Assessing the Accuracy and Long-Term Stability of SeapHOx Oceanographic pH Sensor in Freshwater and Estuarine Environments

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Introduction

pH is an important parameter which helps determine ecosystem health in aquatic environments. In marine environments pH is naturally basic (~8.1) and is controlled by carbon dioxide concentration, temperature, and carbonate buffering (Feely et. al, 2009). In estuaries, pH is controlled by similar variables as marine systems but is also altered by the tides and typically has a pH ranging from 7.0 – 8.5 pH (Hall et. al, 2023). In these environments, pH decreases have been well documented and have demonstrated adverse effects on both heterotrophic and autotrophic species which can have cascading effects through food webs (Kroeker, 2010). Estuaries are especially impacted by acidification because this process decreases the availability of carbonate, which greatly impacts shellfish and other calcifying organisms (Feeley et, al, 2010). In contrast, freshwater environments have a lower buffering capacity leaving temperature as the main driver of pH change (Phillips et al, 2015). Therefore, this results in naturally more acidic waters between pH 6.5 and 7.8 in freshwater ecosystems (US EPA, 2015). In all these environments, surface pH is expected to continue decreasing due to anthropogenic processes and will continue to impact the water chemistry and ecosystems at large (Feeley et. al, 2010).

Measuring pH accurately is critical because small changes can result in significant ecological outcomes. pH is reported on a logarithmic scale so changes between single units indicate a 10X change in hydrogen ion concentration (US EPA, 2015). Over the last 50 - 100 years, marine environments around the world have experienced pH change differently. For example in open water conditions like Hawaii, pH has dropped 0.1 units and continual acidification trends are being monitored through the Hawaii Ocean Time Series (HOTS) (Knor et al., 2025). However in coastal areas with complex geomorphology such as Puget Sound, each basin experiences acidification slightly differently which results in areas that are geographically

close to each other having large differences in pH (Feeley et. al, 2010). In more tropical environments, such as the Great Barrier Reef off the coast of Australia, ocean acidification is already being experienced greatly. pH in this location has decreased by 0.2 pH and is expected to decrease by 0.1 – 0.4 by 2100. This decrease has slowed coral growth and caused the coral in this area to bleach, turning from vibrant colors to muted or white (Guo et al., 2020). High latitude, polar, environments such as Alaska also experience pH decrease and face larger immediate threats because cold water absorbs more carbon dioxide (CO^2) than warm water in equatorial regions. This region has experienced measured decrease in carbonate availability, though the response to pH by organisms is species specific (Goethel et al., 2017). These examples highlight the importance of accurately measuring small pH changes to understand resulting environmental impacts. Doing so, especially in coastal and estuarine environments, can help us better document changes and protect these ecosystems.

Currently, long-term pH monitoring is limited by the reliability, low accuracy, and drift of traditional pH sensor technology. Many oceanographic pH sensors are prone to drift from their initial calibration points and traditional pH sensors, glass bulbs, are particularly sensitive to drift because they interact with hydrogen ions and other chemical ions in an aqueous solution repeatedly to make each measurement (Shahriar Jamasb, Collins, & Smith, 1998). Long term monitoring of these environments has not been instituted due to the limitation of these traditional pH sensors. Less is known about freshwater environment pH and acidification — this is largely because reliable, robust and pressure tolerant pH sensing technology has been typically designed for marine environments. To support much needed long-term monitoring of other environments, we tested a proven pH sensing technology developed for oceanographic settings to determine its accuracy and stability for use in both a freshwater environment and a dynamic tidally influenced

estuarine environment. To conduct this study, we tested the Deep and Shallow SeapHOx™ V2 pH sensor (Fig. 1) which uses Ion Sensitive Field Effect Transistor (ISFET) pH sensing technology. These pH sensors are manufactured by Sea-Bird Scientific and were deployed over three months in freshwater and estuarine environments to assess the accuracy and long-term stability of the pH measurements.

Figure 1: Deep SeapHOx™ V2 pH sensor (left) and Shallow SeapHOx™ V2 pH sensor (right) each coupled with a SBE 37 SMP-ODO MicroCAT that measures temperature, conductivity, pressure and oxygen. Image courtesy of Sea-Bird Scientific

Methods

ISFET Technology

In this study, we tested the Shallow and Deep SeapHOx™ V2 which both utilize ISFET pH sensing technology with integrated conductivity (C), temperature (T), depth measured by pressure (D), and dissolved oxygen (DO). ISFET pH sensing uses H+ ions and a solid-state Cl-

reference electrode to create a solid-state electrochemical cell in which voltage changes when the concentration of H+ ions changes (Branham et al., 2016).

pH Calculation

Multiple equations were utilized to describe the pH response of these sensors. The Deep SeapHOx™ V2 pH response is described in Eq. 1. The Shallow SeapHOx™ V2 uses both an internal and external pH reference source, which are described in Eq.2 and Eq. 3 respectively.

$$pH_{cell} = \frac{v_{ref} - k_0 - k_2 * T - f(P) + \underline{V}_{HCl}\frac{P}{F}}{S_{nernst}} + \log(Cl_T) + 2 * \log(\gamma_{HCl}) - \log(\frac{S_T}{K_s}) \quad \text{(Eq.1)}$$

$$pH_{INTCell} = \frac{V_{INT} - k_{0\,INT} - k_{2\,INT} * t}{S_{nernst}} \quad \text{(Eq.2)}$$

$$pH_{EXT\,Cell} = \frac{V_{INT} - k_{0\,INT} - k_{2\,INT} * t}{S_{nernst}} + \log(Cl_T) + 2 * \log(\gamma_{HCl}) - \log\left(1 + \frac{S_T}{K_s}\right) - \log(\frac{1000 - 1.005 * S}{1000}) \quad \text{(Eq.3)}$$

$$S_{nernst} = \frac{R * T * \ln(10)}{F} \quad \text{(Eq.4)}$$

In these equations, V_{REF} is the electrochemical cell voltage, R is the universal gas constant, T is the temperature in Kelvin, P is the pressure in dbar, S is the salinity in PSU, and F is the Faraday constant. The constants k0 and k2 are the cell standard potential offset and temperature slope respectively and f(P) is the sensor pressure response function. The remaining pH variables, the partial molal volume of HCl (V_{HCl}), total chloride (Cl_T), HCl activity coefficient (γ_{HCl}), total sulfate (S_T), and acid dissociation constant of $HSO4^-$(K_s), are derived from the salinity, temperature, and pressure of the sea water sample (Branham et al., 2016).

Deployment and Sampling

This study was conducted over two different periods and at two different sites, one fresh and one estuarine. The Deep SeapHOx™ V2 was deployed over a period of three months from October 21st, 2024 to January 15th, 2025, in Portage Bay (Lake Union, freshwater), off the

University of Washington's Marine Science Building Dock (47.649451, -122.312563). The sensor package was deployed at three meters depth, and recorded measurements (temperature, pressure, salinity, dissolved oxygen and pH) every 15 minutes. The Shallow SeapHOx™ V2 was deployed over a period of three months from November 5th, 2025, to February 9th, 2026, in the Snohomish River Estuary, off the J Dock in the Port of Everett Marina (48.001864, -122.221659). This location is at the mouth of the Snohomish River which experiences a daily mixed semi-diurnal tidal pattern, making it an ideal deployment location for this study. The sensor was deployed three meters below the floating dock, whose height is tide dependent. The sensor package took measurements (temperature, pressure, salinity, dissolved oxygen and pH) every 15 minutes.

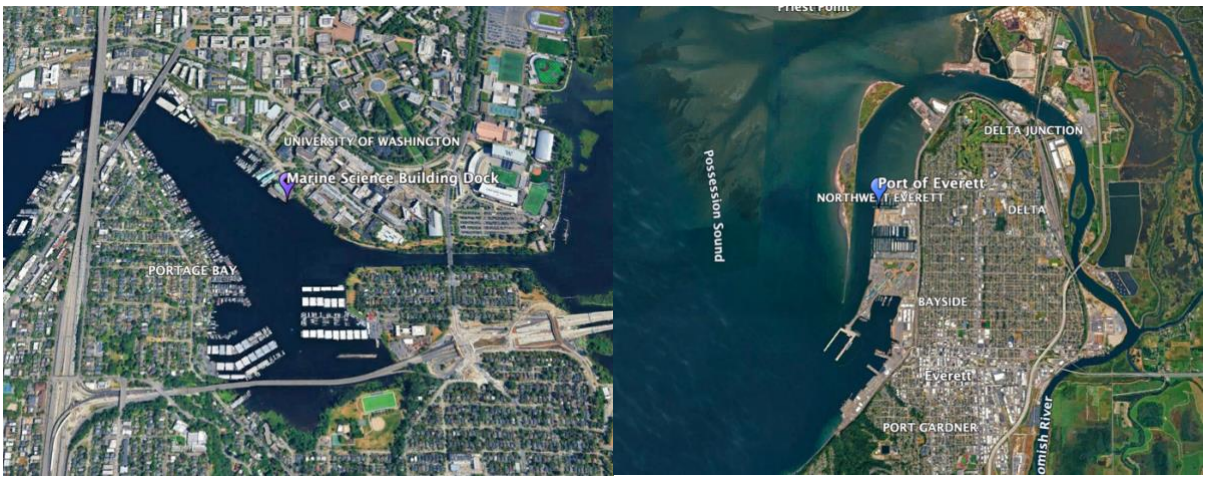

Figure 2: Deep SeapHOx™ V2 pH sensor deployment location, 47.649451, -122.312563 (Left) and Shallow SeapHOx™ V2 pH sensor deployment location, 48.001864, -122.221659 (right).

Sensor data was validated through laboratory analysis. Every two weeks, three replicate water samples were collected using a 3 Liter Niskin bottle at each site. To ensure sample quality we limited ambient oxygen contamination in each sample, sealed them with grease, and kept them at a consistent temperature in a cooler during transportation to the lab. We used

spectrophotometric analysis following the N. K. Douglas freshwater pH determination method for Portage Bay samples and Byrne and Easley method for the estuarine samples (N.K Douglas, 2017; Easley and Byrne, 2015), to determine the true pH of each water sample and ensured that each replicate set was between 0.005 pH of each other. These reference values were then compared to both SeapHOx™ V2 measurements to quantify sensor accuracy and long-term drift.

Post-deployment data processing

With both SeapHOx™ V2 sensor's original factory calibration occurring in salt water, a new k0 constant, which indicates the pH cell's standard potential offset in an aqueous solution, needed to be applied to the freshwater and estuarine data collected (Branham et al., 2016). For both sensors, we performed an in-situ calibration using the first in-situ spectrophotometric pH sample, temperature, salinity and the pH sensor voltage output to correct the factory seawater calibration k0 value and applied this new freshwater k0 value to the subsequent sensor readings using their respective Sea-Bird Scientific pH equations (Sea-Bird Scientific, n.d.). To complete the k0 correction for the Sea-Bird Scientific Deep SeapHOx™ V2, we used Equation. 1 above. For the Shallow SeapHOx™, we utilized Equation 2 for the internal electrode k0 calibration, and Equation 3 for the external electrode k0 calibration. In-situ calibrations were completed using a spectrophotometric pH sample that had been taken after each sensor had chemically equilibrated to the environment (approximately 1 week). A linear regression was performed to help determine if the sensor values experienced drift over the deployment time period. Residuals were then calculated to compare the final corrected sensor values to the spectrophotometric pH values to see if the accuracy fell within the reported accuracy specification of the sensor in salt water (+/- 0.05 pH units). The Shallow SeapHOx™ V2 utilizes both an internal and external pH electrode, so regression analysis, data correction,

and residuals were calculated for each of these datasets. Because the instrument took one week to chemically equilibrate to the deployment environment, all data collected during these weeks of the study period were excluded from our analysis.

Results

Freshwater Data Analysis

We found the Deep SeapHOx™ V2 to accurately measure pH in freshwater environments within the reported specification accuracy of +/- 0.05 pH for seawater but required post-deployment corrections. Two corrections were required: the first to correct the sensor pH readings using a newly calculated freshwater k0 value based on the freshwater spectrophotometric pH analysis, and the second to correct for linear drift of the sensor readings over time (Fig. 3).

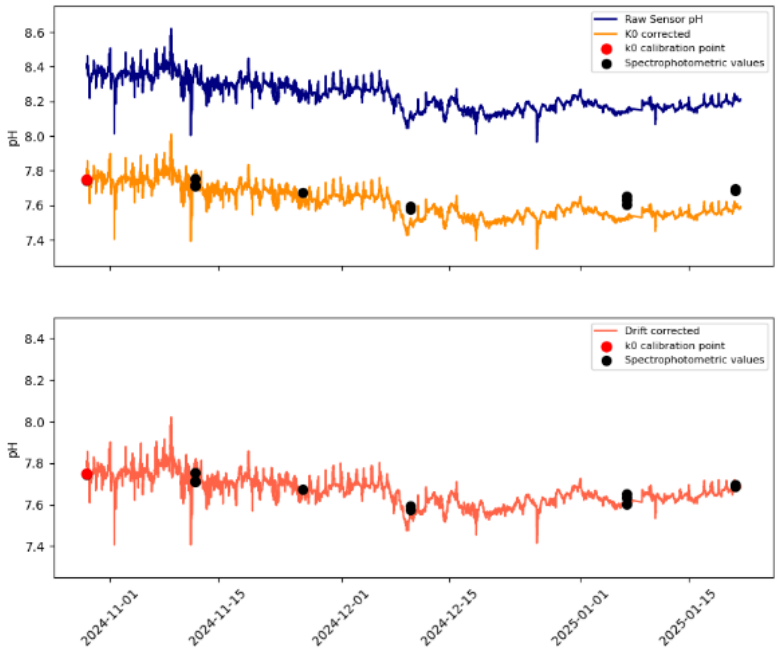

Fig. 3. Time series of sensor data after chemical equilibration with the environment from October 29, 2024 to January 15, 2025 with adjustments from a two-step correction: 1. freshwater k0 correction (top) and 2. linear drift correction (bottom)

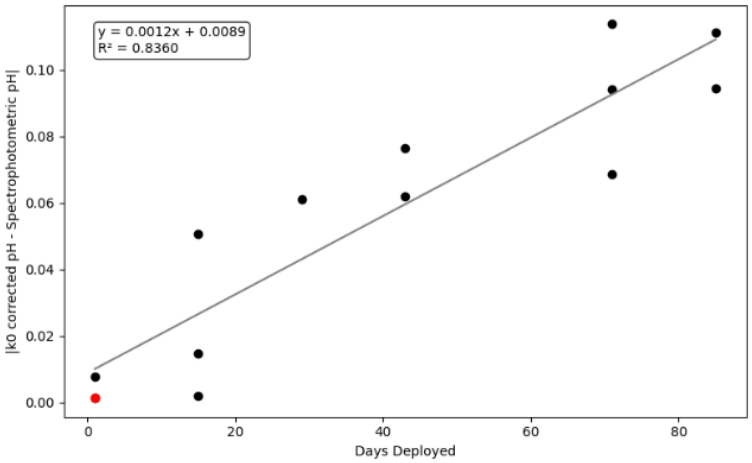

Fig. 4. Difference between freshwater k0 corrected pH values and spectrophotometric pH values relative to days deployed to quantify linear drift over time. Red dot indicates K0 calibration value.

Before the in-situ freshwater k0 calibration occurred, the Deep SeapHOx™ V2 consistently produced readings on average +0.6 pH unit different from the spectrophotometric values (Fig. 3). After the freshwater k0 calibration was applied to collected data, the sensor still exhibited linear drift over time, which was confirmed with a linear regression calculation, so a second correction was applied using the slope of the linear drift: 0.0012 pH units/day (Fig. 4). After both corrections, the residuals show the sensor is accurate to +/- 0.05 pH units in freshwater (Fig. 5).

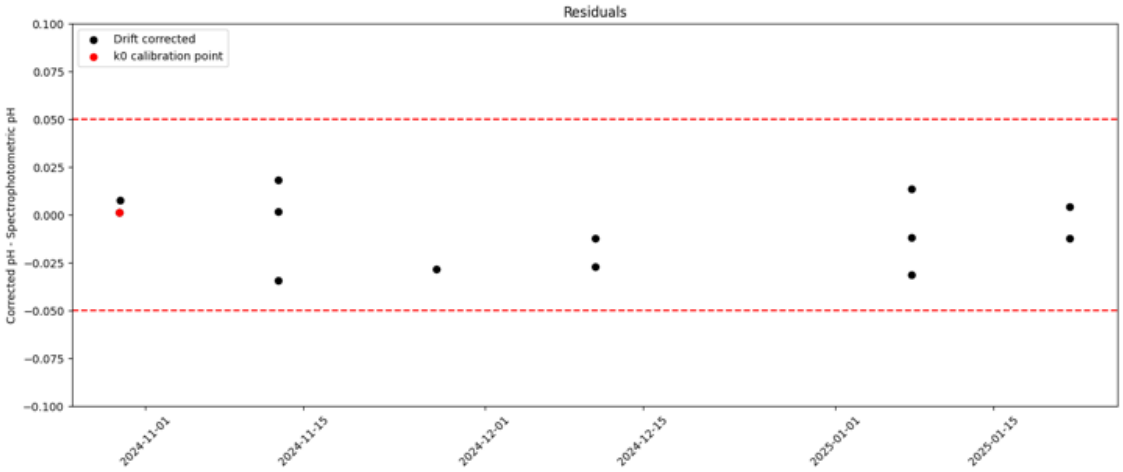

Fig. 5. Difference of final corrected pH values and spectrophotometric pH values over deployment duration. Red bars indicate +/- 0.05 pH units. Red dot indicates K0 calibration value.

Estuarine Data Analysis

The Shallow SeapHOx™ V2 utilizes both an internal and external electrode to collect pH values. Overall, after the one-week equilibration time, these electrodes measured very similarly, especially at points of higher pH. Both electrodes exhibit a large pH fluctuation, going from 6.00 to 7.75 pH (Fig. 6). These oscillations appear to be directly related to the deployment location's tidal cycle. At low tide, pH decreases, while at high tide, pH increases. At the lower pH measurements, these electrodes differed more, with the external reference reading lower than the internal reference. Before post-processing, the spectrophotometric points are more closely aligned with the internal reference pH values.

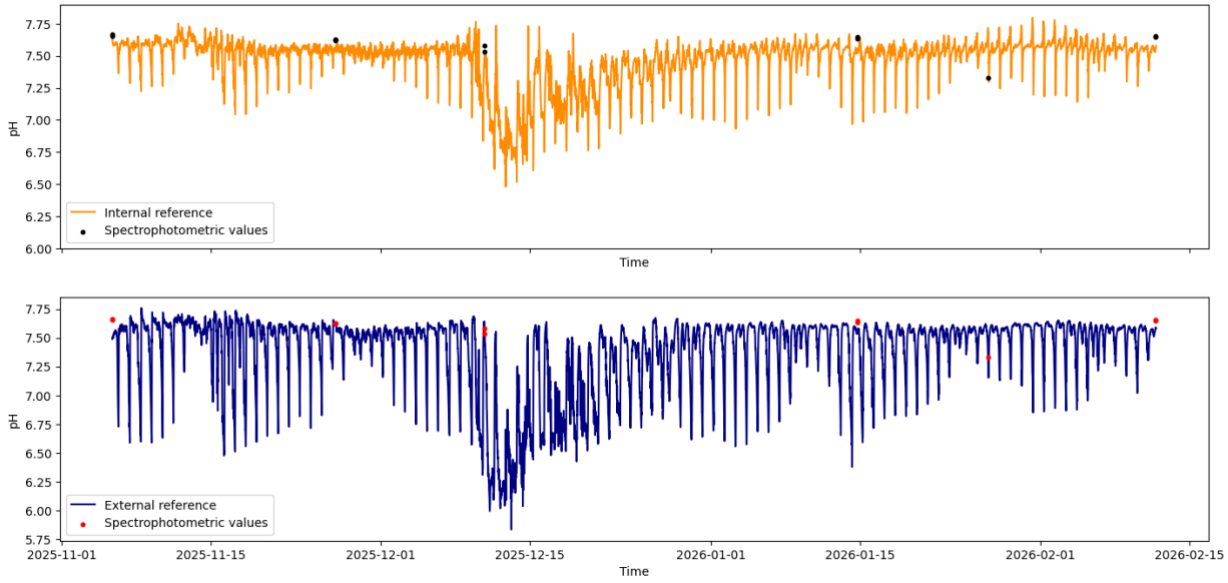

Fig. 6. Comparison of spectrophotometric pH values with the internal (top) and external (bottom) electrode pH measurements before post-processing.

Internal Electrode

We found the Shallow SeapHOx™ V2 to accurately measure pH in estuarine environments within the reported specification accuracy of +/- 0.05 pH, with an applied k0 correction, except for one outlier point which was taken during tidal transition. Raw sensor values consistently measured 0.07 pH lower than the spectrophotometric samples, and this could be corrected with a k0 calibration: this worked to correct the sensor pH readings using a newly calculated k0 value based on the spectrophotometric pH analysis (Eq. 2).

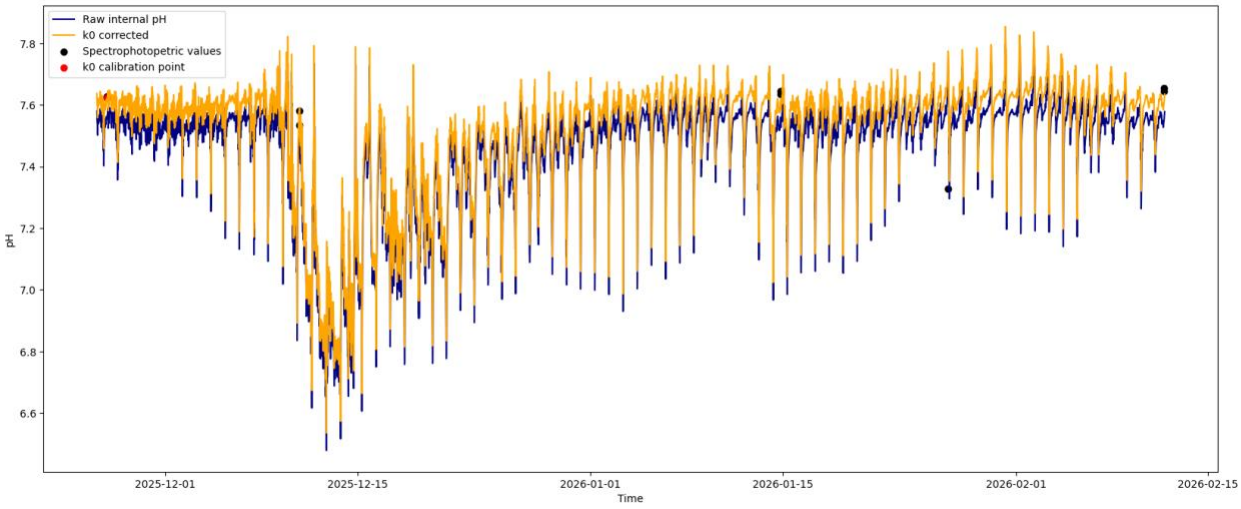

Fig. 7. Time series of internal electrode data after chemical equilibration with the environment from November 13, 2025 to February 9th, 2026 with raw electrode values and k0 correction.

After the k0 calibration was applied to collected data, the sensor maintained stable measurements with a small amount of drift over time, which was confirmed with a linear regression calculation. This determined a drift of 0.0002 pH units a day, with an R^2 value of 0.04, which denotes a negligible correlation between drift as compared to days deployed, therefore we did not apply it to further correct the data. After correction, the residuals show the sensor is accurate to +/- 0.05 pH units in an estuarine environment, except for one outlier (Fig. 8) which was taken during a transition to low tide.

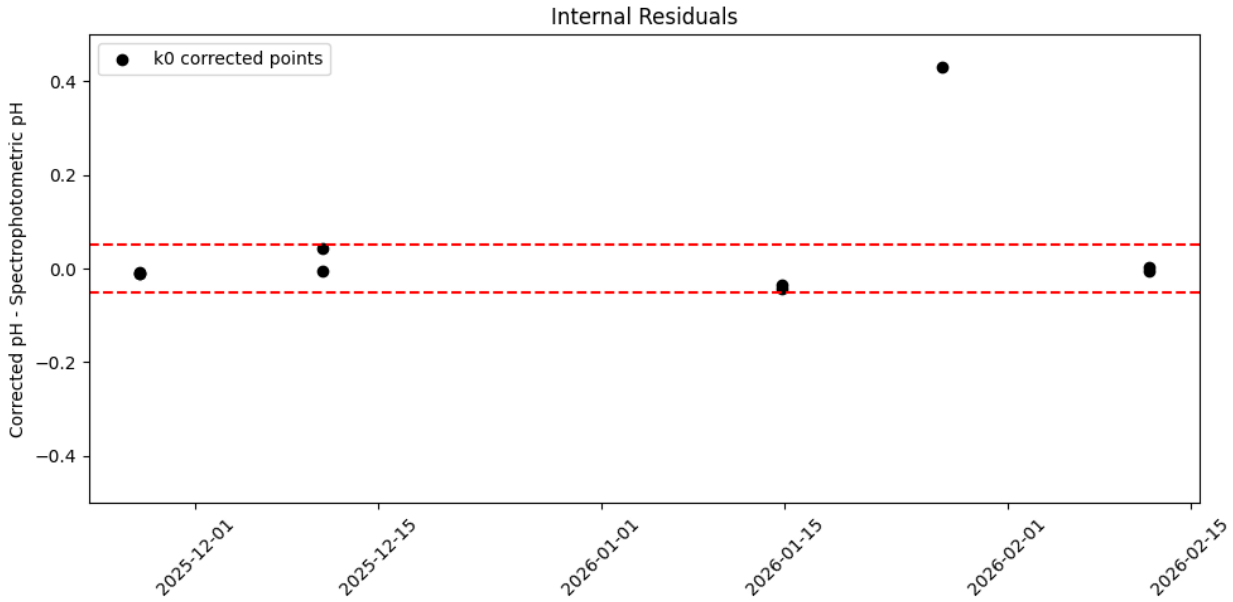

Fig. 8. Difference of final corrected internal electrode pH values and spectrophotometric pH values over deployment duration. Red bars indicate +/- 0.05 pH units.

External Electrode

We found the Shallow SeapHOx™ V2 to accurately measure pH in estuarine environments within the a accuracy of +/- 0.10 pH, with an applied k0 correction. Newly calculated pH values based off the external pH equation (Eq. 3) fell within +/- 0.03 of the raw sensor values, indicating that the factory calibration of this electrode was viable for this environment.

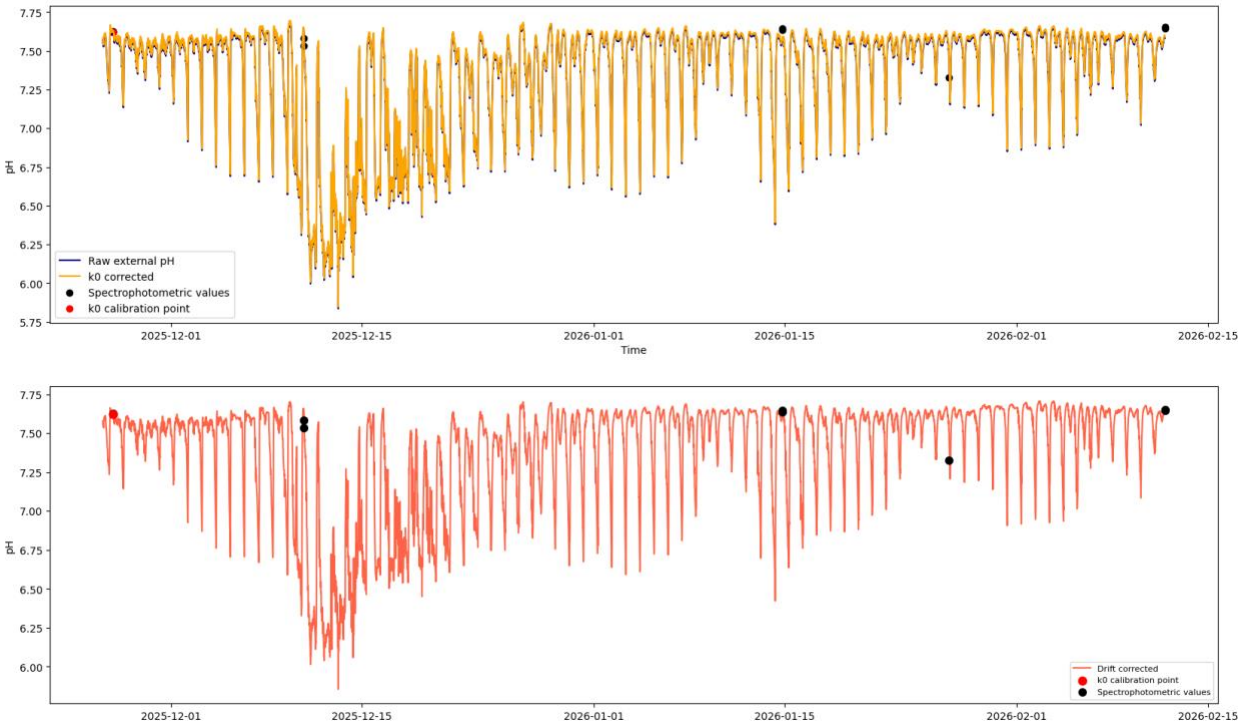

Fig. 9. Time series of external electrode data after chemical equilibration with the environment from November 13, 2025 to February 9th, 2026 with adjustments from a two-step correction: 1. External k0 correction (top) and 2. linear drift correction (bottom)

After the estuarine external k0 calibration was applied to collected data, the sensor still exhibited linear drift over time, which was confirmed with a linear regression calculation. This determined a drift of 0.0007 pH units a day, with an R^2 value of 0.91. This drift was corrected for and after both corrections, the residuals show the sensor is accurate to -0.05 pH units, and +0.10 pH units (Fig. 10). The samples with residuals above 0.05 pH were taken during tidal transitions. External references are known to be less accurate during fast salinity transitions due to their much slower salinity response time as compared to the internal electrode in the same environment.

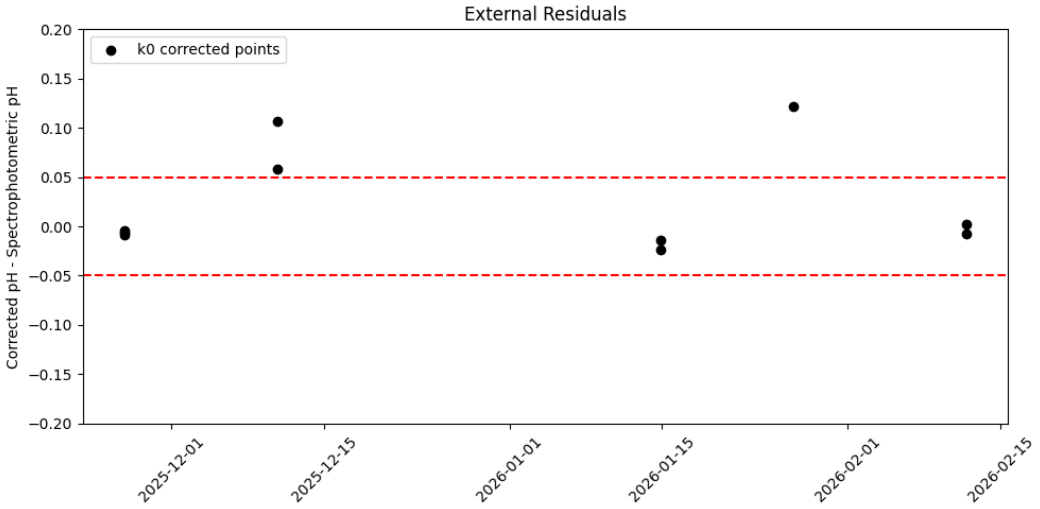

Fig. 10. Difference of final corrected external electrode pH values and spectrophotometric pH values over deployment duration. Red bars indicate +/- 0.05 pH units.

Discussion

Our study demonstrates that the Deep and Shallow SeapHOx™ V2 and ISFET technology is a reliable and accurate tool for long-term aquatic ecosystem monitoring in both fresh and estuarine environments. Although Deep SeapHOx™ V2 sensor measurements were offset by +0.06 pH units and the sensor experienced a linear drift over time, both issues are easily correctable making the Deep SeapHOx™ V2 flexible for both freshwater and saltwater environments. The Shallow SeapHOx™ V2 also maintained reliable estuarine measurements. The internal reference values were determined to be within an accuracy range of +/- 0.05 pH with only a k0 correction necessary, while the external reference was accurate to +/- 0.1 pH with a K0 and a linear drift correction. The Shallow SeapHOx™ pH fluctuations were closely related to Everett's mixed semi-diurnal tidal cycle, measuring higher pH during high tides, and lower pH during low tides.

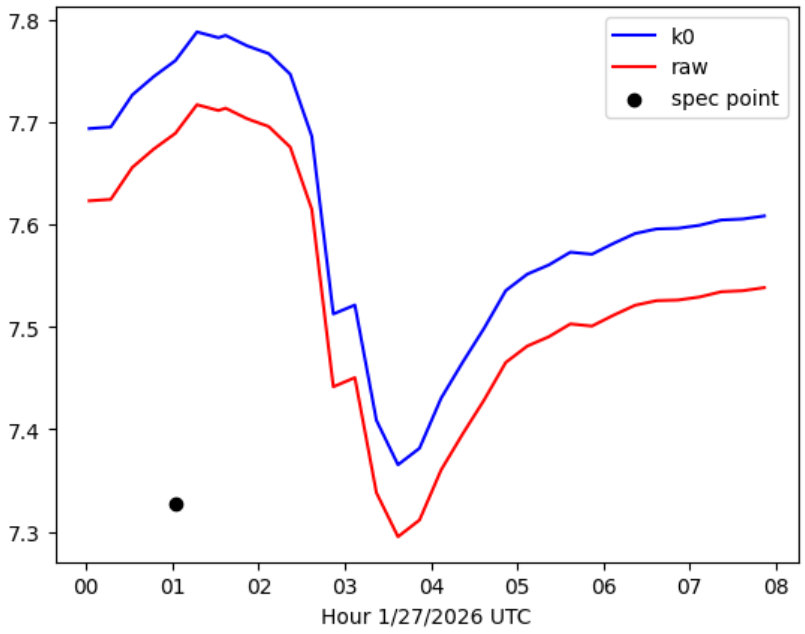

Fig. 11. Time series of internal electrode data on January 27th, 2026 from 00:00 UTC to 8:00 UTC, with raw sensor measurements and adjustments from a k0 correction in relation to the nearest spectrophotometric value.

In the estuarine analysis, there was one outlier within the internal electrode, and two found within the external electrode. These spectrophotometric point outliers were taken specifically during tidal transition periods. For the internal outlier, this point is the only point within the internal reference dataset that does not fall within +/- 0.05 (Fig. 9). The existence of this outlier prompted further investigation. Spectrophotometric samples determined a pH of 7.3 at 1:02 UTC on Jan 27th, 2026, while the sensor did not record this value until 3:39 UTC on Jan 27th , 2026. This indicates a possible 2.5-hour response time in the internal reference, suggesting that this sensor may not respond quickly to rapid pH decreases. This internal reference delay in response time specifically correlated to the periods of pH decrease (Fig. 13). To explore this response time further, more measurements should be taken at low pH periods, that correspond with that needs to be explored further. We found that the external electrode struggled to accurately report the pH during tidal transition periods. This electrode is more exposed to the changing seawater pH and

reliably correlated with spectrophotometric samples taken specifically at high and low tide periods where the water column was more stable.

All pH data across both sensors required a similar data correction method to improve the accuracy of the raw data. This correction included both a k0 calibration and a linear drift correction over the three-month deployment time. After these correction methods, the Deep SeapHOx™ V2 and Internal reference electrode of the Shallow SeapHOx™ were accurate to +/- 0.05 pH of the spectrophotometric samples. The external electrode of the Shallow SeapHOx™ was less accurate in the dynamic estuarine environment, suggesting that the internal electrode is the ideal measurement for these environments.

Future freshwater and estuarine users of this technology should:

1. allow the sensor to chemically equilibrate at the deployment location for one week
2. conduct an in-situ calibration with spectrophotometric pH measurement to calculate a relevant k0 value and apply a correction to all sensor readings
3. correct pH values for drift over time using a calculated linear relationship for freshwater applications

Conclusion

This study expands the environments that the Shallow and Deep SeapHOx™ V2 and ISFET technology can be used and demonstrates its ability to support freshwater and estuarine monitoring studies. Future studies will further explore the possible response time delay in the internal and external electrode of the Shallow SeapHOx™ as well as longer deployment durations to ensure that drift corrections are reliable for instruments deployed over long

timeframes. Following the procedures outlined above, these two sensors can be utilized further in freshwater and estuarine environments to monitor pH change and impacts of acidification.

Acknowledgement

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