



Monterey Bay Aquarium Research Institute

Solutions out of thin air: assessing atmospheric calibration of oxygen sensors for underwater gliders

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ABSTRACT

Advances in autonomous biogeochemical sensing and platform technology have increased our spatial and temporal data collection capabilities, but reliable and practical calibration of such sensors remains a crucial challenge. It has been demonstrated that oxygen optode sensors can be accurately calibrated in situ to several tenths of a percent by making measurements in air due to the virtually constant mole fraction of oxygen in the atmosphere. However, optodes that are in a pumped flow stream such as the SBE63 equipped on Spray Gliders cannot be exposed to the atmosphere while deployed. Alternatively, the optodes could be air-calibrated pre- and post-deployment. Here, we tested a simplified air calibration protocol utilizing inexpensive components without temperature control and compared the results to those obtained from Winkler titrations. We conducted three experiments involving: 1) measurements in a laboratory-controlled environment; 2) an underway line during a week-long cruise, and 3) an underwater glider deployment in Central California. In all cases, the simplified air calibration agreed with Winkler titrations to better than 1%: 0.46% in the laboratory, 0.23% and 0.64% for the underway, and 0.80% on the glider within the surface mixed layer (0 – 20 m). Larger errors were observed when there was active evaporation in the optode flow cell, causing either changes in humidity or air temperature during air calibration.

INTRODUCTION

The ability to measure high resolution biogeochemical data is becoming increasingly important as anthropogenic processes continue to alter major chemical parameters within our oceans. In

particular, the dynamics of dissolved oxygen has significant biological and chemical implications for marine systems. Serving as an essential ingredient for respiratory processes, dissolved oxygen measurements are crucial towards calculating levels of net ecosystem metabolism and carbon export, which ultimately influences the oceanic intake of atmospheric CO₂ (Emerson et al. 1997, Emerson et al. 2008). Moreover, routine oxygen measurements have been key towards understanding and monitoring areas of reduced oxygen concentration such as upwelling regions and other hypoxic/anoxic zones (Kamykowski & Zentara, 1990, Harrison et al. 2016). However, the ocean is highly dynamic and chronically under sampled. Thus, higher spatiotemporal resolution data sets are required to further our current understanding of natural biogeochemical cycles as well as how they are changing in the Anthropocene, especially for dissolved oxygen.

While seawater sampling and analysis using research vessels have and will always serve as prominent tools for data collection, advancements in autonomous sensor and instrumentation technology are increasing our ability to monitor oxygen on growing spatial and temporal scales. In particular, optical oxygen sensors based on dynamic luminescence quenching (referred to as optodes hereafter) are one of the most successful chemical sensors in oceanography. Such systems are especially useful on autonomous platforms such as profiling float networks where large sections of ocean can be continuously monitored (ARGO Network, Jayne et al. 2017; SOCCOM Program, Johnson et al. 2017). In the coastal ocean, autonomous underwater gliders are particularly effective. Gliders can profile and navigate an adjustable route while collecting oceanographic data, which is then sent back to shore when the glider surfaces. Over the years, research groups have spawned various designs ranging from Seagliders (Eriksen et al. 2001), Slocum gliders (Teledyne Webb Research, Schofield et al. 2007), and Spray gliders (Rudnick et al. 2004). Here, we focus our discussion on the Spray glider because it is the type of glider that is operated in the Chemical Sensor and Instrumentation lab at MBARI. These gliders are typically equipped with sensors to measure conductivity, temperature, depth, dissolved oxygen, turbidity, and chlorophyll fluorescence (Rudnick et al. 2004).

Although autonomous sensors have simplified this data collection process in certain areas, accurate measurements require robust calibration. With respect to optode-based dissolved oxygen sensors, exceptional stability has been demonstrated once deployed (Körtzinger et al. 2005, Tengberg et al. 2006); however, they rapidly drift during storage, leading to poor accuracy

when deployed (D'Asaro & McNeil, 2013). Biases as large as 10-15% at the surface have been routinely observed on profiling floats equipped with optodes (Takeshita et al. 2013). In order to account for these unavoidable errors, robust sensor calibration protocols must be an essential part of any observational program. One approach would be to collect discrete samples alongside the glider at the time of deployment or recovery. However, this requires access to research vessels that are capable of conducting hydrocasts, among other potential issues such as spatio-temporal mismatch between hydrocast and glider data and access to Winkler titration equipment. Thus, a simpler approach to calibrating optode sensors is desired.

Numerous lines of evidence suggest that a gain correction is sufficient to accurately correct the drift in optodes (Takeshita et al. 2013, Bittig & Körtzinger, 2015, Johnson et al. 2015):

$$[O_2]_{calibrated} = [O_2]_{raw} * Gain$$

A lack of zero drift of oxygen optodes has been well-verified (Bittig & Körtzinger, 2015), thus a single gain factor is sufficient to correct raw sensor data. Although Winkler titration provides the gold standard for accurate chemical analysis of dissolved oxygen concentration (Emerson et al. 1999, Uchida et al. 2008), they require substantial expertise and instrumentation to conduct properly.

An alternative solution for optode calibration lies with air calibration in which true values of oxygen are calculated via atmospheric measurements instead of Winkler titration. Since the mole fraction of atmospheric oxygen is essentially constant ($\chi_{O_2} = 0.20939$, Khélifa et al. 2007), the atmospheric concentration of oxygen can be calculated as long as atmospheric pressure, relative humidity, and temperature are known. The equilibrium concentration of oxygen depends upon the solubility constant of dissolved oxygen and partial pressure of oxygen, and can be calculated by:

$$\begin{aligned} [O_2] &= K_{sp}(O_2) * pO_2 = K_{sp}(O_2) * \chi_{O_2} * (P_{atm} - pH_2O) \\ &= K_{sp}(O_2) * \chi_{O_2} * (P_{atm} - \varphi * pH_2O_{sat}) \end{aligned}$$

where the mole fraction (χ_{O_2}) is a constant, total atmospheric pressure (P_{atm}) and relative humidity (φ) are measurable parameters, and saturation vapor pressure (pH_2O_{sat}) and the solubility constant of oxygen ($K_{sp}(O_2)$) are temperature-dependent and well-characterized

(Garcia & Gordon 1992). Previous work with optode air calibrations have been quite successful, with gliders and oceanic floats performing mid-deployment air calibrations and demonstrating errors of around 0.5 – 1% (Johnson et al., 2015, Bushinsky et al., 2016, Nicholson & Feen, 2017).

Nevertheless, there are more barriers to air calibration with other ocean glider instruments. Argo floats can surface temporarily, allowing for on-board optodes to make atmospheric measurements during deployment while more specific glider modifications (placing the optode on top of the glider, like in Nicholson & Feen, 2017) can also allow for reliable mid-deployment air calibrations. While possible with these instruments, our Spray gliders are equipped with the SBE63 optode, which is plumbed into a pumped flow stream. Thus, the optode cannot be exposed to the atmosphere at the surface. The benefit of having it pumped is significantly faster response time (Bittig et al. 2014), which is critical for deployments along our coast where sharp oxyclines exist. As such, Spray glider air calibrations would have to occur before or after deployment, a practice that has not been well-documented.

For those reasons, our focus is to assess the uncertainty of conducting pre- and post-deployment atmospheric calibrations on Spray glider oxygen optodes. The optodes themselves, once attached, are difficult to remove from the Spray glider system, thus restricting capabilities for controlled calibrations. Therefore, our attention was centered on whether a more simplified air calibration procedure could produce high quality results observed in previous works, which utilized more detailed and expensive methodology (< 0.1% from Bushinsky & Emerson, 2013; - 0.2% from Bushinsky et al. 2016). We compared our air-calibrated oxygen values to oxygen measurements obtained via Winkler titration to assess its accuracy. Here, we demonstrate a multi-stage procedure to approach this question.

MATERIALS AND METHODS

To assess the viability of our simplified air calibration design, we compared the accuracy of air-calibrated sensor readings to Winkler titrated measurements under three different experimental settings: laboratory-control, shipboard underway, and spray glider. In all settings, Winkler calibrations involved the collection of raw oxygen optode values in sampled water alongside Winkler titrations. Winklers and raw outputs are temporally aligned and compared to produce a Winkler gain value. Air calibrations were conducted with the optode exposed to flowing air. Raw

outputs from the optode as well as barometric data are collected in order to calculate the air calibration gain. Specific air calibration gain calculations were based upon the formulation used in Johnson et al. 2015:

$$Gain = \frac{K_{sp}(O_2) * (P_{atm} - \varphi * pH_2O_{sat})}{[O_{2,raw} * (1013.25 - pH_2O_{sat})]}$$

where total atmospheric pressure (P_{atm}), relative humidity (φ), and temperature (used to calculate pH_2O_{sat} and $K_{sp}(O_2)$) were used to calculate atmospheric oxygen concentration, which, when divided by the raw oxygen output from the optode ($O_{2,raw}$) and air pressure at 1 atm, provides the gain factor.



Figure 1: Depictions of lab-controlled calibration experiments consisting of Winkler-based calibration (left) and simplified air calibration (right).

LABORATORY-CONTROLLED CALIBRATION

An Aanderaa 4835 oxygen optode (Aanderaa Data Instruments AS) was placed within a 20L Nalgene Rectangular Autoclavable PPCO Carboy with a spigot (Fisher Scientific, USA). The carboy was filled with deionized Milli-Q water, which spent several days equilibrating at lab temperature. A Uniclife UL40 aquarium pump (Uniclife) was used to bubble air near the surface to keep the water at a stable oxygen concentration; the solution was stirred with a stir bar (*Figure 1*). Tera Term was used to record optode readings in the carboy every 5 seconds. Once optode readings were stable to $< 1\mu\text{M}$ over 12 hours, Winkler samples were collected using a spigot attached to an adapter and silicone tubing following best practices (Dickson, 1996). Winkler

oxygen values were then compared to the raw oxygen output from the optode to calculate the Winkler gain factor.

Air calibrations were carried out by placing the Aanderaa 4835 in a custom-built flow cell (*Figure 1*). Ambient air was then run through the flow cell using a UL40 aquarium pump. Raw optode oxygen concentration, air saturation, and temperature values were monitored for stability using Tera Term software. Once stable values were achieved, a \$130 Digi-Sense Digital Barometer with NIST-Traceable calibration (± 4 mbar, $\pm 3\%$ (RH), $\pm 0.4^\circ\text{C}$, Cole-Parmer) was used to collect atmospheric pressure, relative humidity, and temperature measurements.

Air calibration system precision and repeatability was tested by conducting multiple air calibrations in different locations at the Monterey Bay Aquarium Research Institute from mid-July to early August. Air calibrations were conducted at the main laboratory ($n = 9$), in a nearby staff kitchen ($n = 2$), and on the balcony of one of the buildings on campus ($n = 2$). Just like before, air calibrations began when optode readings (oxygen, air saturation) stabilized, and several minutes of optode oxygen and temperature data were averaged and combined with the barometer readings to calculate the true atmospheric oxygen concentration.

FIELD CALIBRATIONS: SHIPBOARD OPTODES

To assess the effectiveness of an air calibration in the field, 2 SeapHOx (sp1 & sp3) sensors were connected to the underway flow system of the *R/V Western Flyer* during the Central California Carbon, pH, and Oxygen (C3PO) cruise from July 23-29, 2019 (*Figure 2*). The cruise's mission was to conduct CTD casts at all stations in CalCOFI lines 67, 73, and 80 (California Cooperative Oceanic Fisheries Investigations). The optodes within both SeapHOx sensors were calibrated before and after the cruise using the air



Figure 2: The route taken by the *R/V Western Flyer* during the July 2019 C3PO cruise (red line). Individual markers represent locations where CTD casts and/or underway seawater samples were taken and processed.

calibration procedure explained above. Optode oxygen concentration and temperature was measured every 15 minutes. Winkler values were generated by sampling and titrating seawater through a lab sink which was also connected to the ship's underway system ($n = 23$). The sensor timestamp was interpolated to the time discrete samples were collected for comparison.

FIELD CALIBRATIONS: SPRAY GLIDER

A Spray glider was deployed on July 16, 2019 at sampling station C1 using the *R/V Paragon*. The glider's SBE63 (Sea-Bird Scientific, USA) oxygen optode was calibrated prior to deployment using our simplified air calibration method. From there, the glider continued westward along CalCOFI survey line 67, where it would make more than 100 dives reaching depths up to 1000m (*Figure 3*). A CTD cast was conducted near station

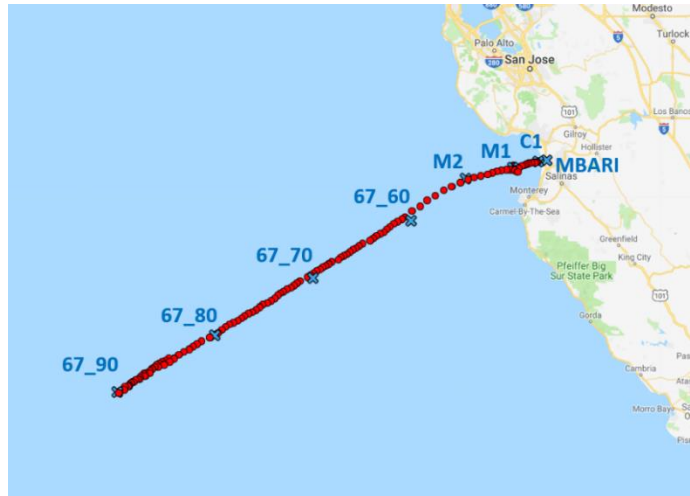


Figure 3: The route taken by the Spray glider deployed prior to the C3PO cruise. Each red dot represents the location where the glider surfaced while CTD cast stations are shown in blue.

67-70 to collect discrete samples alongside a glider profile ($n = 12$) to a depth of 500 m; the shipboard cast and glider profile were within 500 m and 1 hour. Real time data transmitted by the glider was used for the comparison.

RESULTS

LABORATORY-CONTROLLED CALIBRATION

Our in-lab tests served to not only constrain the accuracy of our air calibration system with respect to Winkler calibration values but also assess the precision of our simplified air calibration method over time and within different

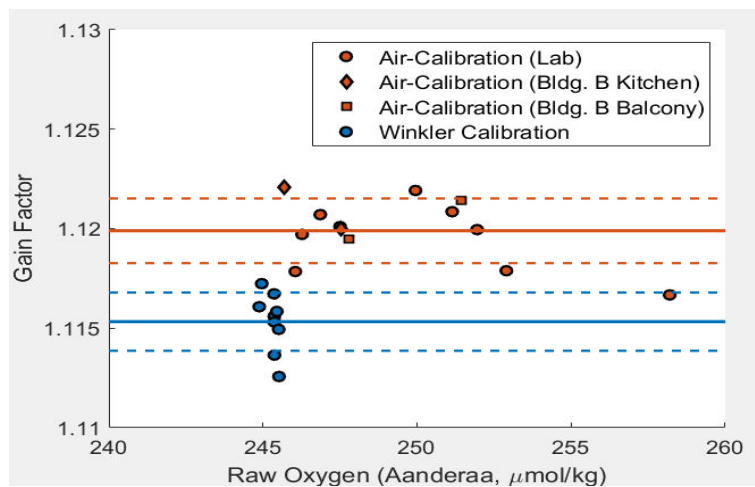


Figure 4: A comparison of calculated gain factors from lab-based Winkler and air calibrations, separated by color. Different shapes indicate different locations where air calibrations took place. Mean gains are represented by the solid line while dotted lines represent the standard deviation.

locations. Air calibration temperature readings from three locations around MBARI ranged from 19.96 to 22.14°C while measured oxygen concentration ranged from 245.69 to 258.2 μM. The mean gains based on air calibrations and Winkler's were 1.120 ± 0.0016 (1σ) and 1.115 ± 0.0015 (1σ), respectively (Figure 4). There were no clear patterns of gain obtained from different locations at MBARI. The average difference between air and Winkler based gains was only 0.46%.

SHIPBOARD OPTODES

Pre-cruise calibrations yielded gain values of 1.066 and 1.041 for sp1 and sp3 respectively while post-cruise calibration gains increased to 1.073 and 1.064 respectively. For both sensors, a significant increase in gain was observed for the post deployment calibration. Winkler gain values were 1.063 ± 0.004 for sp1 and 1.047 ± 0.007 for sp3 and did not appear to experience significant drift over time for both sensors ($\text{Gain}_{\text{sp1,slope}} = -0.00017 \pm 0.00069$, $R^2 = 0.0032$, $P = 0.80$; $\text{Gain}_{\text{sp3,slope}} = 0.0019 \pm 0.0010$, $R^2 = 0.14$, $P = 0.087$; Figure 5). Pre- and post-cruise gains were applied to the underway data to determine which calibration gain yielded the most accurate oxygen values. Pre-cruise gains were more consistent with Winkler gains and differed by only 0.23% for sp1 and by 0.64% for sp3. Meanwhile, post-cruise gains differed by 0.97% and 1.63% for sp1 and sp3 respectively.

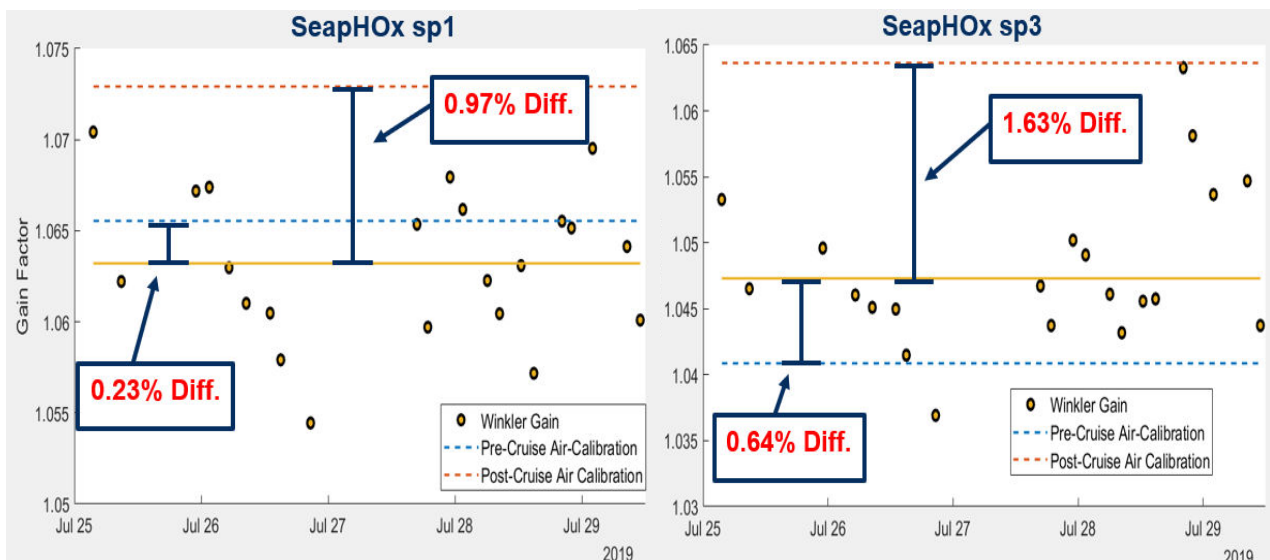


Figure 5: Comparisons of calculated gain factors among Winkler, pre-cruise air, and post-cruise air calibration methods for both deployed SeapHOx sensors. Individual Winkler gain values are represented by yellow markers while lines represent mean gain values (coordinated by color).

SPRAY GLIDER

Spray glider oxygen data were aligned to cast oxygen by linearly interpolating based on pressure measurements taken at each Niskin bottle depth. For the entire profile, air-calibrated oxygen readings from the spray glider were far more offset from Winkler values than in our previous experimental stages ($\Delta(\text{AirCal} - \text{Winkler}) = 9.01 \pm 10.61 \mu\text{mol/kg}$, Error = $6.91 \pm 8.94\%$) though they were still improvements over raw output readings ($\Delta(\text{Raw} - \text{Winkler}) = 11.51 \pm 12.25 \mu\text{mol/kg}$, Error = $7.61 \pm 9.89\%$, *Figure 6*). This resulted in a gain difference of $5.60 \pm 14.91\%$ between Winkler and air calibration. However, if we were to examine values from only the top 20 m of the water column, the previous gain differences are noticeably reduced ($\Delta(\text{AirCal} - \text{Winkler}) = 1.94 \pm 0.12 \mu\text{mol/kg}$, Error = $0.77 \pm 0.050\%$; $\Delta\text{Gain} = 0.80 \pm 0.052\%$). Interestingly, increases in accuracy are observed at depth (300-500m) as well ($\Delta\text{Gain} = 0.043 \pm 0.022\%$).

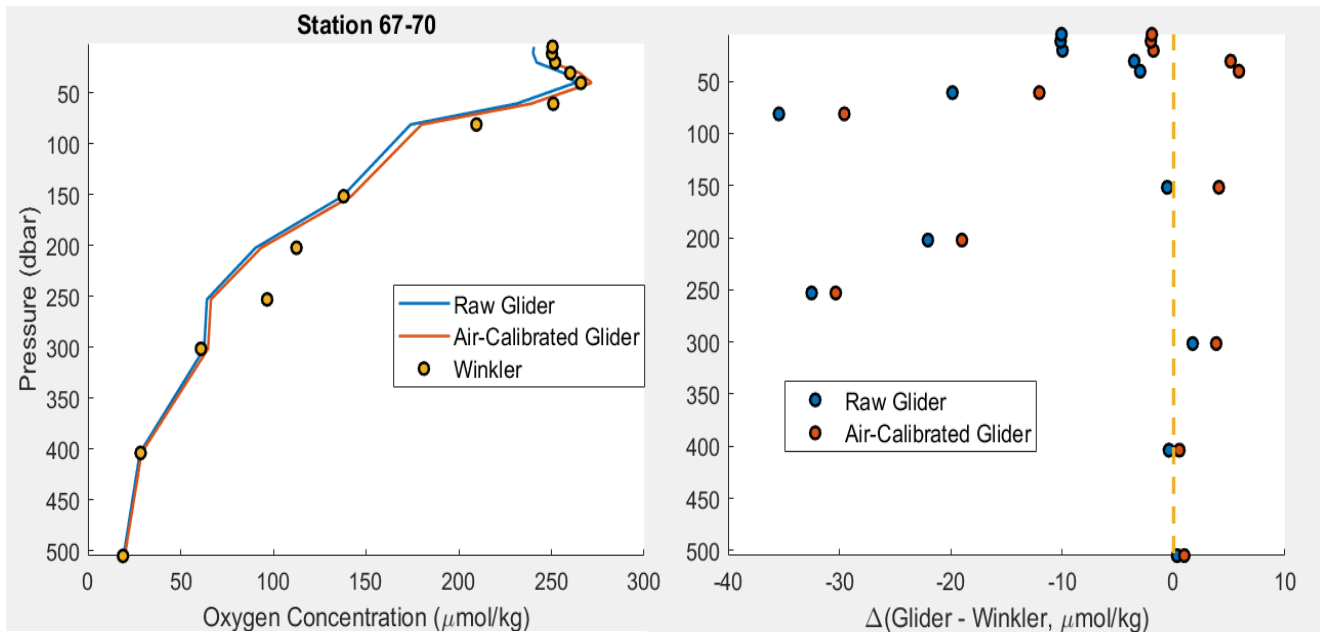


Figure 6: On the left, a pressure-based depth profile displaying oxygen concentrations measured from CTD cast-based Winkler titrations as well as from raw and air-calibrated Spray glider data (interpolated to CTD cast depths). On the right, a residual plot (flipped to portray the pressure/depth profile) compares differences of raw and air-calibrated Spray glider oxygen concentrations from Winkler-based concentrations.

DISCUSSION

The overlying goal of this three-stage project was to constrain and assess the performance and accuracy of a simplified air calibration procedure on different oxygen optodes within different experimental settings. The final stage was to test this method on a spray glider optode in order to

verify whether such a procedure would generate results with comparable accuracy to previous works.

Our air calibrations involved several major shortcuts with respect to previous assessments such as in Bushinsky & Emerson, 2013. Firstly, there was no temperature control implemented during the lab-control testing. It has been demonstrated that changes in temperature can influence the accuracy of an air-calibrated value with respect to the true oxygen concentration, with errors increasing by about 1.5% with a temperature drop from 25°C to 10°C (Bushinsky & Emerson, 2013). Although our lab-controlled stage did not experience such sheer changes in ambient temperature, it should be acknowledged that changes of a degree or two could potentially lead to around 0.5% error in our accuracy. Another shortcut results from a relatively cheaper barometer, which measured conditions outside of the optode flow cell. The Digi-Sense barometer could measure atmospheric pressure with around 0.4% error and relative humidity at around 5% error (“Digi-Sense Traceable”). However, Bushinsky & Emerson, 2013 used a Paroscientific Model 223A-102 Pressure Transducer ($\pm 0.005\%$, Payne, 1995) and ensured that air calibrations were always conducted at 100% humidity. Similarly, Bushinsky et al. 2016 used this approach with a Model 223A-101 ($\pm 0.01\%$ accuracy, Wearn & Larson, 1982). Most importantly, their pressure sensor was placed within the optode flow cell while our barometer was separate, thus eliminating potential inaccuracies that our system might have encountered. Although our lab-based accuracy of 0.46% is impressive considering the relatively simple nature of this lab-based setup, it should be reiterated that even more accurate results are not only expected but have been documented using more intricate and expensive methods ($< 0.1\%$ from Bushinsky & Emerson, 2013; -0.2% from Bushinsky et al., 2016).

In-situ results from air-calibrating SeapHOx optodes showed similar accuracies (0.23% for sp1, 0.64% for sp3) using the same simplified air calibration procedure. These specific percentages were obtained from the pre-cruise air calibration, which displayed noticeably better accuracy than the post-cruise air calibration (0.97% for sp1, 1.63% for sp3). This is likely not due to sensor drift because Winkler gains displayed little evidence of drift over the cruise, but a bias that results from the methodology. A likely explanation for such an offset in air calibration values (separated only by a week) could be from errors in relative humidity. During pre-cruise calibrations, the SeapHOx flow cell had not been exposed to water for some time and was

properly dried out. On the other hand, post-cruise calibrations were carried out immediately after the end of the cruise. Although care was taken to dry the flow cell before air calibration, it is possible that some residual seawater could have introduced errors in measured relative humidity. When gains are calculated assuming 100% relative humidity for the post-deployment calibration, it agrees better with both the pre-deployment and Winkler gains. This type of error could be avoided by either integrating our barometric sensors within the optode flow cell itself, ensuring a dry flow cell, or using 100% humid air for the calibration by bubbling it through water first.

Interpolated Spray glider measurements were highly accurate to Winkler-based CTD cast data near the surface (0-20 meters) and at depth (300-500 meters). This is likely because these are sections of the water column that do not have a large vertical gradient in oxygen. Raw and calibrated glider oxygen readings were both highly accurate to Winkler readings from 300 to 500 meters though raw readings appeared slightly more accurate than calibrated ones at the 300- and 500-meter marks (*Figure 6*). Nevertheless, the small sample size of this observed behavior ($n = 2$) and the expected errors involved with Winkler titration measurements (around 0.3 – 0.5 $\mu\text{mol/kg}$, K. Conner, unpublished data) prevent us from concluding whether air calibration worsened glider oxygen accuracy at depth. The large discrepancy between glider and shipboard oxygen between 20-300 m is likely caused either by the large vertical gradient in oxygen or that they were in different water masses. During strong vertical oxygen gradients, larger residuals are expected due to sensor response time (Takeshita et al. 2013). Although we matched the CTD cast and Spray glider dive spatially (~500 meters) and temporally (~1 hour) to the best of our abilities, it is quite possible that different water bodies were sampled between the CTD and glider. If anything, these results not only demonstrate the viability of simple air calibrations towards correcting surface seawater measurements, but also the difficulty of calibrating oxygen readings in the oxycline.

CONCLUSIONS/RECOMMENDATIONS

Using a simplified air calibration procedure without temperature control and a relatively cheap barometer not integrated within the optode flow cell, we were able to obtain highly accurate air-calibrated oxygen readings that were within 1% of corresponding Winkler titration-based values. Specifically, lab-controlled gains differed by only 0.46% while in-situ SeapHOx-based gains differed by 0.23% and surface spray glider gains by 0.80%. Although our air-calibrated Spray

glider readings were accurate near the surface, obtaining accurate midwater comparisons were still difficult. Nevertheless, this simplified air calibration method has been demonstrated to provide quality calibration results for near-surface oxygen optode sensor measurements. To obtain the most accurate atmospheric air calibration measurements, we recommend that barometric sensors be integrated within the optode flow cell. If not, optode flow cells should be properly dried in lab for around a day before conducting pre- or post-deployment calibrations.

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