, and was 4.8 in 2001. The dissolved oxygen deficit is almost twice as high in January 2001 as it was in the next highest year (January 1999) when it was 2.7.



She concludes that: *Measured dissolved oxygen values are lower in January 2001 than in January 1998-2000. Water temperature and saturated dissolved oxygen values are about the same, so the decrease in dissolved oxygen is not related to a change in water temperature.*



Therefore her hypothesis that warmer water was causing the lower dissolved oxygen value was rejected. It is all right to disprove your hypothesis. Scientists do this all the time. Often in finding out that our hypothesis is not correct, we come up with alternatives that lead to a better understanding of the problem at hand.



Future Research

There is nothing in this data to suggest WHY the dissolved oxygen is so much lower in winter 2001 than during the 3 previous years. The student does notice that the 2000-2001 winter seems longer in duration than the other winters but cannot think of why that might affect dissolved oxygen levels later in the winter. She also notices that the summer dissolved oxygen data in 2000 appear more variable than in previous years. Perhaps something else has changed in the river to cause a higher demand for dissolved oxygen. One reason might be that more bacteria, such as those associated with decaying organic matter from sewage, might be present in the water. A student might investigate whether there have been external changes in the watershed.

Figure HY-DO-1a

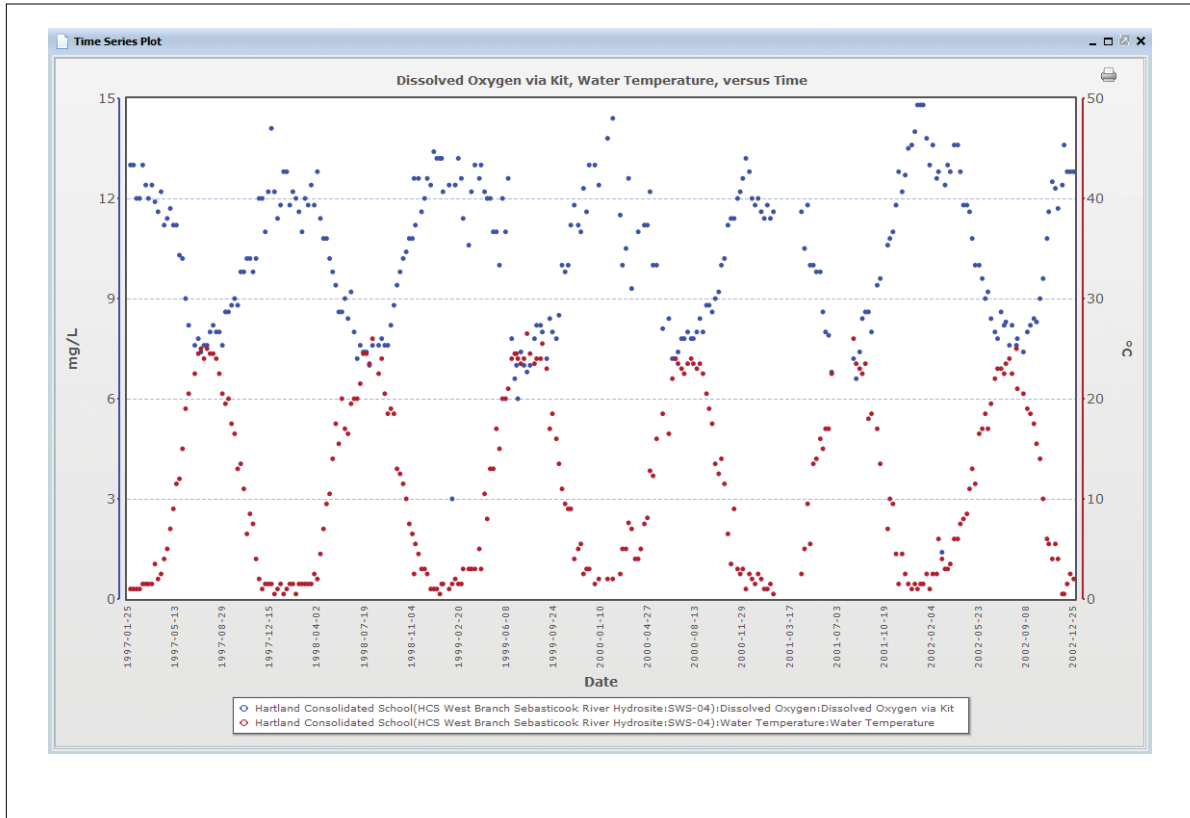


Figure HY-DO-1b

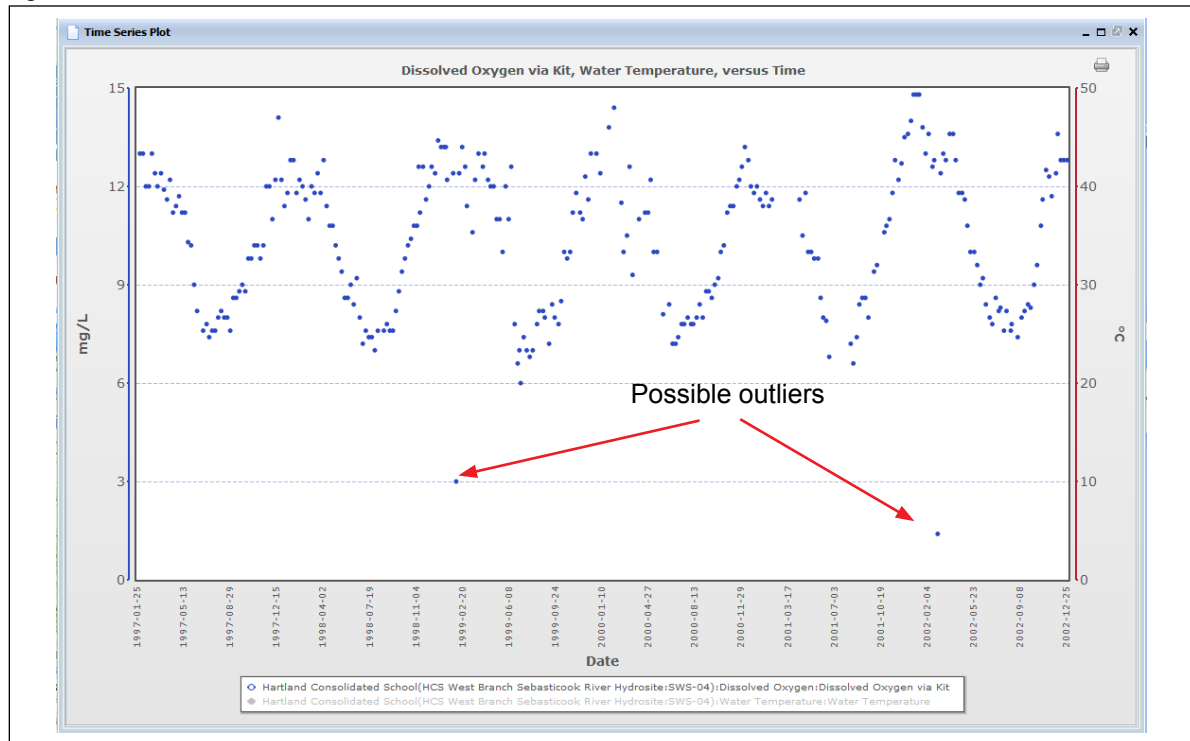


Figure HY-DO-2

