Introduction

Total dissolved gas (TDG) supersaturation caused by hydroelectric dams can result in harm to aquatic animals. Water becomes supersaturated with TDG when air mixes with water at depth, equilibrates under pressure, and moves to a shallower depth. In these conditions air can remain dissolved at levels that are greater than saturation. Aquatic animals equilibrate with the respective TDG and, if TDG levels are sufficiently high, bubbles can form in their tissues and cause damage, known as gas bubble trauma (GBT) (Pleizier, Algera, et al., 2020). Weiss saturometers have been used to monitor TDG since 1971 but methods for field calibration are rarely reported. Regular calibration, accurate measurement procedures, and standardized TDG reporting are important for generating reliable measurements that are easily interpreted. We provide a description of low-cost calibration equipment that practitioners can build themselves and recommend a specific two-point calibration protocol. We also recommend methods for the more accurate measurement and reporting of TDG.

1. Introduction

Total dissolved gas (TDG) supersaturation caused by hydroelectric dams can result in harm to aquatic animals. Water becomes supersaturated with TDG when air mixes with water at depth, equilibrates under pressure, and moves to a shallower depth. In these conditions air can remain dissolved at levels that are greater than saturation. Aquatic animals equilibrate with the respective TDG and, if TDG levels are sufficiently high, bubbles can form in their tissues and cause damage, known as gas bubble trauma (GBT) (Pleizier, Algera, et al., 2020). Weiss saturometers have been used to monitor TDG since 1971 but methods for field calibration are rarely reported. Regular calibration of all measurement equipment is crucial for making accurate, reliable measurements, yet TDG probe manufacturers do not always provide information on adequate calibration procedures. There is an alarming possibility that TDG meters are not being field calibrated in many cases. Commercially available equipment exists for performing calibrations of saturometers but can be prohibitively expensive. We describe a novel, low-cost alternative to commercial calibration equipment that can easily be constructed, and we propose a simple method for calibrating Weiss saturometers.

Measuring TDG supersaturation poses unique challenges because the combined pressure of multiple supersaturated dissolved gases must be measured. Levels of constituent gases are important in predicting the effects of TDG on aquatic animals; for example, increased oxygen to nitrogen ratios in TDG supersaturated water reduces harmful effects on fish (Jensen, 1988; Nebeker et al., 1976, 1979). However, the combined pressure of all dissolved gases must also be measured to assess the effects of TDG on aquatic animals.
TDG supersaturation is commonly measured using a Weiss saturometer. Other methods include the Van Slyke method and gas chromatography, but the Weiss saturometer is preferred because TDG can be measured in situ. Weiss saturometers have different names, including tensiometer, gasometer, membrane-diffusion method, and total gas pressure meter, but to our knowledge the basic construction is similar for all (Figure 1). The probe consists of a sealed coil of silastic tubing which is permeable to gases and water vapor but not to liquid water. A pressure gauge inside the TDG probe measures the gas pressure in the silastic tubing (which we refer to as the TDG pressure), while a separate pressure gauge in the dry portion of the saturometer measures aerial atmospheric pressure. Percent (%) supersaturation is calculated based on the difference between the TDG pressure of the water being measured and the surface aerial atmospheric pressure and is reported as %TDG. Weiss saturometers have been used in numerous studies, but methods for their calibration and use are rarely described in detail in either the gray or published literature (although detailed protocols can be found in American Public Health Association, 2017; Arntzen et al., 2009; Tanner & Johnston, 2001). Calibration is critical for making accurate measurements. For example, the authors use two saturometers; the TDG pressure gauge reading was 21 mmHg (3% TDG) lower than the actual pressure for one of these upon purchase and the other had a TDG pressure gauge reading that drifted by −10 mmHg (−1% TDG) within a week of purchase. Given that 110% TDG is frequently used as a management guideline for the protection of aquatic animals from GBT, inaccuracies in TDG measurements of this magnitude represent a potentially important error. The lack of information on the calibration and use of Weiss saturometers means that those conducting environmental monitoring and research are left to discover best practices by trial and error, which may lead to measurements with low accuracy.

Here we provide a design for an inexpensive calibration chamber and recommend best practices for calibrating Weiss saturometers. We also describe methods for taking accurate TDG measurements, including multiple different methods for preventing bubbles from forming on or adhering to the TDG probe. Air bubbles in contact with the silastic tubing tend to result in TDG readings that are below the ambient TDG in the water (American Public Health Association, 2017). We also recommend that TDG supersaturation be reported as both a change in units of absolute pressure (i.e., ΔmmHg) and %TDG, so that measurements can be compared between studies. We hope these suggestions will be helpful to dam operators, environmental consultants, environmental agencies, and researchers engaged in TDG monitoring and research.

2. Calibration Chamber Design

A calibration chamber for the TDG probe can be constructed from readily available materials. The calibration chamber must be air-tight and equipped with an air valve, a pump, and a pressure gauge. We constructed such a pressure chamber from PVC pipe (Figure 2). A removable cap attaches to the threaded end of the pipe so that the chamber can be opened and the TDG probe placed inside. The other end of the chamber is covered with rigid acrylic. The cord of the TDG probe exits through an opening in the rigid acrylic at the end of the chamber; this opening is sealed with an O-ring which is in contact with the TDG probe housing to prevent air leaks. A plastic ring surrounding the cord of the TDG probe is screwed onto the end of the pipe to prevent the probe from being forced out of the chamber when it is pressurized. We attached a Schrader air valve to a threaded opening in the chamber. A pressure gauge was attached to a separate opening (model DPGA-05, Dwyer Instruments Inc.); this gauge can easily be removed for factory calibration or replacement. All openings, except the one equipped with the O-ring, were sealed with thread seal tape. The chamber can be pressurized via the air valve using a bicycle pump.
3. Calibration Procedure

As described above, a Weiss saturometer contains two pressure gauges that must be calibrated (Figure 1). Our calibration procedures are based on those reported by Tanner and Johnston (2001). It can be difficult to conduct a two-point calibration on the atmospheric pressure gauge of a saturometer, so we propose a one-point calibration by correcting the reading to current atmospheric pressure. Practitioners can measure the current atmospheric pressure using a separate barometer or they can access the most recent atmospheric pressure reported by a nearby weather station if they are located at sea-level. Weather stations generally correct atmospheric pressure to sea-level and thus atmospheric measurements from these sources will be inaccurate at other altitudes. The TDG pressure gauge can be calibrated using a two-point calibration on a dry TDG probe. Bouck (1982) describes a calibration protocol for a wet probe, but because of difficulties ensuring complete gas transfer in a wet calibration chamber, we prefer a dry calibration method. The first point can be measured at atmospheric pressure. The TDG probe must be pressurized to measure the second calibration point, which can be accomplished using a commercial pressure calibrator or by constructing one’s own pressure chamber (Figure 2, described above). We recommend choosing a second calibration point with a pressure that is greater than the maximum TDG that one anticipates measuring with the probe. The TDG probe should not be exposed to pressure exceeding the maximum specified by the manufacturer or there is a risk of damaging the probe. The probe will take several minutes to equilibrate to the increased pressure; once it has equilibrated it can be compared to the pressure measured by a separate gauge attached to the pressure calibrator or the pressure chamber. The regression between the two point-measurements of the TDG gauge of the saturometer and the actual pressure can be used to correct the readings of the TDG gauge (Figure 3).

In addition to calibration it is necessary to test for tears in the silastic tubing of the TDG probe during field sampling because debris and aquatic organisms can cause damage. Tanner and Johnston (2001) describe submerging the TDG probe in carbonated water to test for tears; they report that the TDG reading will increase gradually with an intact probe but rapidly in a probe with damaged silastic tubing where carbonated water quickly enters the tubing.

4. TDG Measurement

We recommend deploying TDG probes below the compensation depth for bubble growth. The primary concern with deploying saturometer probes is bubbles being in contact with the silastic tubing when the probe is above the compensation depth, which will result in an underestimation of TDG (American Public Health Association, 2017). There are multiple

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**Figure 2.** Calibration chamber. (a) Schematic of the calibration chamber. (b) Open calibration chamber with total dissolved gas probe. (c) Closed chamber with pump attached to the air valve. (d) Legend of the chamber components.

**Figure 3.** The relationship between the actual pressure generated in the calibration chamber above ambient (ΔP in mmHg), and that measured by the total dissolved gas probe above ambient (ΔP in mmHg) (Pleizier, Cooke, & Brauner, 2021). Six measurements were made at each pressure (n = 6) but the variation between measurements are contained within the diameter of the symbol and are not visible due to the low variation between readings.
methods for minimizing this problem; the preferred method is to measure TDG below the compensation depth. The compensation depth for bubbles suspended in the water column can be defined as the depth at which hydrostatic pressure is equal to, or even slightly greater than, the TDG pressure in the water (Figure 4). This is different from the compensation depth for bubble growth in fish tissues, which depends in part on conditions inside the fish’s body (Fidler, 1985; Pleizier, Nelson, et al., 2020). Hydrostatic pressure can be defined by the following equation,

\[ P = \rho \times g \times h \]  

(1)

In which \( P \) is the hydrostatic pressure, \( \rho \) is the density of water, and \( g \) is gravitational acceleration (9.81 m/s²), and \( h \) is the depth. The density of water (\( \rho \)) changes with temperature, but changes in the density of water are negligible in the range of temperatures experienced by most fish in rivers. Other factors that can affect water density include large volumes of bubbles in the water, which reduces the density of the water, and dissolved solids such as silt and salt, which can increase water density. In the absence of these factors, hydrostatic pressure increases by 9.7% of an atmosphere per meter depth in freshwater. According to Henry’s Law, the amount of dissolved gas that will be contained in a solvent at a given temperature is directly proportional to partial pressure of the gases in the bubble at that depth. For this reason, air bubbles that have been mixed with water will dissolve and the total pressure of gases dissolved in the water will be in proportion to the hydrostatic pressure exerted on the bubbles at that depth. As a result, more gases can be contained in water as depth increases. Bubbles will collapse when the ambient pressure is greater than the pressure inside the bubble and this prevents bubbles from forming or adhering on the TDG probe and thus improves the accuracy of measurements.

We recommend mechanically removing bubbles from TDG probes when it is not feasible to deploy the TDG probe below the compensation depth. This is especially important if probes are to be deployed for continuous measurements. Options for bubble removal include deploying the probe in flowing water that is strong enough to remove bubbles, attaching the probe to a mechanical shaker, shaking the probe manually during measurements, or using a saturometer that is equipped with a mechanical stirrer strong enough to remove bubbles.

Another consideration for deployment is the time necessary for TDG to equilibrate across the silastic tubing of the probe. Unlike other probes, such as dissolved oxygen probes that give stable measurements after several seconds, TDG probes can take 15–30 min to equilibrate, depending on the temperature and the TDG level. If the probe remains in TDG supersaturated water between measurements the time to equilibrate is reduced. When making measurements we recommend waiting until the %TDG reading is stable for two minutes before recording the reading, as described in Tanner and Johnston (2001).

5. Reporting

We recommend reporting TDG in both units of absolute pressure and as a % of aerial atmospheric pressure (%TDG). Percent TDG is calculated as a percent of aerial atmospheric pressure using the following equation:

\[ \% \text{TDG} = \left( \frac{\Delta P + P_{\text{am}}}{P_{\text{am}}} \right) \times 100 \]  

(2)

In which \( \Delta P \) is the difference between the TDG pressure measured in the water and \( P_{\text{am}} \) (ambient aerial atmospheric pressure). The equation illustrates how a constant \( \Delta P \) can result in differences in %TDG, if studies are conducted in locations with different ambient atmospheric pressure. For example, at sea level, water with a \( \Delta P \) that is 76 millimeters of mercury (mmHg) greater than \( P_{\text{am}} \) (where \( P_{\text{am}} \) is about 760 mmHg) will be 110% TDG. Whereas at an altitude of 2,000 m above sea level (where \( P_{\text{am}} \) is ~600 mmHg), a similar \( \Delta P \) of 76 mmHg will result in about 113% TDG. This is of significance as it is the \( \Delta P \) across a bubble or membrane.
that is the driving force for gas diffusion and eventual equilibration. In the previous exercise $\Delta P$ is constant ($\Delta P = 76$ mmHg), as would be the potential for bubble growth and GBT, however, $\%$TDG differs. For this reason, Colt (1983) recommends reporting levels of supersaturation as $\Delta P$ rather than $\%$TDG. We recommend reporting TDG as both $\Delta P$ and $\%$TDG, or at least reporting $P_{atm}$ of the study to allow others to calculate $\Delta P$. Unfortunately, most of the TDG literature does not report $P_{atm}$ which likely leads to some of the variation in the relationship between $\%$TDG and symptoms of GBT. Out of convention, $\Delta P$ has been reported in mmHg in the TDG literature (where 1 mmHg = 0.133 kPa). Figure 5 shows a comparison of various units of pressure.

A few older studies report $\%$TDG as a $\%$ of saturation relative to the depth of measurement, which is a potential source of confusion. As discussed above, as water depth increases so does hydrostatic pressure and, as a result, the amount of gas that can be dissolved at that depth (Henry’s law). However, $\%$TDG as measured by a Weiss saturometer does not change with depth as it is calculated as a $\%$ of atmospheric pressure, not as a $\%$ of saturation. According to the common convention of reporting $\%$TDG as a $\%$ of atmospheric pressure, water in a well mixed water column at sea level with a $\Delta P$ of 76 mmHg would be reported as 110% TDG throughout the water column. This is convenient, not only because this is the value that is reported by the saturometer, but also because neither water nor aquatic animals tend to remain at a constant depth over time. Unless animals or water always remain below the compensation depth, at 110% TDG there is the potential for bubble formation in surface waters and in animal tissues at the surface, which can lead to GBT. Thus, expression of TDG levels relative to $P_{atm}$ provides a conservative indication of the potential for negative effects related to elevated TDG. As more is known about the depth inhabited by an animal over time, then some modification factor can be introduced to account for this. Regardless of the convention used, the method of calculating $\%$TDG should be stated explicitly to prevent any confusion.

6. Conclusions

By regularly calibrating equipment for measuring TDG practitioners can ensure that TDG measurements are accurate. In addition, ensuring that bubbles do not adhere to the silastic tubing of the TDG probe and reporting TDG measurements in a standardized manner will produce results that are easy to interpret and compare between studies. It is our hope that these practices and a general theoretical description of why they are important will result in more reliable data, which is beneficial for both research and decision-making. For example, repeatable TDG measurements will make it easier to identify thresholds of effect of TDG supersaturation on aquatic animals (Pleizier, Algera, et al., 2020). We hope that clarifying best practices will also encourage others to undertake TDG and GBT monitoring and research, as these continue to be environmental challenges downstream of features such as dams.

Figure 5. Relative scales of different units of pressure. Units are atmospheres (atm), kilopascals (kPa), pounds per square inch, millimeters of mercury (mmHg), and percent total dissolved gas ($\%$TDG) at an atmospheric pressure of 760 mmHg.
**Data Availability Statement**

The calibration data in Figure 1e can be found in the UBC Research Data Collection, Scholars Portal Dataverse [https://doi.org/10.5683/SP2/S6QTE8].

**References**


