



PERFORMANCE VERIFICATION STATEMENT For Onset's HOBO U26 Dissolved Oxygen Sensors



TECHNOLOGY TYPE:	Dissolved Oxygen sensors
APPLICATION:	In situ estimates of DO for coastal moored deployments
PARAMETERS EVALUATED:	Response linearity, accuracy, precision and reliability
TYPE OF EVALUATION:	Laboratory and Field Performance Verification
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EXECUTIVE SUMMARY

The Alliance for Coastal Technology (ACT) conducted a sensor verification study of in situ dissolved oxygen sensors during 2015-2016 to characterize performance measures of accuracy and reliability in a series of controlled laboratory studies and field mooring tests in diverse coastal environments. The verification including several months of Laboratory testing along with three field deployments covering freshwater, estuarine, and oceanic environments. Laboratory tests of accuracy, precision, response time, and stability were conducted at Moss Landing Marine Lab. A series of nine accuracy and precision tests were conducted at three fixed salinity levels (0, 10, 35) at each of three fixed temperatures (5, 15, 30 °C). A laboratory based stability test was conducted over 56 days using deionized water to examine performance consistency without active biofouling. A response test was conducted to examine equilibration times across an oxygen gradient of 8mg/L at a constant temperature of 15 °C. Three field-mooring tests were conducted to examine the ability of test instruments to consistently track natural changes in dissolved oxygen over extended deployments of 12-16 weeks. Deployments were conducted at: (1) Lake Superior, Houghton, MI from 9Jan – 22Apr, (2) Chesapeake Bay, Solomons, MD from 20May – 5Aug, and (3) Kaneohe Bay, Kaneohe, HI from 24Sep – 21Jan. Instrument performance was evaluated against reference samples collected and analyzed on site by ACT staff using Winkler titrations following the methods of Carignan et.al. 1998. A total of 725 reference samples were collected during the laboratory tests and between 118 – 142 reference samples were collected for each mooring test. This document presents the performance results of Onset’s HOBO U26 Dissolved Oxygen Logger using the RDO® Basic Technology developed by In-Situ, Inc.

Instrument accuracy and precision for the HOBO U26 was tested under nine combinations of temperature and salinity over a range of DO concentrations from 10% to 120% of saturation. The means of the difference between the HOBO U26 and reference measurement ranged from 0.047 to 0.314 mg/L over all nine trials. There was a slight decrease in the magnitude of the differences with increase of both temperature and salinity levels. A linear regression of instrument and reference measurements for all trials combined ($n=358$; $r^2 = 1.00$; $p<0.0001$) produced a slope of 1.037 and intercept of -0.084. The absolute precision, estimated as the standard deviation (s.d.) around the mean, ranged from 0.003 – 0.013 mg/L across trials with an overall average of 0.006 mg/L. Relative precision, estimated as the coefficient of variation ($CV\% = (s.d./mean)\times 100$), ranged from 0.036 – 0.268 percent across trials with an overall average of 0.074%.

Instrument accuracy was assessed under a 56 day lab stability test in a deionized water bath cycling temperature and ambient DO saturation on a daily basis. The overall mean of differences between instrument and reference measurements was 0.041 (s.d. = 0.339) mg/L for 154 comparisons (out of a potential total of 156). A regression of measurement difference over time showed a minor drift in response with a slope of -0.004 mg/L/d ($r^2 = 0.049$; $p=0.006$). But if two large negative responses near the end of the test are omitted the regression is not significant

A functional response time test was conducted by examining instrument response when rapidly transitioning between adjacent high (9.6 mg/L) and low (2.0 mg/L) DO water baths, maintained commonly at 15 °C. The calculated τ_{90} was 27 s during high to low transitions and 26 s for low to high transitions covering the 8 mg/L DO range.

At Houghton, MI a field deployment test was conducted under the ice over 104 days with a mean temperature and salinity of 0.7 °C and 0.01. The HOBO U26 operated successfully throughout the entire 15 week deployment and generated 9859 observations based on its 15 minute sampling interval for a data completion result of 100%. The average and standard deviation of the measurement difference over the total deployment was 0.855 ± 0.116 mg/L with a total range of 0.533 to 1.030 mg/L. The drift rate of instrument offset, estimated by linear regression ($r^2=0.73$; $p<0.0001$), was 0.003 mg/L/d. This rate would include any biofouling effects as well as any electronic or calibration drift. A linear regression of the instrument versus reference measurements over the first month ($r^2 = 0.98$; $p<0.0001$) produced a slope of 0.855 and intercept of 2.64 indicating mostly an initial calibration offset.

At Chesapeake Biological Lab, a field deployment test was conducted over 78 days with a mean temperature and salinity of 25.6 °C and 10.9. The HOBO U26 generated 7270 observations over the 11 week deployment, however, only 5314 of the measurements were considered acceptable based on values that were less than 2 mg/L from any reference sample over a similar timeframe for a data completion result of 73%. The average and standard deviation of the difference between instrument and reference measurements for the deployment was -0.211 ± 0.860 mg/L, with the total range of differences between -1.85 to 0.94 mg/L. The calculated drift rate in instrument response for the entire deployment period using the accepted data was -0.079 mg/L/d ($r^2 = 0.77$; $p<0.001$). A linear regression of all data from the first month ($r^2 = 0.262$; $p<0.001$) produced a slope of 0.594 and intercept of 2.93. The high variability indicate that biofouling impacts were likely present along with an initial calibration offset.

At Kaneohe Bay, HI a field deployment test was conducted over 121 days with a mean temperature and salinity of 25.8 and 33.4 °C. The HOBO U26 reported 11296 observations based on its 15 minute sampling interval, however, only 11179 of the measurements were considered acceptable based on excluded values that were more than 2 mg/L from reference samples over a similar timeframe, for a data completion result of 99%. The average and standard deviation of the differences between instrument and reference readings (limited to ± 2.0 mg/L DO; $n=107$ of 129 potential observations) were $0.090 \pm .576$ mg/L, with a total range in the differences of -1.195 to 1.808 mg/L. There was a small, but statistically significant, drift in instrument offset (slope = 0.009 mg/L/d; $r^2 = 0.18$; $p=0.009$) throughout the deployment period but the scatter resulted in a very low goodness of fit in the regression. This rate would include any biofouling effects as well as any electronic or calibration drift. A linear regression of the instrument versus reference measurements ($r^2 = 0.550$; $p<0.0001$) for the first month of data had a slope of 1.595 and intercept of -3.102.

Overall, the response of the HOBO U26 RDO sensor during field testing showed good linearity across all three salinity ranges including freshwater, brackish water, and oceanic water. The response curves were generally consistent across the concentration ranges observed within each test site (although more variable at CBL) and relatively consistent over the wide range of DO conditions (4 - 14 mg/L) across sites. A linear regression of the combined one-month field deployment measurements ($r^2 = 0.94$; $p<0.0001$) had a slope of 1.106 and intercept of -0.895.

The HOBO U26 was evaluated in a profiling field test in the Great Lakes at two separate locations in order to experience transitions from surface waters into both normoxic and hypoxic hypolimnion. In Muskegon Lake, the temperature ranged from 21.0 °C at the surface to 13.5 °C in the hypolimnion, with corresponding DO concentrations of 7.8 and 2.8 mg/L, respectively. In Lake Michigan, the temperature ranged from 21.0 °C at the surface to 4.1 °C in the hypolimnion,

with corresponding DO concentrations of 8.6 and 12.6 mg/L, respectively. Two profiling trials were conducted at each location. The first trial involved equilibrating test instruments at the surface (3m) for ten minutes and then collecting three Niskin bottle samples at one minute intervals. Following the third sample, the rosette was quickly profiled into the hypolimnion where samples were collected immediately upon arrival and then each minute for the next 6 minutes. The second trial was performed in the reverse direction. For Muskegon Lake, the range in measurement differences between instrument and reference was -0.15 to 1.78 mg/L for cast 2 and -0.92 to -0.17 mg/L for cast 3 (cast 1 was aborted and redone as cast 3. For Lake Michigan, the range in measurement differences was -3.03 to 0.40 mg/L for cast 1 and -0.81 to 3.05 mg/L for cast 2. The equilibration rate of the sensor differed across each of the profiles with no obvious trend across temperature or DO levels.

BACKGROUND AND OBJECTIVES

Instrument performance verification is necessary so that effective existing technologies can be recognized and so that promising new technologies can be made available to support coastal science, resource management and ocean observing systems. To this end, the NOAA-funded Alliance for Coastal Technologies (ACT) serves as an unbiased, third party testbed for evaluating sensors and sensor platforms for use in coastal environments. ACT also serves as a comprehensive data and information clearinghouse on coastal technologies and a forum for capacity building through workshops on specific technology topics (visit www.act-us.info).

As part of our service to the coastal community, ACT conducted a performance verification of commercially available, in situ dissolved oxygen (DO) sensors through the evaluation of objective and quality assured data. The goal of ACT's evaluation program is to provide technology users with an independent and credible assessment of instrument performance in a variety of environments and applications. To this end, the data and information on performance characteristics were focused on the types of information users most need.

The fundamental objectives of this Performance Verification were to: (1) highlight the potential capabilities of particular in situ DO sensors by demonstrating their utility in a range of coastal environments; (2) verify the claims of manufacturers on the performance characteristics of commercially available DO sensors when tested in a controlled laboratory setting, and (3) verify performance characteristics of commercially available DO sensors when applied in real world applications in a diverse range of coastal environments.

INSTRUMENT TECHNOLOGY TESTED

Onset's HOBO U26 Dissolved Oxygen Logger uses the RDO® Basic Technology developed by In-Situ, Inc. This technology represents the latest advancements in optical Dissolved Oxygen (DO) measurement. By improving upon the breakthrough optical DO sensor technology that In-Situ brought to the environmental market in early 2004, the RDO Basic sensor provides higher quality data and more robust performance than traditional methods. The "Basic" version of the RDO used in the HOBO U26 provides the RDO advantages in an affordably-priced stand-alone data logger.

Optical sensor technology uses LED lights, a lumiphore and a detector. A source of light excites a lumiphore which luminesces, emitting a light of a different wavelength. Typically,

luminescence lifetime methods are used to determine DO concentration. However, optical technologies differ in measurement method. Luminescence lifetime can be measured using either the time domain method or a frequency domain method.

1. Time Domain Method – Uses a pulsed measurement method to measure a single or an average of a series of exponential decay events. This method is susceptible to drift and interference from stray light.
2. Frequency Domain Method – Measures the phase shift between the entire signal and reference wave forms across a number of cycles. This method, which is used by the RDO Basic sensor, delivers the highest accuracy across the widest operating range.

When the RDO Basic sensor initiates a reading, a blue LED emits blue light, which excites the lumiphore molecules. Excited lumiphore molecules emit red light, which is detected by a photodiode. Oxygen molecules quench the excited lumiphore molecules and prevent the emission of red light. This process is called “dynamic luminescence quenching.” The RDO Basic sensor measures a phase shift between the red returned light and a red reference light. DO concentration and red returned light are inversely proportional. Optical electronics calculate DO concentration and report results in mg/L. DO determination by luminescence quenching has a linear response over a broad range of concentrations and offers a high degree of accuracy and stability.

In addition, the RDO Basic technology improves deployments by providing the following:

- The sensor has virtually no drift over the life of the sensor cap. And, unlike membrane-based sensors, the RDO Basic sensor excels in hypoxic conditions. No sample flow or stirring is required.
- The sensor face requires periodic cleaning, but no hydration, conditioning, or special storage is required. Membranes and electrolyte/filling solution are eliminated.
- The abrasion-resistant sensing element withstands fouling, high sediment loads, and rapid flow rates. The lumiphore is not affected by photo bleaching or stray light. In addition, unlike membrane-based sensors, the RDO Basic sensor is unaffected by sulfides, sulfates, hydrogen sulfide, carbon dioxide, ammonia, pH, chloride, and other interferences.

The HOBO U26 DO Logger is part of the HOBO family of data loggers which feature optical communication for reliable offload in harsh field conditions and easy-to-use HOBOware software for logger setup, data analysis and data export.

PERFORMANCE EVALUTION TEST PLAN

Laboratory Tests

Laboratory tests of accuracy, precision, response time, and stability were conducted at Moss Landing Marine Lab. All tests were run under ambient pressure (logged hourly from a barometer at the laboratory) and involved the comparison of dissolved oxygen concentration reported by the instrument versus Winkler titration values of water samples taken from the test baths. All tests were run in thermally controlled tanks at specific temperature, salinity, and DO concentrations. Tanks were well mixed with four submersible Aquatic Ecosystem Model 5 pumps with flow rates of 25 L/min. Temperatures were controlled to within approximately 0.2°C of set point using Thermo Digital One Neslab RTE 17 circulating thermostats flowing through closed

coils distributed within the tank. Four RBR temperature loggers were deployed within the tank to verify actual temperature to better than 0.02°C. Salinity was varied by addition of commercial salts (Instant Ocean) to Type 1 deionized water. Salinity was verified at the beginning and end of each test condition by analysis on a calibrated CTD. Dissolved oxygen concentrations were controlled by use of compressed gases of known oxygen concentration sparging through diffusers within the tank. Tanks were covered with a layer of floating closed-cell plastic insulation that continuously sealed the water surface and to minimize atmospheric exchange. If required by the manufacturer, instruments were only calibrated prior to the start of the first lab test, and then again prior to the stability test which began one month later. The following series of tests were conducted in the laboratory trials:

Accuracy at various T/S and DO conditions

A series of measurements were conducted under 36 discrete conditions to target 3 temperatures, 3 salinities, and at least 4 DO concentrations as follows:

- Temperature Conditions: 4, 15, 30°C
- 3 Salinity Conditions: 0, 10, 34
- Dissolved oxygen,(% air saturation): 0% (hypoxic), 20 – 30%, 100% and $\geq 120\%$, (levels were achieved by mixing pure O₂ and N₂ sources with pure N₂ was used for the 0% O₂ concentration)

Tests were run such that all 4-6 DO concentrations were tested for a fixed temperature and salinity on the same day. The tests began at ambient, near air saturation, conditions following overnight equilibration of tank water to the test salinity and temperature. Subsequently DO were dropped to near 0 mg/L and increased stepwise to the highest concentration. Instruments were allowed to equilibrate at the fixed temperature and salinity for 1 h before the start of that day's trial. Sparging with each DO gas concentration was conducted for a minimum of 60 minutes prior to the start of data collection and reference sampling. For each test condition, the test instruments were programmed to sample at no slower than 1 minute intervals and reference samples were collected at 6 timepoints spaced 5 minutes apart for each of the fixed conditions. For three of the timepoints duplicate samples were collected from two different sampling ports mounted at opposite ends of the tank to access heterogeneity within the tank. Inlets of sampling ports were positioned at the depth of the sensor heads (ca. -0.5m). All reference samples were collected while the gas sparging was off and took approximately 1 minute to complete. Reference samples were processed and analyzed as defined below. The order of the test conditions were 15 then 5 then 30°C, going from 0 then 10 then 34 salinity at each temperature.

Precision Test at various DO concentrations

Instrument precision was evaluated under stable conditions generally achieved at the start of each trial's day. Instruments were equilibrated to each test condition for a minimum of one hour prior to testing. The sampling frequency for test instruments was 1 minute with reference samples matching instrument sampling to monitor for drift in tank DO. At least 6 reference samples were collected over a 30 minute instrument precision evaluation trial. Reference samples were processed and analyzed as defined below.

Functional Response Time Test

A response time test was conducted by examining measurements during a rapid exchange across a large gradient in dissolved oxygen for a fixed temperature (15 °C) in deionized water,

following the approach described in Bittig et al. 2014. The reservoirs of the thermostat baths were constantly bubbled with either N₂ gas or air to maintain discrete DO levels. A submersible pump was added to each bath to ensure uniform flow and oxygen conditions and instruments were mounted at a fixed position within the baths to minimize variance due to instrument manipulation. Instruments were programmed to measure every 10s continuously (or their fastest possible rate) for eight minutes following the exchange. For instruments with the capability, real-time monitoring of instrument output was monitored to verify a steady state reading had been obtained. Instruments were moved from the high DO concentration to the low DO concentration and subsequently reversed to check for response hysteresis. During transitions, care was taken to minimize carryover by shaking off residual water. The sensor was then carefully inserted into the new bucket and mixed by hand to ensure no bubble entrapment and full exposure to the new solution. Reference samples from each reservoir were taken at the beginning and end of the exposure. The test instrument was equilibrated in the high DO reservoir for at least 30 min prior to the exchange to ensure temperature equilibration.

Lab-based Stability Test

A laboratory stability test was conducted to examine potential instrument drift in a non-biofouling environment. These results are contrasted to the stability of measurement accuracy observed in the long-term field mooring deployments. The test occurred over 56 days, with daily temperature fluctuations of approximately 10°C, achieved by alternating the set point of the recirculation chiller. Reference samples were collected at minimum and maximum temperatures at least 3 times per week. The test was conducted in deionized water at saturated air conditions. Tanks were well circulated and open to the atmosphere. Water in the test tank was exchanged as needed if there was any indication of biological growth. Instruments stayed continuously submerged and were not exposed to air during any water exchange. The goal of comparisons of accuracy over time between the field and a sensor deployed similarly in the laboratory is intended to provide insight into drift and reliability intrinsic to the instrument relative to changes that may result from biofouling.

Moored Field Tests

Field Deployment Sites and Conditions

A four month moored deployment was conducted at Michigan Technological University's Great Lakes Research Center dock in Houghton, MI. Instruments were deployed in January and kept under ice cover until April. Instruments were programmed to sample at a minimum frequency of once per hour. ACT collected reference samples twice per day for 4 days per week during the entire deployment. Instruments were moored at approximately 4m depth and surface access through the ice was maintained by gentle circulation with a propeller to allow deployment of the Van Dorn sampling bottle. The goal of this test application was to demonstrate instrument performance (reliability, accuracy, and stability) in winter-time environmental conditions and to demonstrate the ability to operate continuous observations under ice.

A three month moored deployment was conducted at the Chesapeake Biological Lab Pier, Solomons, MD. Instruments were deployed between May and August during a period of warming temperatures and high biological production. Instruments were moored at fixed depth of 1m on a floating dock. Instruments were programmed to sample at a minimum frequency of once per hour. ACT collected reference samples twice per day for 3 days per week and collected six samples on

one day per week during the entire deployment. The intensive sampling was spaced to capture the maximum range of expected diurnal variation in dissolved oxygen concentrations. The goal of this test application was to demonstrate instrument performance (reliability, accuracy, and stability) under high biofouling conditions and over a range of salinity and temperature conditions in a coastal estuarine environment.

A four month moored deployment was conducted in a shore patch reef at the Hawaii Institute of Marine Biology (HIMB), Coconut Island, Kaneohe, HI. Instruments were deployed between September and January. Instruments were moored at approximately 1m depth on a bottom mounted PVC rack and were programmed to sample at a minimum frequency of once per hour. Some manufacturers chose to sample more frequently to demonstrate that capability. ACT collected reference samples twice per day for 3 days per week and collected six samples on one day per week during the entire deployment. The intensive sampling was spaced to capture the maximum range of expected diurnal variation in dissolved oxygen concentrations. The goal of this test application was to demonstrate instrument performance (reliability, accuracy, and stability) under high biofouling conditions in warm, full salinity coastal ocean conditions.

Field Testing Procedures

The moored deployments were run sequentially, and instrument packages were returned to manufacturers for reconditioning and calibration in between each successive field test. Prior to each deployment, instruments were set-up and calibrated if required, as directed by the manufacturer and demonstrated at a prior training workshop. Sensors were programmed to record dissolved oxygen data at a minimum of once per hour at the top of the hour for the duration of the planned deployment. All instrument internal clocks were set to local time and updated before programming using www.time.gov as the time standard. A photograph of each individual sensor and the entire sensor rack was taken just prior to deployment and just after recovery to provide a qualitative estimate of biofouling during the field tests. In the final step before deployment, instruments were placed in a well aerated fresh water bath, with a known temperature, for 45 minutes and allowed to record three data points as a baseline reference. Reference samples were drawn at the corresponding sampling times and analyzed for dissolved oxygen using Winkler titration method described below.

All instrument packages were deployed on a single box shaped rack that allowed all sensor heads to be at the same depth, with instruments side by side and all sensor heads deployed at the closest proximity feasible. The rack was deployed so that all sensor heads remained at a fixed depth of 1 m below the water surface, except as noted above. A standard and calibrated CTD package was deployed at each test site and programmed to provide an independent record of conductivity and temperature at the sensor rack during each instrument sampling event. At least four additional RBR temperature loggers were placed on the rack to capture any spatial variation in the temperature across the rack.

A standard 4 L Van Dorn bottle was used at each test site to collect water samples for Winkler titrations. The bottles were lowered into the center of the sensor rack, at the same depth and as close as physically and safely possible to the sensor heads. The bottle was triggered to close at the same time as the instruments were measuring to ensure that the same water mass was compared for DO content. Three replicate 125 ml BOD bottles were filled from each reference sample and immediately fixed in the field for subsequent Winkler titration analysis as described

below. The order of each sub-sample was recorded and tracked to examine any variation that arose from sample handling. Approximately 10 - 12 independent sampling events were conducted each week. At least once per week an intensive sampling event was conducted to capture the maximum diurnal range of dissolved oxygen concentrations. Once per week field duplicates were collected to examine fine-scale variability around the mooring site. Approximately 120 comparative reference samples were collected over the 3 - 4 month-long deployments.

In conjunction with each water sample collection, each deployment site also recorded site-specific conditions. The following information, logged on standardized datasheets were transmitted electronically on a weekly basis to the ACT Chief Scientist, for data archiving and site performance review:

- Date, time (local) of water sample collection.
- Barometric pressure from nearest weather station at time of water sample collection.
- Weather conditions (e.g., haze, % cloud cover, rain, wind speed/direction) and air temperature at time of water sample collection.
- Recent large weather event or other potential natural or anthropogenic disturbances.
- Tidal state and distance from bottom of sensor rack at time of water sample collection.
- Any obvious problems or failures with instruments.

ACT was responsible for accurately characterizing temperature and salinity surrounding the mooring with the goal of characterizing micro-stratification or heterogeneity surrounding the mooring. Four RBR Solo temperature loggers and two SeaBird CTDs were deployed at each mooring site. Sensors were mounted both at the instrument sampling depth and approximately 0.5 m above the sampling depth

Water-Column Profiling Test Procedures

Instruments were tested in a profiling application on a CTD rosette aboard the R/V Laurentian in the Great Lakes. Profiling tests were conducted during strong thermal stratification (late August, thermal gradient of >15 °C) and in two different regions including a normoxic and hypoxic hypolimnion. The normoxic hypolimnion site was in Lake Michigan within a 100m deep water column approximately 15 km offshore of Muskegon, MI. The hypoxic site profiling was conducted in Muskegon Lake, a drowned river mouth lake adjacent to Lake Michigan.

Two full water-column CTD casts were conducted at each test site. The first trial involved equilibrating test instruments at the surface (3m) for ten minutes and then collecting three Niskin bottle samples at one minute intervals. Following the third sample, the rosette was quickly profiled into the hypolimnion where samples were collected immediately upon arrival and then each minute for the next 6 minutes. The second trial was performed in the reverse direction where instruments were equilibrated for 10 minutes within the hypolimnion, three samples collected, and then profiled into the surface and sampled at one minute intervals over the next 7 minutes. The CTD was then immediately returned to the ship for sample processing. Triplicate BOD bottles were filled from each Niskin and immediately fixed for Winkler titrations.

Reference Sample Analysis

The Winkler titration for quantifying dissolved oxygen was used as the standard for comparison. The specific method is described in detail below and is based on the procedures described in, *Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method* (Carignan et. al. 1998). All Winkler titrations were done at the individual laboratory and field sites by trained ACT staff using standardized techniques and equipment.

Initial Preparation

The volumes of each BOD bottles (≈ 125 mL) were determined with a precision better than 0.005%. The volume of each bottle was measured gravimetrically (± 0.01 mL) near 20°C, after filling with degassed (boiled 10 min and cooled) distilled water. Since the procedure's precision approaches $1 \mu\text{g O}_2 \cdot \text{L}^{-1}$, particular care was taken to avoid contamination of the glassware and working space from any trace amounts of thiosulfate, iodate, I_2 , and manganese. Reagents recommended by Carritt and Carpenter (1966) were used and whole bottles titrated to minimize the loss of volatile I_2 and the oxidation of iodide to I_2 at low pH.

Reagents

- (1) Manganese chloride solution (3M Mn^{2+}): dissolve 300 g of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in 300 mL of distilled water. Bring to 500 mL.
- (2) Alkaline iodide solution (8M OH^- , 4M I^-): separately dissolve 160 g of NaOH and 300 g of NaI in ca 160 mL of distilled water. Mix with stirring and bring to 500 mL.
- (3) 23N Sulfuric acid solution: slowly add 313 mL of concentrated H_2SO_4 to 175 mL of distilled water. Carefully mix and cool and bring to 500 mL.
- (4) Thiosulfate titrant 0.03N: add 300 mL 0.1N $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (Fisher SS368-1) to 700 mL DI. The thiosulfate is standardized daily with KIO_3 according to the procedure described below. Note: The normality of thiosulfate will be adjusted to ensure that a complete sample can be titrated within one burette volume (less than 10 mLs), but kept as low as possible to maximize precision.
- (5) Potassium iodate standard, 0.1000N $\pm 0.005\text{N}$ commercially available stock (Fisher SP232-1).

Sample Fixing Procedures

- (1) Samples were fixed immediately after collection into the BOD bottles. Filling order was noted on log sheets along with bottle and sample IDs. 1.0 ± 0.05 mL of MnCl_2 was dispensed just below the water surface, followed by 1.0 ± 0.05 mL of alkaline iodide using positive displacement pipettors. The pipettors were washed with distilled water every day to prevent valve and plunger malfunction due to salt crystallization.
- (2) The bottle was immediately closed and shaken vigorously. The precipitate was allowed to settle for about two thirds of the bottle and shaken again to re-suspend the precipitate a second time. A water seal was immediately added to the neck of the bottle to prevent air suction by the contained water sample.
- (3) Samples were stored in the dark and room temperature (ca. 20°C) and temperature variations were minimized. Samples were titrated within 18 - 24 hours of being fixed.
- (4) Samples were acidified just prior to titration. With the precipitate settled to the lower third of the bottle, 1.0 ± 0.05 mL of 23N H_2SO_4 was added. The H_2SO_4 was allowed to flow gently along the neck of the bottle. The bottle was closed and shaken vigorously, until precipitate was dissolved
- (5) If titration was delayed beyond the 24 hour window, the fixed sample remained stored in darkness and at a temperature equal to or slightly lower than the temperature of the samples, with a water seal maintained at all times. The sample was acidified only immediately before titration.

Storage at temperatures above the sample temperature cause the loss of I₂ due to the thermal expansion of the solution of 0.025 mL · °C⁻¹ for a 125 ml sample (Carignan et.al. 1998).

Sample Titration Procedures

Whole bottles were titrated using a Metrohm automated model 916 Ti-Touch titrator equipped with a 10-mL burette and a Metrohm Pt ITrode. The Pt ring of the electrode was polished weekly. The titrator was used in the dynamic equivalence point titration (DET) mode, with a measuring point density of 4, a 1.0-µL minimum increment, and a 2 mV·min⁻¹ signal drift condition. In this method, the solution's potential (controlled by the I₂/I⁻ and S₂O₃²⁻/S₄O₆²⁻ – redox couples) was monitored after successive additions of titrant, where optimal increment volumes are calculated by the titrator's software. During titration, the size and rotation speed of the magnetic stirring bar was controlled in such a way that complete mixing of the I₂ generated during standardization occurred within 3 - 4 s, without vortex formation. To reduce the titration time (3 - 4 min) and I₂ volatilization, an initial volume of titrant equivalent to 85–90% of the expected O₂ concentration was added at the beginning of the titration. Because the molar volume of water and the normality of the titrant vary appreciably with temperature, care was taken to standardize the titrant and conduct all titrations of a given batch of samples at constant temperature ($\pm 1^{\circ}\text{C}$).

- (1) The stopper of the BOD bottle was removed and, using a wash bottle fitted with a 200-µL pipette tip, the I₂ present on the side and conical part of the stopper was rinsed into the BOD bottle with 1 - 2 mL of distilled water.
- (2) BOD bottles (Corning No. 5400-125) had been selected to accommodate the displacement of the electrode without having to remove any volume of the fixed sample.
- (3) The stirring bar was inserted into the bottle using plastic or stainless steel forceps.
- (4) The delivery tip and the electrode were immersed, the stirrer turned on and the titration begun. The electrode was not allowed to touch the neck of the bottle.
- (5) Once the titration was complete, the equivalence point volume (VT) was noted

Thiosulfate Standardization

The Thiosulfate was standardized at room temperature as the first and last step in daily analysis. Either triplicate assays of a fixed volume of iodate standard was run, or a range of volumes (≥ 3) bracketing the normal sample titration range (e.g. 0.500, 1.000, 1.500, 2.000 mL for well oxygenated waters.) A clean BOD bottle and clean glassware were dedicated to this purpose.

- (1) Insert a stirring bar into a 200 mL beaker.
- (2) With mixing add 1.0 mL of the H₂SO₄ reagent followed by 1.0 mL of the alkaline iodide and then 1.0 mL Mn²⁺ reagent.
- (3) Using a gravimetrically calibrated pipet add a suitable volume of the KIO₃ standard to the stirring solution
- (4) Insert the electrode and delivery tube and immediately begin titration
- (5) The normality of the thiosulfate is calculated from the equivalence point volume as Vol_{KIO₃} / Vol_{Thio}) * N KIO₃ using replicates of single KIO₃ volume additions or from the slope of a range of KIO₃ addition volumes.

Blank determination

Reagent blanks were determined as follows:

- (1) A volume of 1-2 L of site water was brought to a boil in a clean glass reagent bottle.
- (2) Boiled, degassed water was cooled and poured into 125 ml sample flasks and sparged with N₂

for no less than 30 minutes.

(3) The sample was then rapidly fixed as a normal sample, and on the auto titrator.

(4) A global reagent blank taken as the mean of samples fixed at each test site (0.078 ± 0.020 , n=5) and used to correct all reference values.

RESULTS of LABORATORY TESTING

Instrument accuracy for the HOBO U26 RDO sensor was tested under nine combinations of temperature and salinity over a range of DO concentrations from 10% to 120% of saturation. Specific test conditions are summarized in Table 1. Results are plotted as a time series of instrument readings against the time series of comparative Winkler reference samples (Figures A-I). The bottom panel of each figure shows the time series of the difference in instrument measurement versus corresponding reference sample (denoted as Delta's). Comparisons of tank duplicates taken at opposite sides of the tank from between 9-18 of the timepoints during each trial showed a mean difference of 0.017 mg/L, with a range over the nine trials of 0.006 – 0.038 mg/L. Those values are within the analytical precision of the reference method and indicate conditions throughout the tank were very homogeneous. However, small changes in measured DO concentrations did occur during some of the sampling phases indicating the tank was slightly moderating after sparging was stopped. Those small drifts in DO concentrations were clearly captured by both instrument and reference sample measurements.

Table 1. Dissolved oxygen temperature and salinity challenge trial conditions. For each trial pre and post measurements of tank temperature (°C) and salinity (S) were made with an calibrated SBE26+4M CTD, equilibrated in well mixed tank for 20 min until stable readings obtained.

Trial ID	Mean Temperature °C	S.D. Temperature °C	Mean Salinity PSU	S.D. Salinity PSU	Levels of DO tested mg/L	Figure for HOBO
L_T15_S00	15.44	0.03	0.00	0.000	0, 2, 5, 9, 10, 14	A
L_T15_S10	15.47	0.01	8.82	0.003	0, 2, 8, 9, 13	B
L_T15_S35	15.39	0.03	34.20	0.009	0, 2, 6, 8, 12	C
L_T04_S00	5.40	0.08	0.00	0.000	0, 4, 12, 17	D
L_T04_S10	5.30	0.03	8.98	0.009	0, 5, 12, 16	E
L_T04_S35	5.23	0.07	34.77	0.073	0, 4, 10, 14	F
L_T30_S00	30.22	0.03	0.00	0.000	0, 3, 5, 9	G
L_T30_S10	30.51	0.12	9.28	0.036	0, 3, 7, 10	H
L_T30_S35	30.61	0.07	34.43	0.050	0, 2, 6, 9	I

The mean and standard deviation of the differences between the HOBO U26 RDO and reference measurements for each trial (n = 36 - 51) are presented in Table 2. Explanations for the difference between observed and possible comparative observations for the Lab trials are noted in the respective figure legends. Mean difference among trials ranged from 0.047 to 0.314 mg/L over all nine trials. There was a slight decrease in the magnitude of the differences across both temperature and salinity levels (Table 2).

Table 2. Summary of the mean and standard deviation of offsets between paired HOBO sensor measures and Winkler reference DO measures during laboratory trials.

Trial ID	Instrument – Winkler DO			
	<i>mean</i>	<i>s.d.</i>	<i>Observed n</i>	<i>Possible n</i>
L_T15_S00	0.192	0.214	51	51
L_T15_S10	0.086	0.147	39	39
L_T15_S35	0.047	0.113	38	38
L_T04_S00	0.314	0.324	39	39
L_T04_S10	0.208	0.218	36	36
L_T04_S35	0.111	0.140	37	37
L_T30_S00	0.079	0.149	40	40
L_T30_S10	0.114	0.145	39	39
L_T30_S35	0.102	0.128	40	40

The precision of the HOBO U26 sensor was also characterized for each of the nine temperatures and salinity trials (Table 3). Precision trials were conducted at the start of each new tank test when conditions were most stable. Instruments were equilibrated in test tanks at indicated temperature and salinities for 45 min then the subsequent 31 one-minute measurements were used to estimate average tank DO (mg/L) and its variation over that interval. The absolute precision, estimated as the standard deviation (s.d.) around the mean, ranged from 0.003 – 0.013 mg/L across trials with an overall average of 0.006 mg/L. Relative precision, estimated as the coefficient of variation ($CV\% = (s.d./mean)\times 100$), ranged from 0.036 – 0.268 percent across trials with an overall average of 0.074%.

Table 3. Characterization of the precision of the HOBO U26 sensor over a range of temperatures and salinities test conditions.

Trial ID	Temperature	Salinity	Dissolved Oxygen Reading			
			<i>mg/L mean</i>	<i>mg/L s.d.</i>	<i>CV%</i>	<i>n</i>
L_T15_S00	15.44	0.00	9.478	0.006	0.065	31
L_T15_S10	15.47	8.82	9.570	0.004	0.038	31
L_T15_S35	15.39	34.20	8.349	0.003	0.036	31
L_T04_S00	5.40	0.00	12.696	0.006	0.044	31
L_T04_S10	5.30	8.98	12.384	0.007	0.054	31
L_T04_S35	5.23	34.77	10.813	0.006	0.054	31
L_T30_S00	30.22	0.00	4.913	0.013	0.268	31
L_T30_S10	30.51	9.28	7.048	0.004	0.057	31
L_T30_S35	30.61	34.43	5.961	0.003	0.050	31

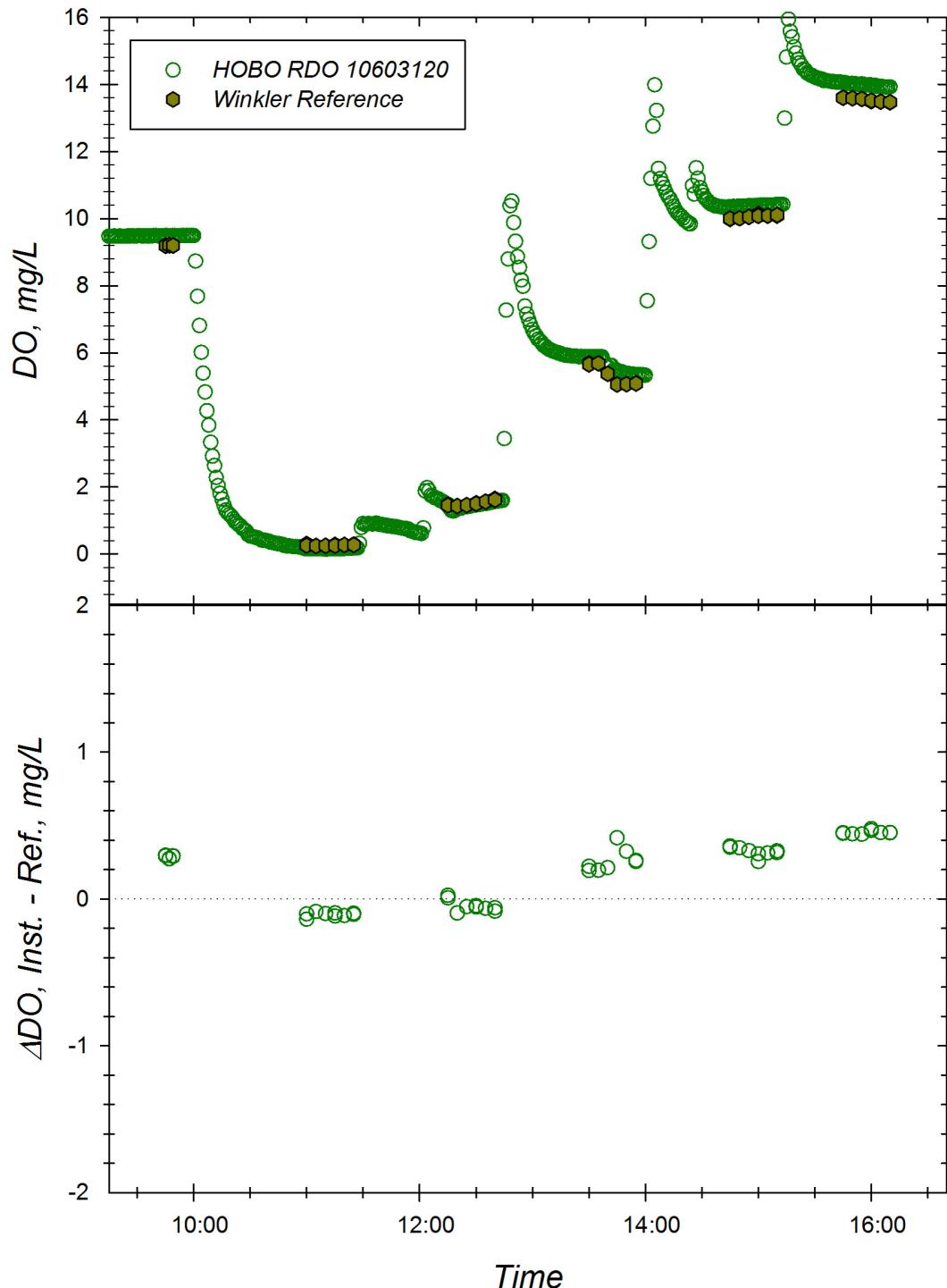


Figure A. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 15.4°C and S=0.00. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference.

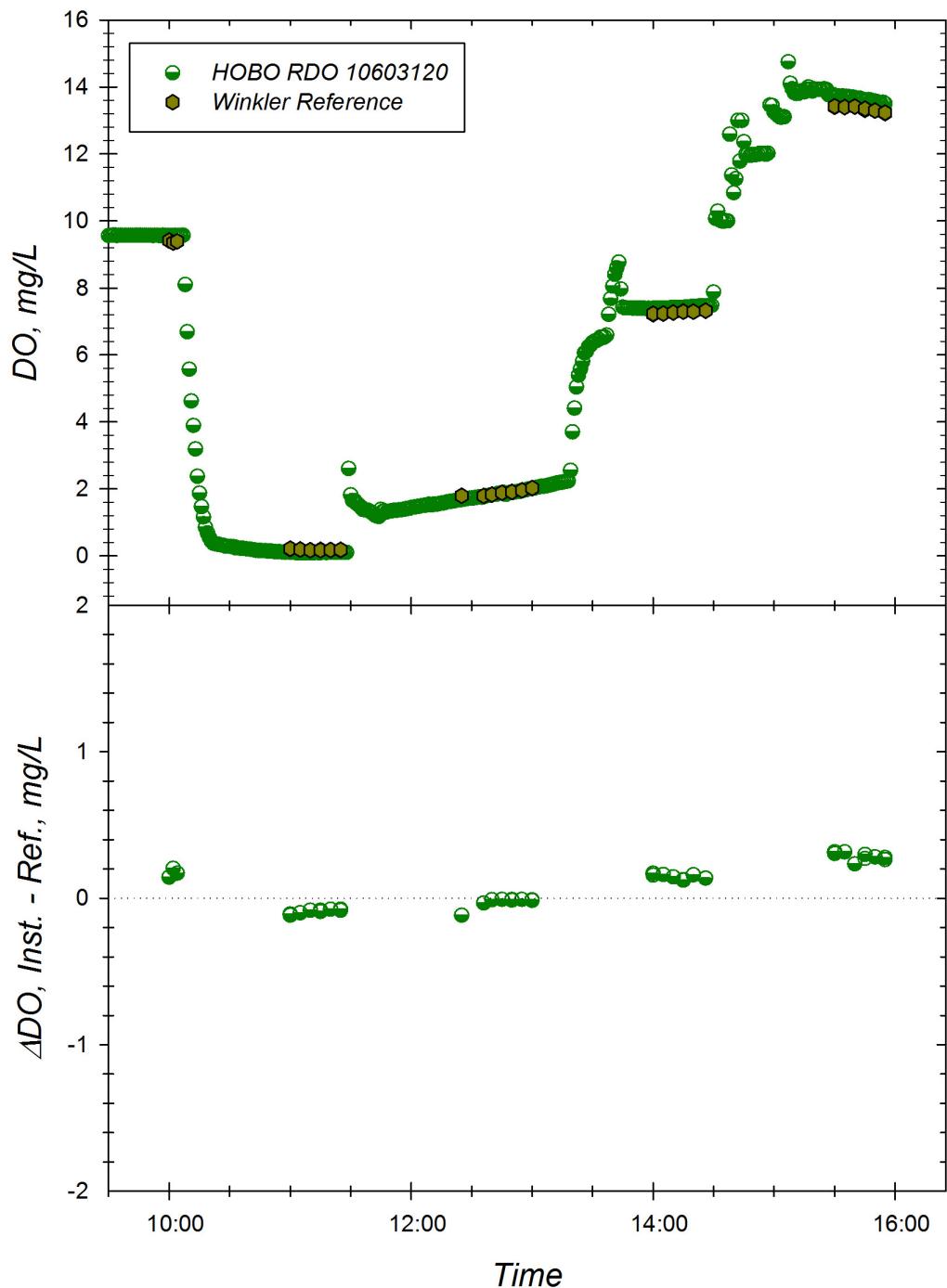


Figure B. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 15.4°C and S=8.82. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference. Instrument reads were adjusted to bath salinity using HOBOware's DO Assistant processing feature.

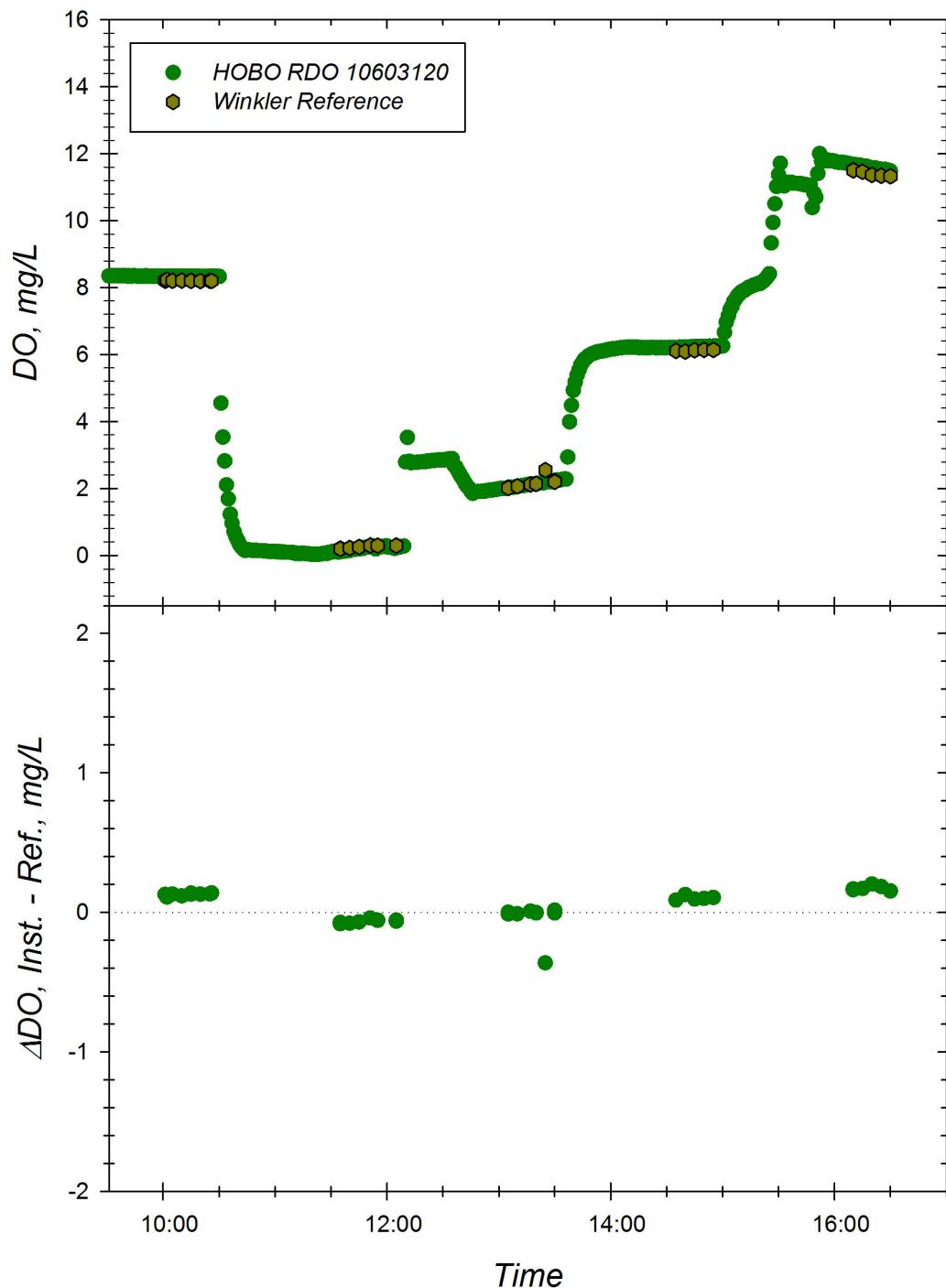


Figure C. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 15.4°C and S=34.20. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference. Instrument reads were adjusted to bath salinity using HOBOware's DO Assistant processing feature.

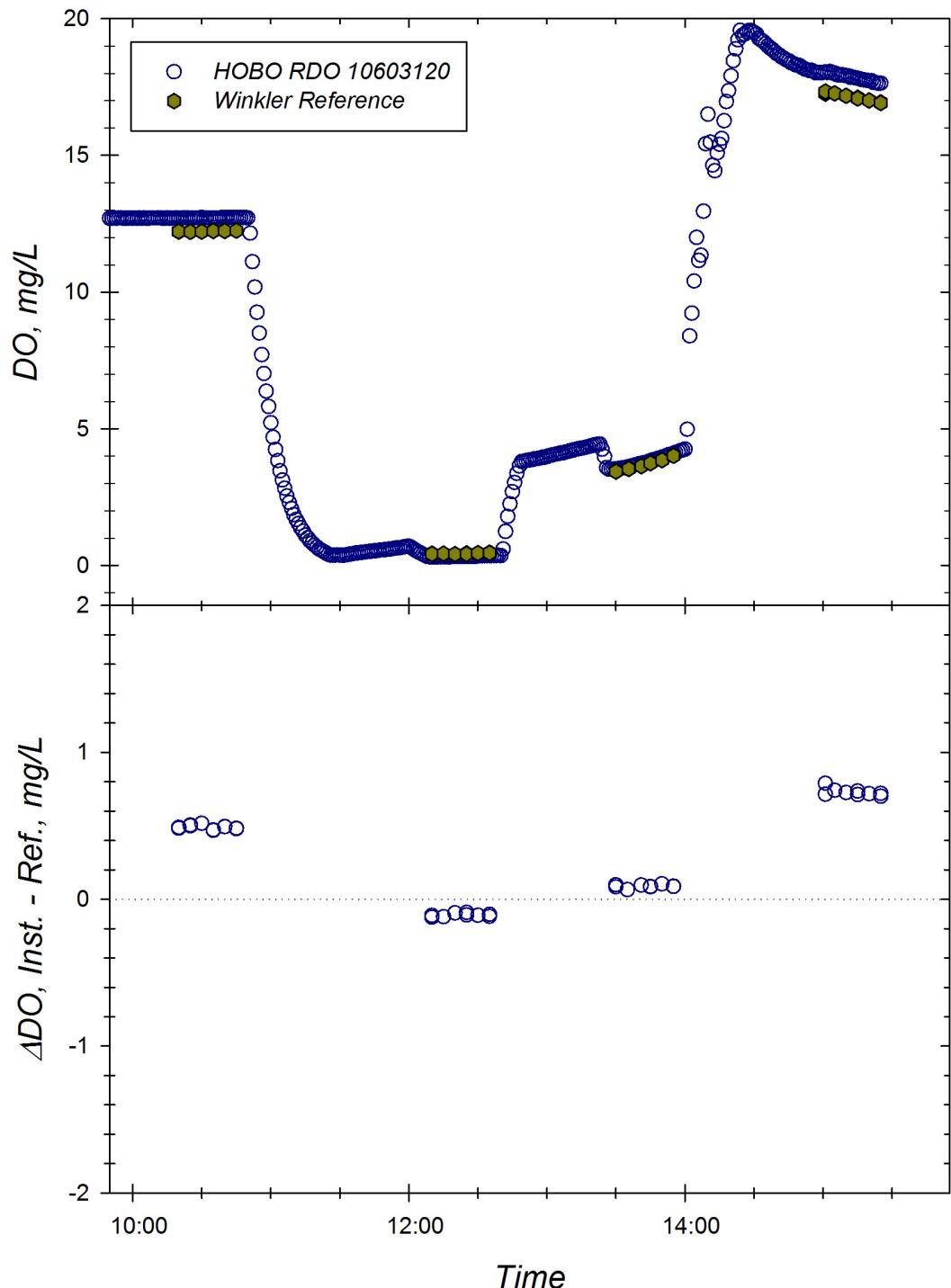


Figure D. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 5.4°C and S=0.00. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference.

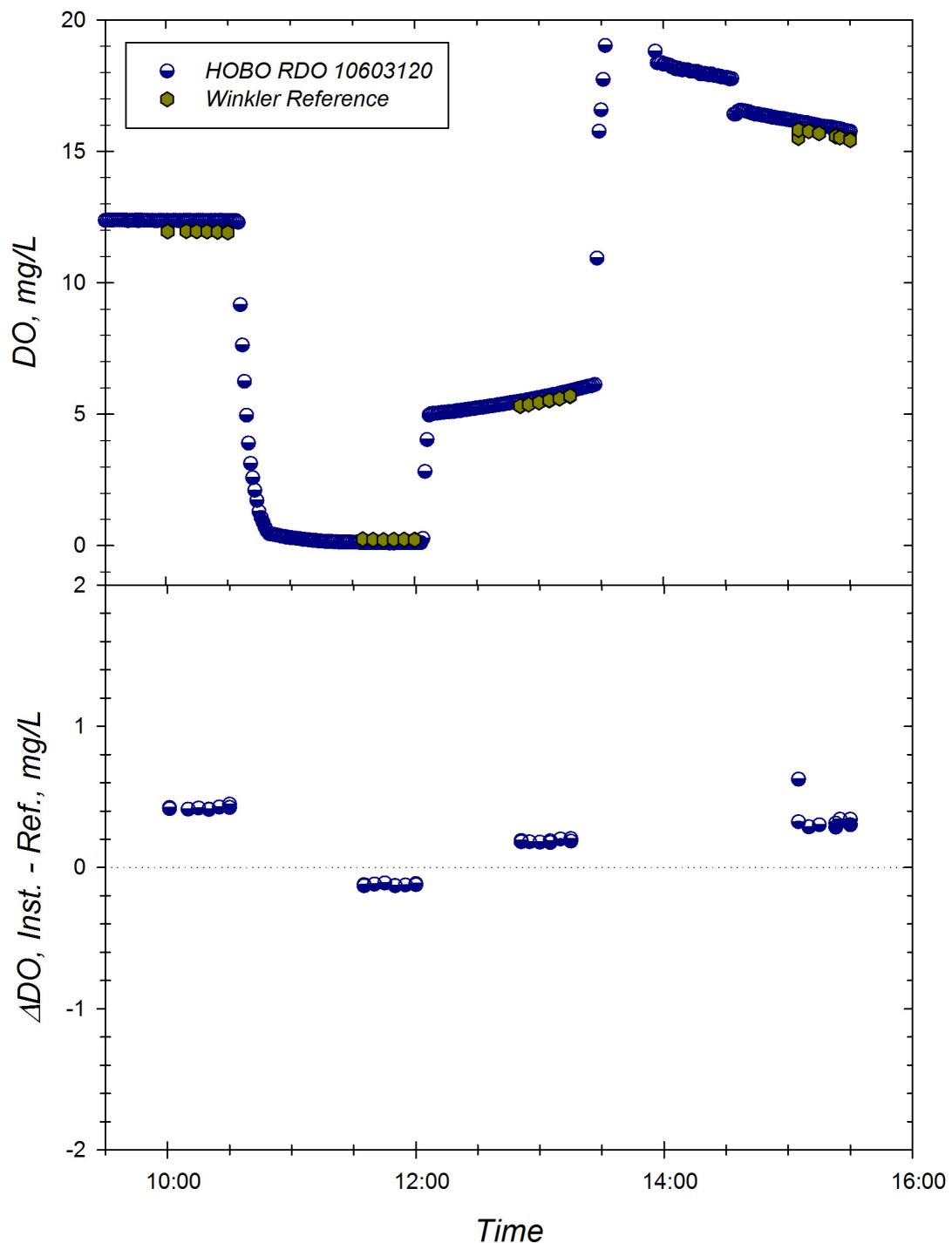


Figure E. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 5.3°C and S=8.98. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference. Delta plots limited to ± 2 mg/L for clarity. Instrument reads were adjusted to bath salinity using HOBOware's DO Assistant processing feature.

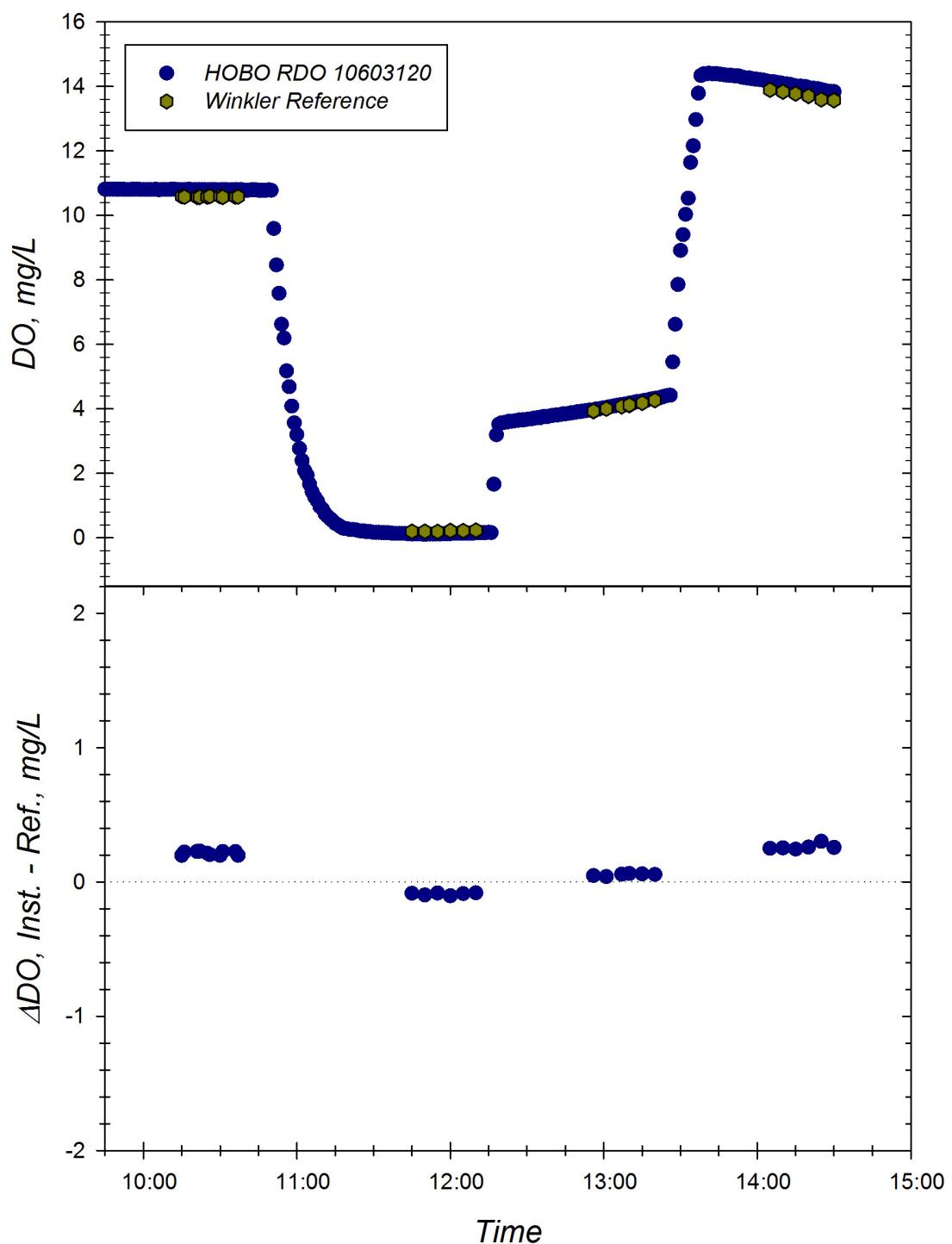


Figure F. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 5.2°C and S=34.77. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference. Delta plots limited to ± 2 mg/L

for clarity. Instrument reads were adjusted to bath salinity using HOBOware's DO Assistant processing feature.

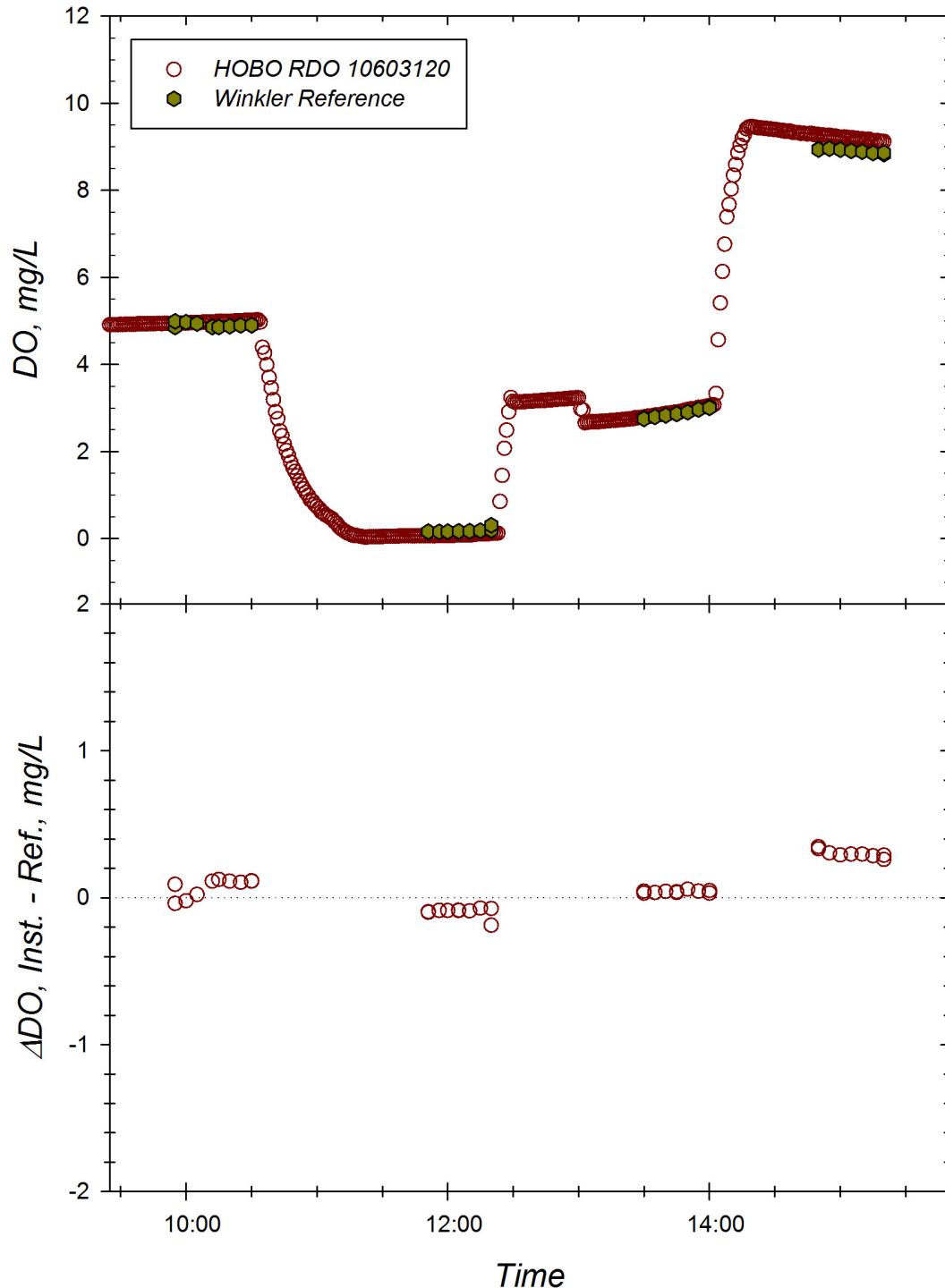


Figure G. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 30.2°C and S=0.00. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference.

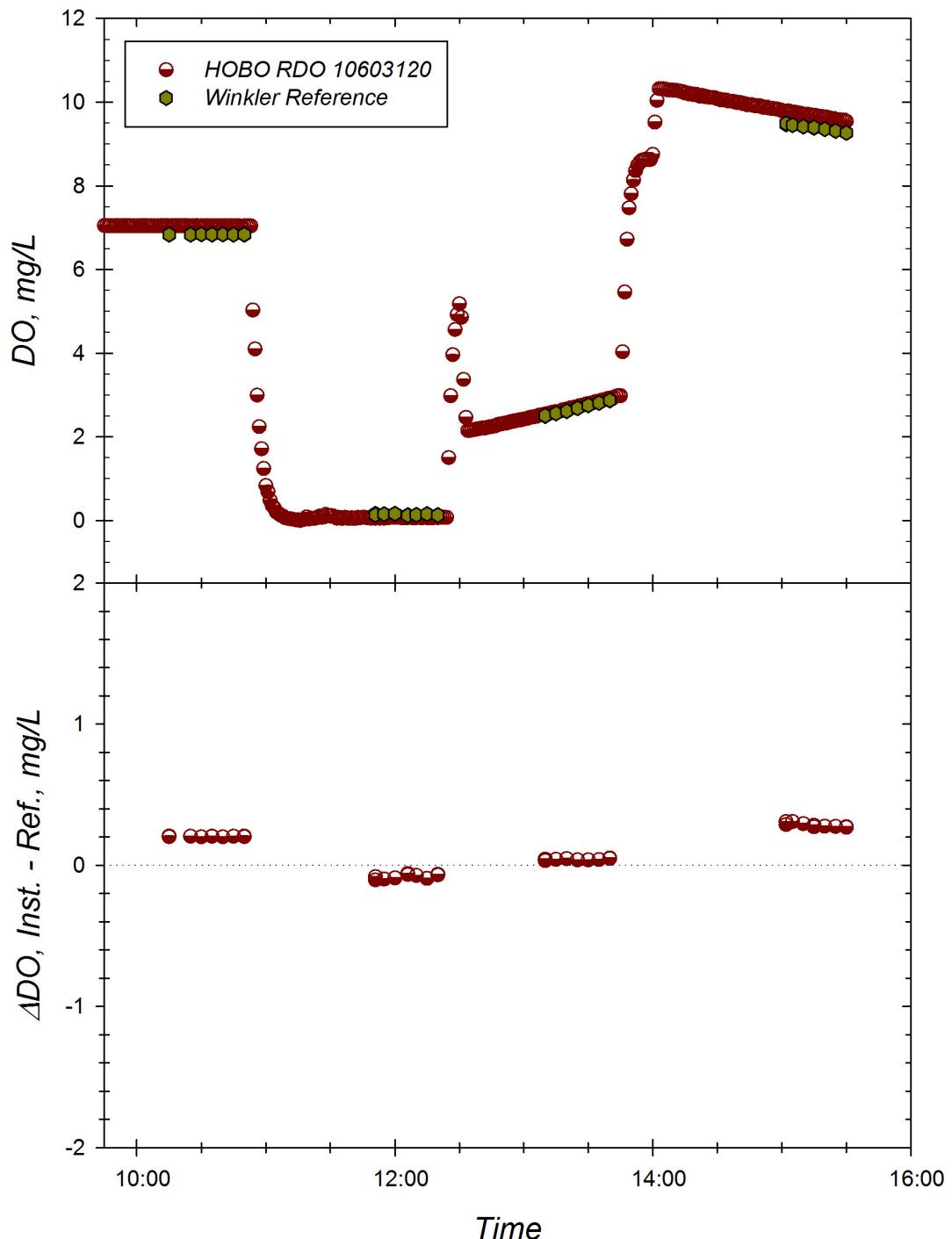


Figure H. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at 30.5 °C and S=9.28. *Top Panel:* Time series of instrument readings and Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:*

Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference. Instrument reads were adjusted to bath salinity using HOBOware's DO Assistant processing feature.

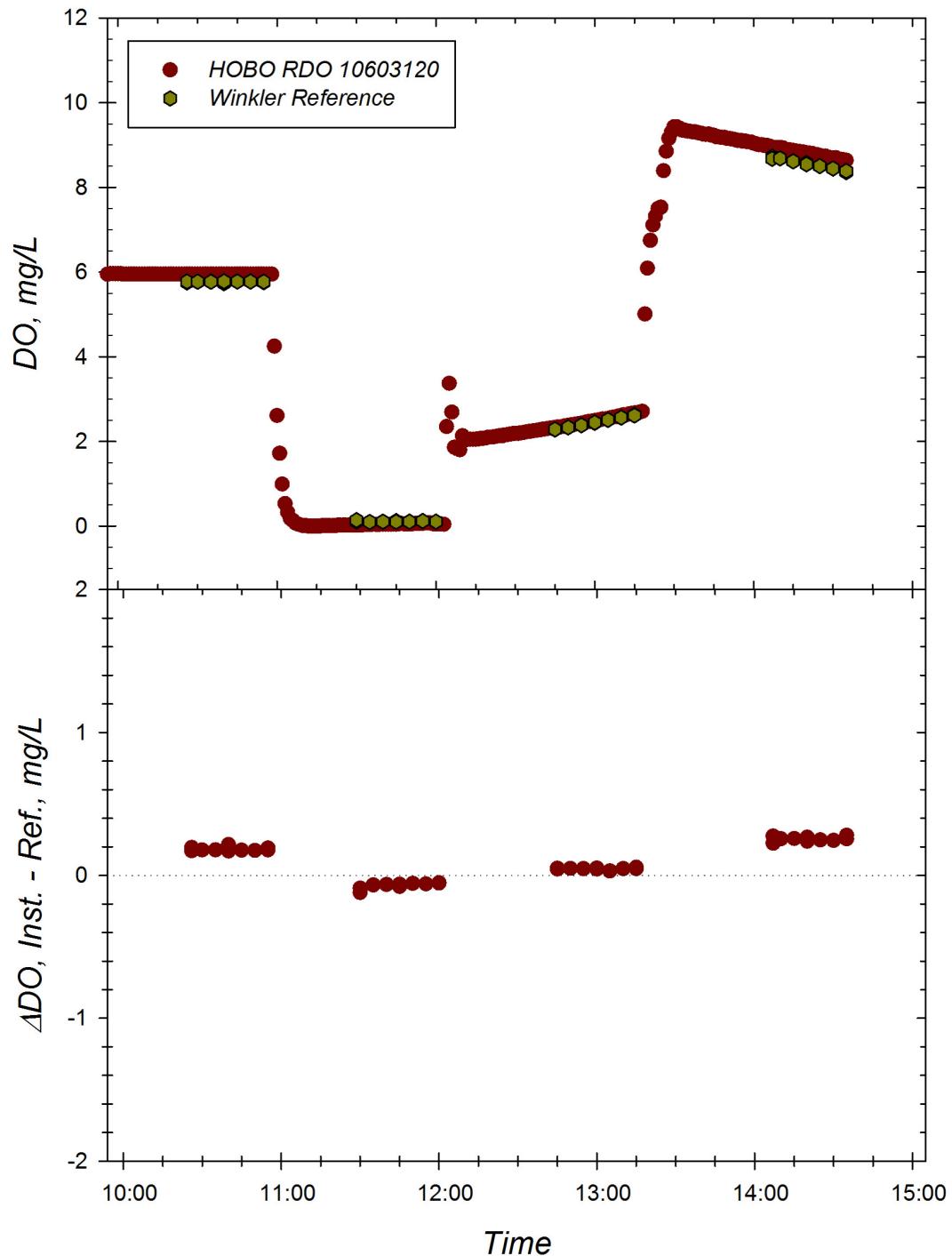


Figure I. Response of the Onset HOBO logger with RDO (sn 10603120) to changing dissolved oxygen concentrations at $30.6^{\circ}C$ and $S=34.43$. *Top Panel:* Time series of instrument readings and

Winkler reference samples drawn at level of sensor foil in well-mixed test tanks. *Bottom Panel:* Corresponding offset between instrument reading and Winkler DO reference sample during exposure trial. Dotted line represents zero offset from reference. Instrument reads were adjusted to bath salinity using HOBOware's DO Assistant processing feature.

The global response of the HOBO U26 for all nine temperature-salinity trials is shown in figure J. This plot includes all instrument-reference comparisons. In general instrument readings were well correlated across the entire range of DO concentrations (hypoxic to supraoxic) covering all temperature and salinity ranges tested. A linear regression of the instrument versus reference measurements ($n=358$; $r^2 = 1.00$; $p<0.0001$) produced a slope of 1.037 and intercept of -0.084

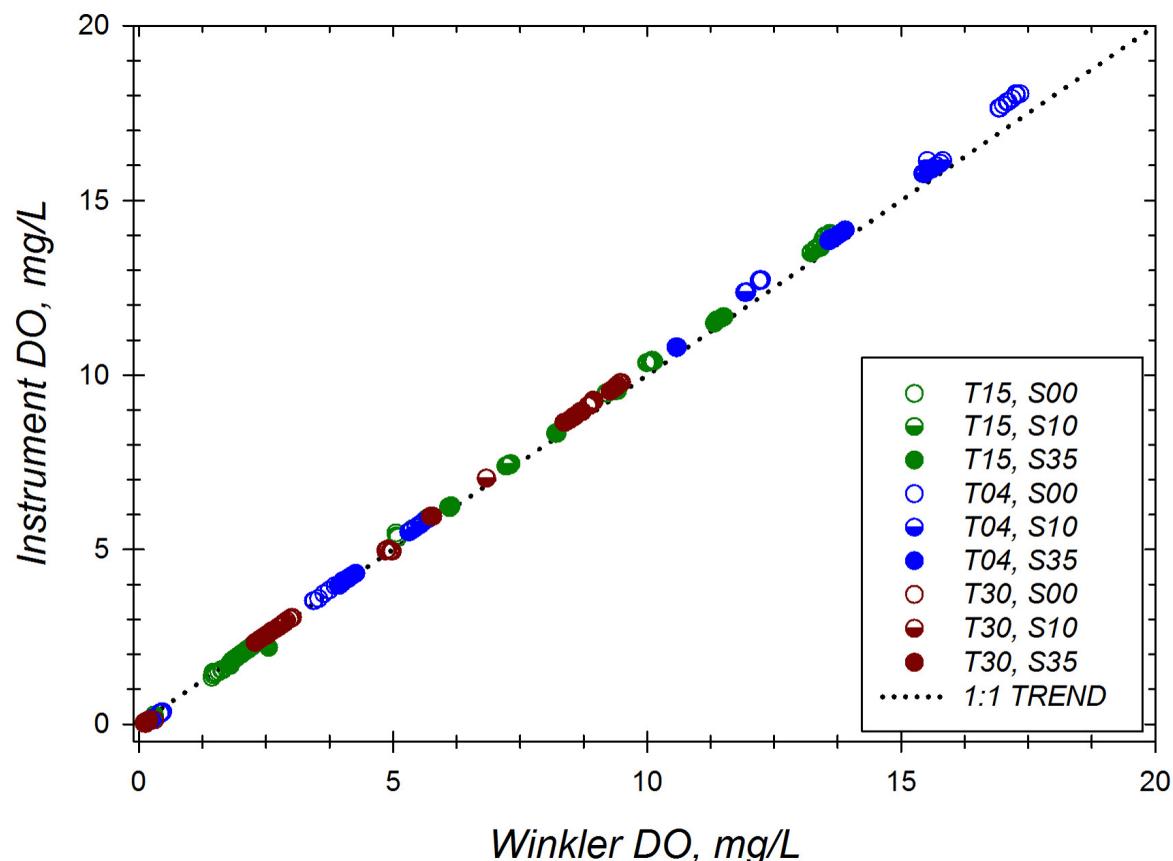


Figure J. Concordance of the Onset HOBO logger with RDO (sn 10603120) oxygen measures paired with discrete Winkler DO reference samples. In general readings are well correlated across a range of DO concentrations (hypoxic to supraoxic). Offsets in instrument readings increase at higher DO levels and with salinity. Dotted line represents perfect agreement between the two measures.

Individual response slopes and intercepts of instrument measurement versus reference DO for each of the laboratory trials at a fixed temperature and salinity level are summarized in Table 4. The response curves of the HOBO U26 exhibited no consistent trends in the response slopes across the temperature and salinity levels.

Table 4. Summary of regression statistics for the HOBO U26 versus reference sample response curves for each of the nine laboratory trials.

Test ID	R2	y Intercept	Slope
LT15S00	1.000	-0.074	1.041
LT15S10	1.000	-0.083	1.028
LT15S35	1.000	-0.071	1.023
LT04S00	1.000	-0.115	1.049
LT04S10	1.000	-0.061	1.032
LT04S35	1.000	-0.076	1.026
LT30S00	1.000	-0.108	1.044
LT30S10	1.000	-0.075	1.039
LT30S35	1.000	-0.060	1.039

Results of the 56 day long-term stability and thermal stress challenge for the HOBO U26 RDO are shown in figure K. The instrument was maintained in a well circulated tank and oxygen content manipulated by alternately varying water temperature set point between 15 and 25°C several times per week of deployment. The time series of instrument readings at 15min intervals is plotted against discrete values for Winkler reference samples (*top panel*) along with the time series of the difference between instrument and reference measurements (*bottom panel*). The overall mean of differences between measurements was 0.041 (s.d. = 0.339) mg/L for 154 comparisons (out of a potential total of 156). If the outliers near the end of the test are omitted there was no significant change in accuracy over time (slope = -0.002 mg/L/d; r^2 = 0.011; p=0.206). For all data, the slope increased to -0.004 mg/L/d (r^2 = 0.049; p=0.006) reflecting the influence of the two large negative responses near the end of the test.

Results for a functional sensor response time assessment of the HOBO U26 are shown in figure L. The top panel depicts the time series of 60s instrument reads during transfers between adjacent high (9.6 mg/L) and low (2.0 mg/L) DO water baths, maintained commonly at 15 °C. The bottom panel (*lower left*) depicts results fit with a 3 parameter exponential decay function: $DO_{rel} = DO_{relMin} + ae^{-bt}$ and indicated τ calculated from fit. Data for low DO to high DO transitions (*lower right*) were treated similarly but normalized to steady state value in subsequent high DO tank and subsequently fit with an analogous 3 parameter exponential rise function: $DO_{rel} = DO_{relMin} + a(1-e^{-bt})$ with indicated τ being directly calculated from fit. The calculated τ_{90} was 27 s during high to low transitions and 26 s for low to high transitions covering a DO range of approximately 8 mg/L at a constant 15°C.

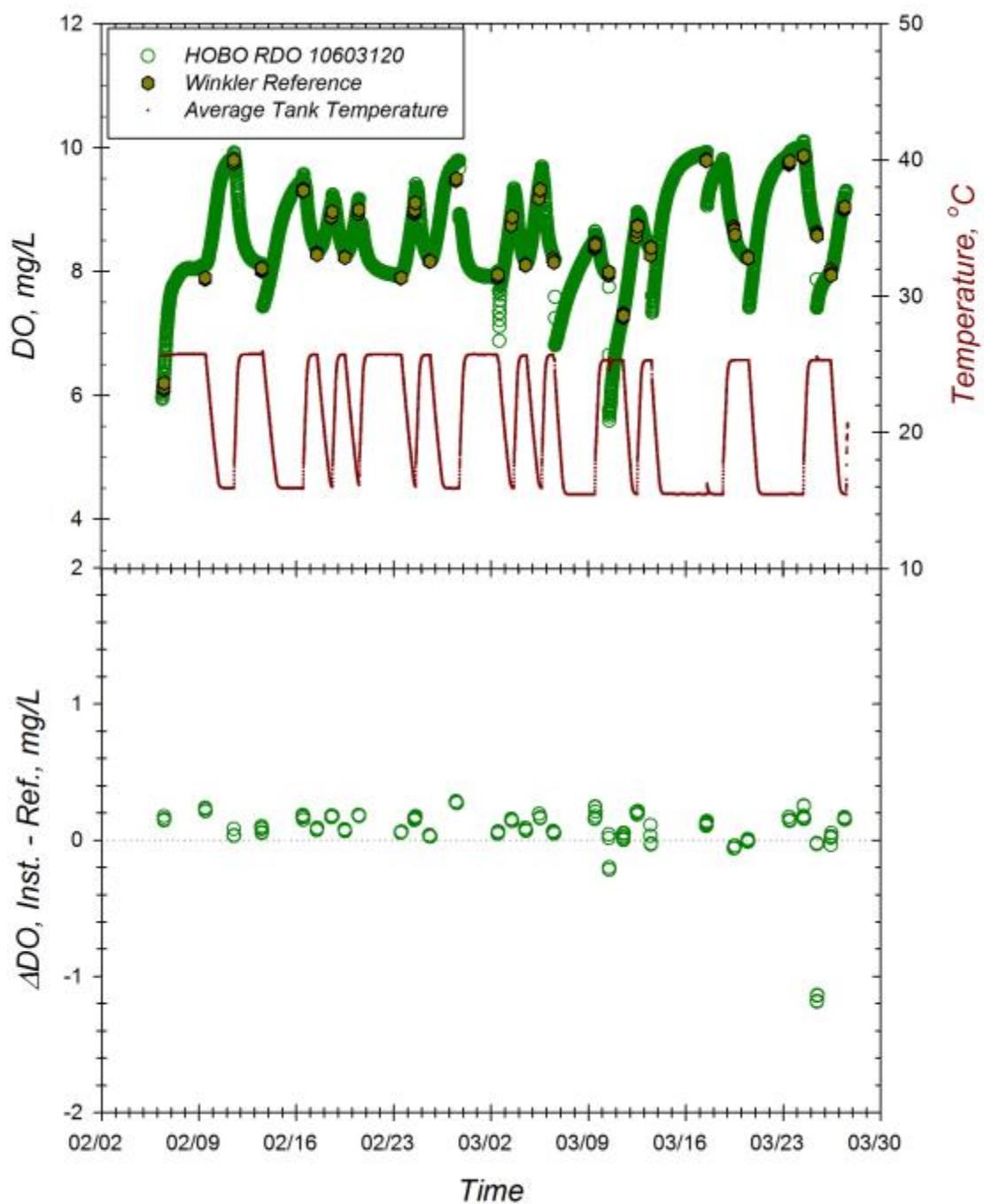


Figure K. Long-term stability and thermal stress challenge of the Onset HOBO logger with RDO (sn 10603120). Instrument was maintained in well circulated tank and oxygen content manipulated by alternately varying water temperature set point between 15 and 25 °C several times per week of deployment. *Top Panel:* Time series of the instrument readings at 15min intervals with discrete values for Winkler assay of grab samples taken at sensor level. *Bottom Panel:* Offset in paired DO readings of and Winkler grab samples.

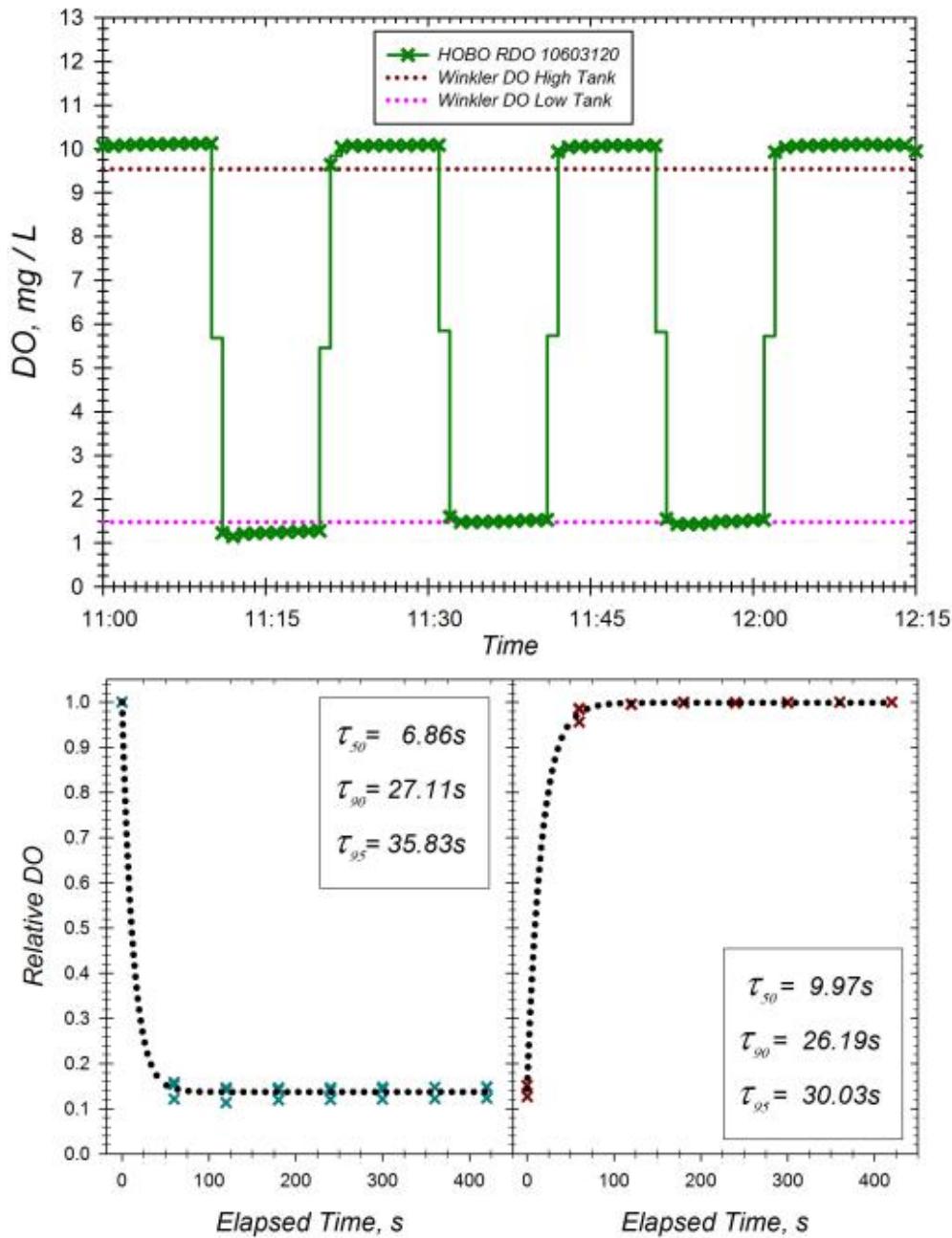


Figure L. Assessment of the sensor response time for the Onset HOBO logger with RDO (sn 10603120). *Top Panel:* Time series of 10s reads of instruments during transfers between high and low DO buckets maintained adjacent in a common water bath equilibrated at 15 °C. Indicated DO levels were maintained by airstone bubblers (high DO) or air+N₂ (low DO) and verified by Winkler assays. *Bottom Panels:* All high DO to low DO transitions were normalized to last reading before condition switch for the indicated time post transition (left). Data was fit with a 3 parameter exponential decay function: $\text{DO}_{\text{rel}} = \text{DO}_{\text{relMin}} + ae^{-bt}$ and indicated τ calculated from fit. Data for low DO to high DO transitions (*lower right*) were treated similarly but normalized to steady state value in subsequent high DO tank and subsequently fit with an analogous 3 parameter exponential rise function: $\text{DO}_{\text{rel}} = \text{DO}_{\text{relMin}} + a(1-e^{-bt})$ with indicated τ being directly calculated from fit.

RESULTS of MOORED FIELD TESTS

Moored field tests were conducted to examine the performance of the HOBO U26 RDO to consistently track natural changes in dissolved oxygen over extended deployment durations of 11–18 weeks. In addition, field tests examined the reliability of the instrument, i.e., the ability to maintain integrity or stability of data collection over time. Reliability was determined by quantifying the percent of expected data that was recovered and useable. The performance of the HOBO was examined in three separate field deployment tests at various ACT Partner sites to include a range of biogeochemical conditions. The range and mean for temperature and salinity for each test site is presented in Table 4. The final reference temperature data was computed from the mean of two RBR thermistors and a SeaBird SBE that were mounted at the same sampling depth as the test instrument. Immediately before each deployment the HOBO was exposed to a laboratory reference tank for 3–4 measurements to confirm good working status and provide an initial offset against its latest calibration (Table 5). In all cases the HOBO was calibrated by ACT personnel according to manufacturer instructions just prior to the pre-deployment check.

Table 4. Range and average for temperature, and salinity at each of the test sites during the sensor field deployments. Temperature and salinity were measured by at least 2 RBR temperature loggers and a SeaBird SBE 26 (or SBE26plus) mounted on the instrument rack for the duration of the deployment.

SITE (deployment period/duration)		Temperature °C)	Salinity
Keweenaw Waterway	Min.	0.04	0.00
9Jan – 22Apr	Max.	5.2	0.01
(n = 104 days)	Mean	0.7	0.01
Chesapeake Bay	Min.	19.3	4.7
20May – 5Aug	Max.	36.7	13.7
(n = 78 days)	Mean	25.6	10.9
Kaneohe Bay	Min.	23.1	27.3
24Sep – 21Jan	Max.	29.1	34.7
(n = 121 days)	Mean	25.8	33.4

Table 5. Results of the pre-deployment check for the HOBO U26 for each deployment site. The DO of the reference solution is based on the mean of three Winkler titrations of the reference tank during the exposure. Ambient water was used for the reference solution at each deployment site.

Deployment Site	Reference Solution	Measurement Temperature	Reference Solution DO (mg/L)	Instrument DO (mg/L)
MTU	Site Water	11.7°C	10.193	10.67
CBL	Site Water	21.6°C	7.983	8.17
HIMB	Site Water	26.6°C	6.547	6.71

Michigan Tech Great Lakes Research Center Field Deployment Site

A 15 week deployment under ice took place from January 9 through April 22 in the Keweenaw Waterway adjacent to the Great Lakes Research Center in Houghton, MI. The deployment site was located at 47.12° N, 88.55° W, at the end of the pier at the Great Lakes Research Center docks. This site is located on the south side of the Keweenaw Waterway, and is connected to Lake Superior in both the NW and SE directions. The instrumentation rack was lowered off of the end of the pier with a ½ ton crane and rested on the bottom, under the ice, in 4.5m of water. A small shelter was constructed at the end of the pier to provide shelter for processing the reference samples during winter sampling efforts.



Photo 1. Aerial view of the Keweenaw Waterway (left) and dockside mooring deployment (right).

Time series results of ambient conditions for temperature and specific conductivity are given in figure 1. Temperature ranged from 0.04 - 5.3°C and specific conductivity from 87 - 137 $\mu\text{S}/\text{cm}$ over the duration of the field test. The bottom panel displays the maximum difference recorded between all reference thermistors mounted at the same depth as the sensors sampling intakes as well as a meter above, at different locations across the mooring rack. The average temperature difference observed across the space of the mooring rack was 0.01°C with a maximum of 0.98°C. Differences between instrument and reference readings resulting from this variability should be minimized as the sampling bottle integrates across the mooring space.

Unexpected shifts between adjacent reference samples were noted on three occasions during the test. Upon inspection it was determined that these excursions occurred during changes in the batches of Winkler reagents. A correction to reference values was subsequently made based on the magnitude of change observed between the adjacent Winkler measurements after adjusting for ambient changes determined by the average of all seven DO sensors deployed on the mooring. Adjusted values are noted within each figure.

The HOBO U26 operated successfully throughout the entire 15 week deployment and generated 9859 observations based on its 15 minute sampling interval for a data completion result of 100%. Time series results of the HOBO and corresponding reference DO results are given in figure 2 (top panel). Ambient DO measured by the HOBO ranged from 9.41 to 14.65 mg/L compared to the range captured by reference samples of 10.918 to 14.007 mg/L. The sharp

excursion between April 8 - 9 was real and was picked up by all instruments and correspondingly seen in specific conductance and temperature variability across the instrument rack (Fig. 1).

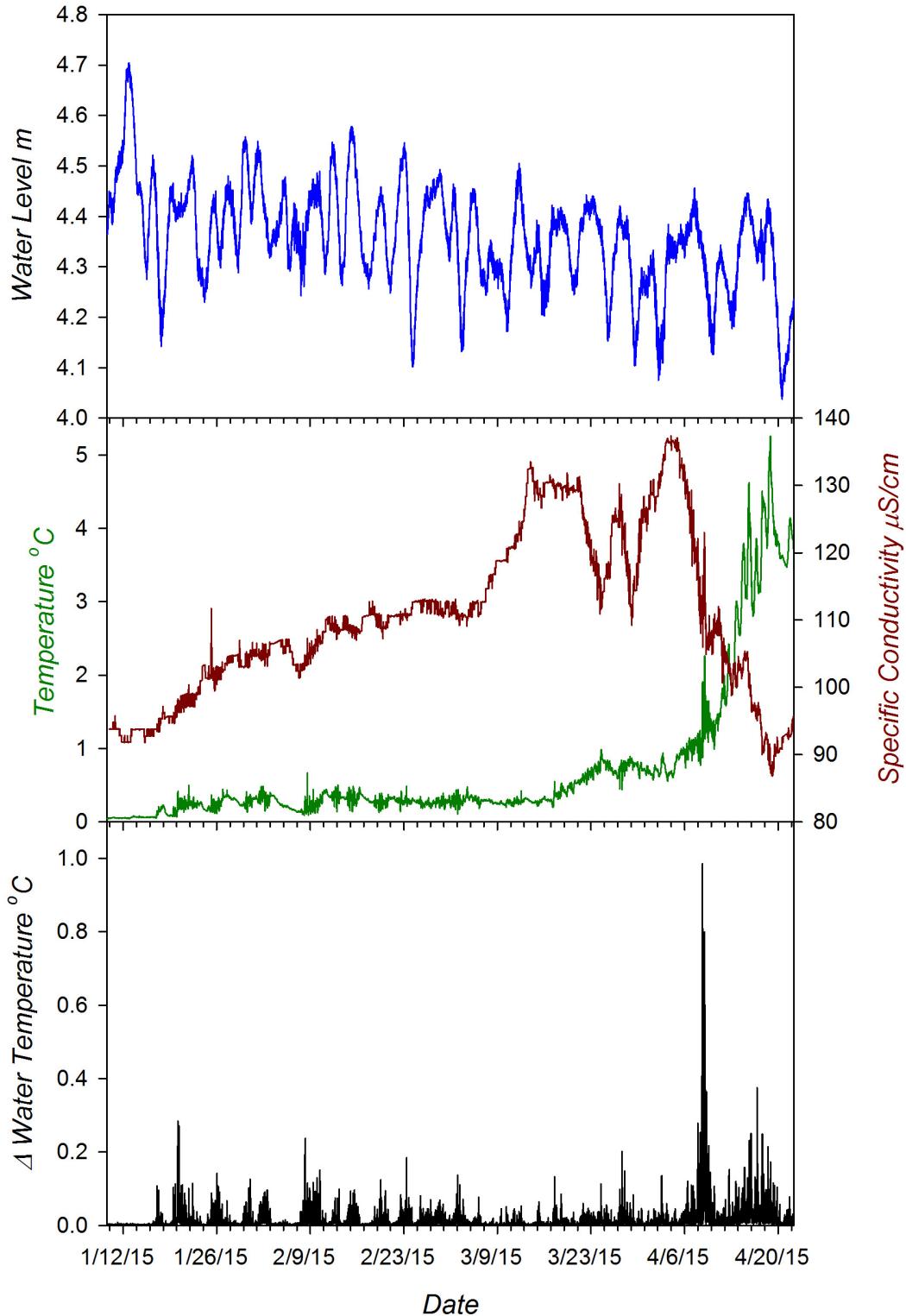


Figure 1. Environmental conditions encountered during deployment at the MTU site. Test sensor array deployed at 4.5 m fixed depth, variation in local water levels indicate active water flow around instruments (*Top Panel*). Variation in specific conductivity (red) and temperature (green) at depth of instrument sensor detected by an SBE 26 and two RBR Solo thermistors (*Middle Panel*). Temperature range determined from max-min temperatures detected by 4 RBR thermistors spanning instrument sensor array (*Bottom Panel*).

The time series of the difference between instrument and reference DO measurements for each matched pair ($n=118$ observations) is given in the bottom panel of figure 2. The average and standard deviation of the measurement difference over the total deployment was 0.855 ± 0.116 mg/L with a total range of 0.533 to 1.030 mg/L. The drift rate of instrument offset, estimated by linear regression ($r^2=0.73$; $p<0.0001$), was 0.003 mg/L/d. This rate would include any biofouling effects as well as any electronic or calibration drift.

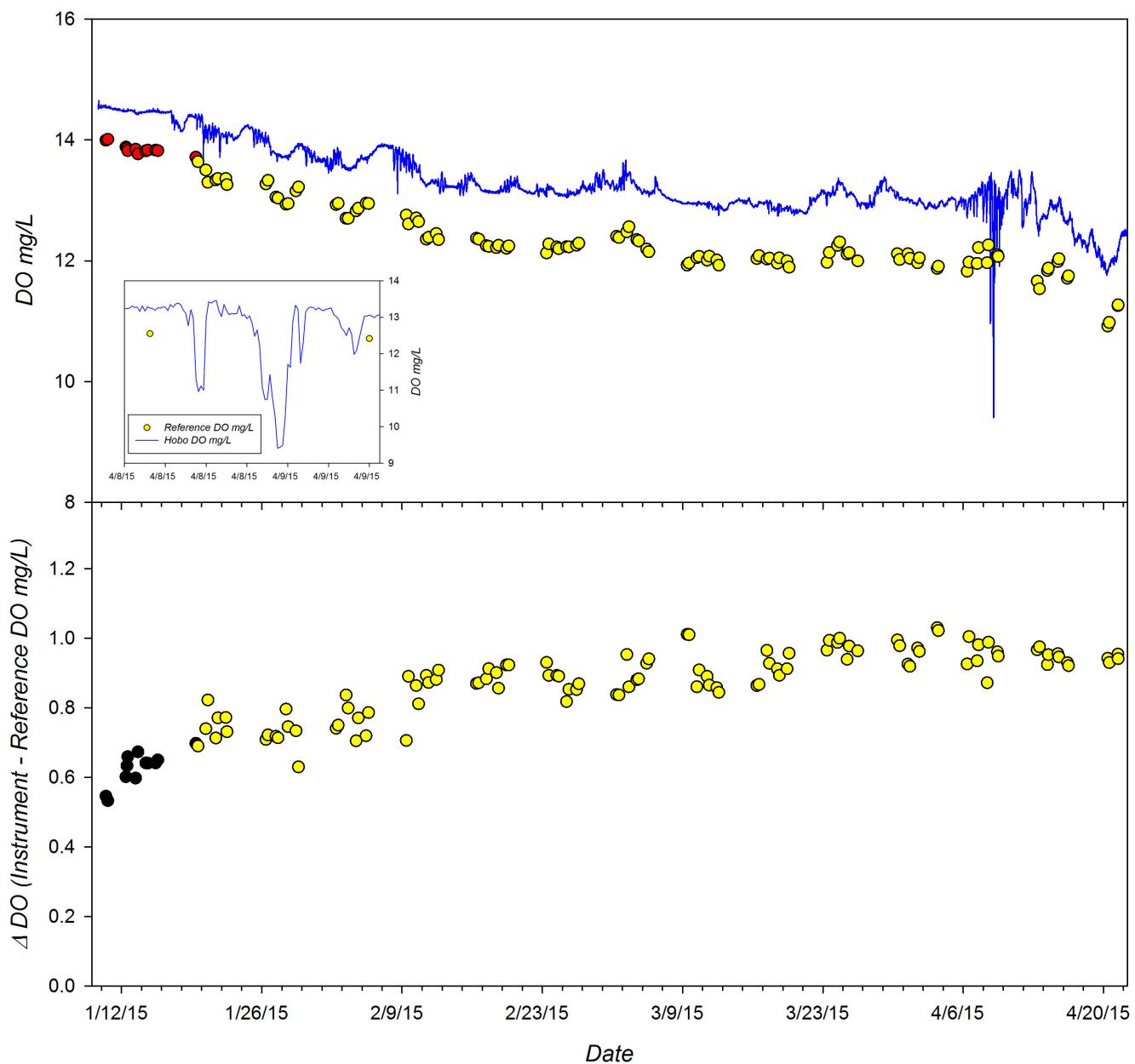


Figure 2. Time series of DO measured detected by HOBO U26 deployed during the 15 week Great Lakes field trial. *Top Panel:* Continuous DO recordings from instrument (blue line) and DO of adjacent grab

samples determined by Winkler titration (red circles, yellow circles represent adjusted reference values). *Bottom Panel:* Difference in measured DO relative to reference samples (Instrument DO mg/L – Reference DO mg/L) observed during deployment. *Insert:* Close up of excursion that occurred 4/8-4/9. No reference samples were collected during this time period.

A cross-plot of the matched observations during the first month of deployment is given in figure 3. Comparisons were limited to one month as a representation of a typical field deployment and to focus on initial instrument offset versus impacts of biofouling during an extended deployment. A linear regression of the instrument versus reference measurements ($r^2 = 0.98$; $p < 0.0001$) produced a slope of 0.855 and intercept of 2.64 indicating mostly an initial calibration offset. The calibration offset was also noticed in the original tank test before deployment.

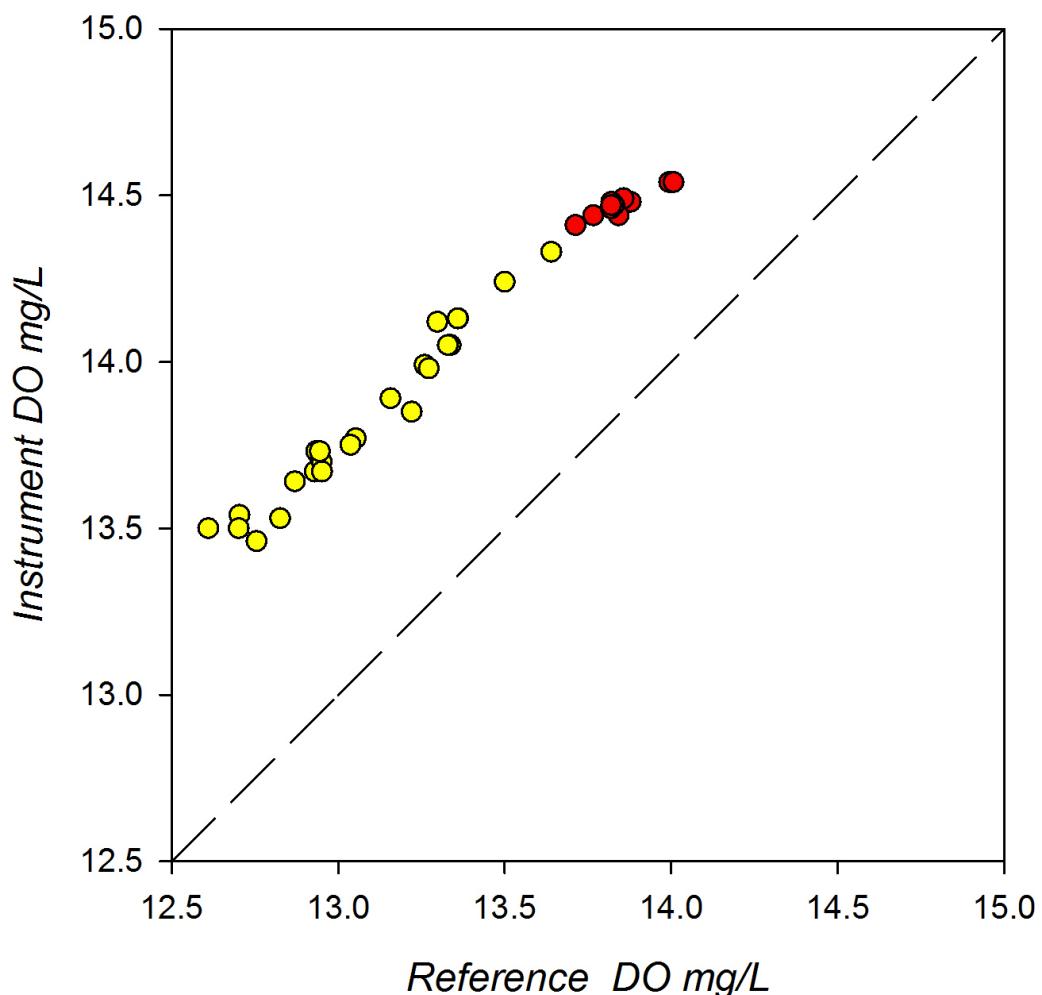


Figure 3. MTU GLRI field response plot for the first month of deployment of HOBO instrument compared to reference DO samples. The plotted line represents a 1:1 correspondence. (Yellow circles denote adjusted reference values).

Photos of test instrument before and after the field deployment to indicate potential impact of biofouling (Photo 2).



Photo 2. HOBO U26 prior to and following 15 week deployment under ice for the MTU field test.

Moored Deployment at Chesapeake Biological Laboratory (CBL)

An eleven week moored field test was conducted in Chesapeake Bay from May 20 to August 5, 2015. The deployment was located at 38.32°N, 76.45°W attached to the side of a pier at the mouth of the Patuxent River (Photo 3.) The site was brackish with an average water depth of the test site was 2.2 m.

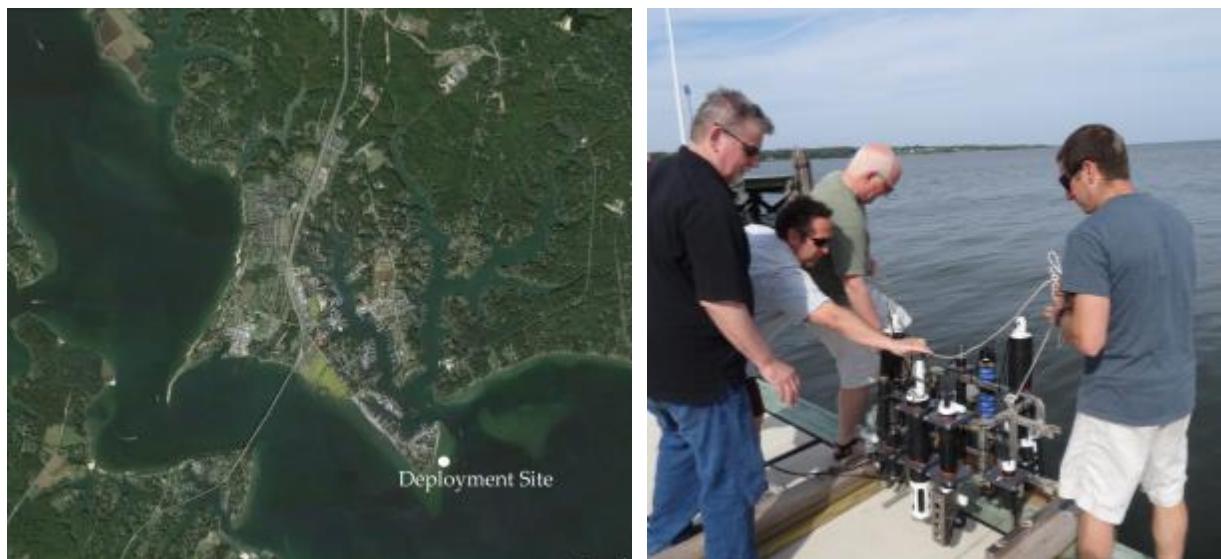


Photo 3. Aerial view of CBL deployment site (left) and duplicate sampling at mooring rack.

Time series results of ambient conditions for tidal height, temperature, and salinity are given in figure 4. Temperature ranged from 19.2 to 30.1 and salinity from 4.7 to 13.7 over the duration of the field test. The bottom panel displays the maximum difference recorded between all reference thermistors (RBR Solo and SBE26) mounted at the same depth and different locations across the mooring rack. The average temperature difference observed across the space of the mooring rack was 0.21 ± 0.25 °C, with a maximum of 3.26°C. Differences between instrument and reference readings resulting from this variability should be minimized as the sampling bottle integrates across the mooring space.

The HOBO U26 generated 7270 from a possible 7270 observations over the 11 week deployment from May 20 through August 4 based on its 15 minute sampling interval. However, only 5314 of the measurements were considered acceptable based on values that were less than 2 mg/L from any reference sample over a similar timeframe and from continuously monitored DO from a nearby independent data sonde. The accepted data resulted in a data completion rate for this deployment of 73%. Time series results of the HOBO U26 and corresponding reference DO results are given in the top panel of figure 5. Ambient DO measured by the HOBO ranged from 2.00 to 11.81 mg/L compared to the range captured by the reference measurements of 4.370 to 10.85 mg/L. The bottom panel presents the time series of the difference between the HOBO and reference DO for each matched pair (limited to ± 2.0 mg/L; n=75 observations out of a total of 142). The average and standard deviation of the measurement difference for the deployment was -0.211 ± 0.860 mg/L, with the total range of differences between -1.85 to 0.94 mg/L.

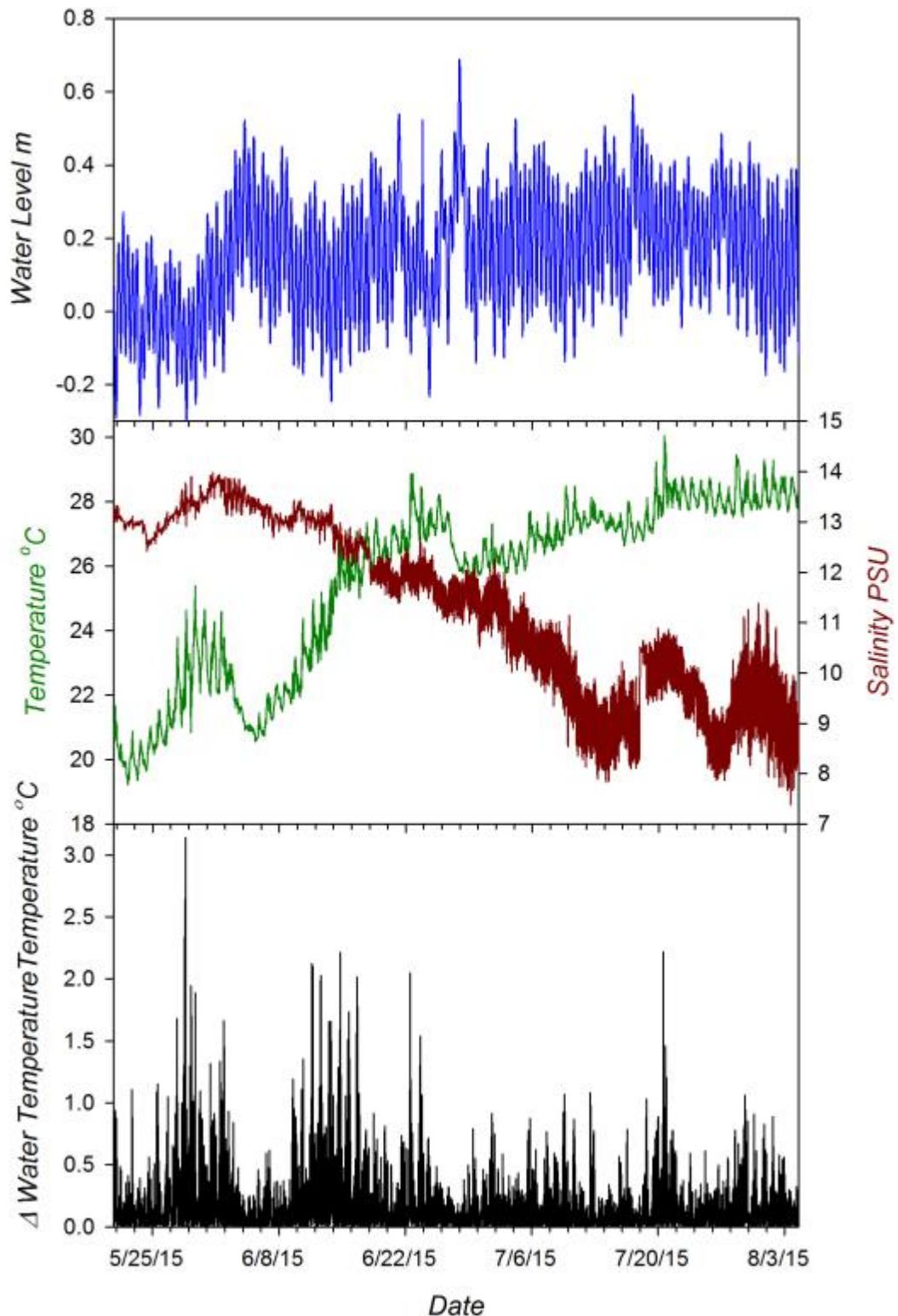


Figure 4. Environmental conditions encountered during the 11 week CBL floating dock deployment. Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in salinity (red) and temperature (green) at depth of instrument sensor detected by an SBE 26 and two RBR Solo thermistors (*Middle Panel*). Temperature range determined from max-min temperatures detected by RBR thermistors spanning instrument sensor array (*Bottom Panel*).

The calculated drift rate in instrument response for the entire deployment period (using the accepted data in Fig 5) was -0.079 mg/L/d ($r^2 = 0.77$; $p < 0.001$). If we consider only the first 35 days of the deployment before any indication of a malfunction, the drift rate was -0.075 mg/L/d ($r^2 = 0.67$; $p < 0.001$). In both cases the rate would include any biofouling effects as well as any electronic or calibration drift.

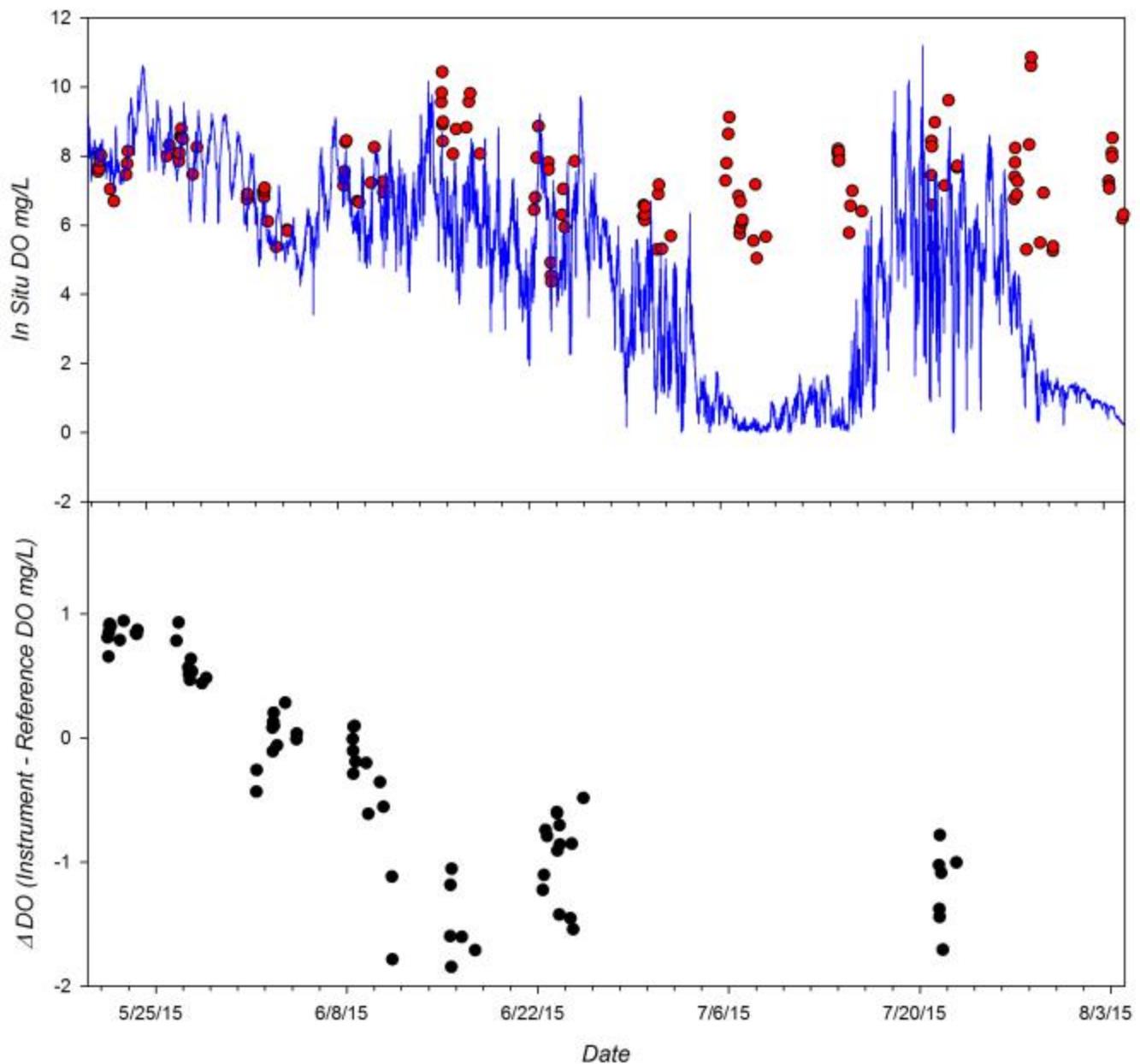


Figure 5. Time series of DO measured by the HOBO during the 12 week CBL field trial. *Top Panel:* Continuous DO recordings from instrument (blue line) and DO of adjacent grab samples determined by Winkler titration (red circles). *Bottom Panel:* Difference in measured DO relative to reference samples (Instrument DO mg/L – Reference DO mg/L) observed during deployment.

A cross-plot of all matched observations for the first month of the deployment is given in figure 6. Comparisons were limited to one month as a representation of a typical field deployment and to focus on initial instrument offset versus impacts of biofouling during an extended deployment. However, it should be noted results include data that were outside of the 2 mg/L acceptance criteria and the high degree of scatter indicates the instrument was impacted by biofouling within this first 30 days (see photo 4 below). A linear regression of the data ($r^2 = 0.262$; $p < 0.001$) produced a slope of 0.594 and intercept of 2.93.

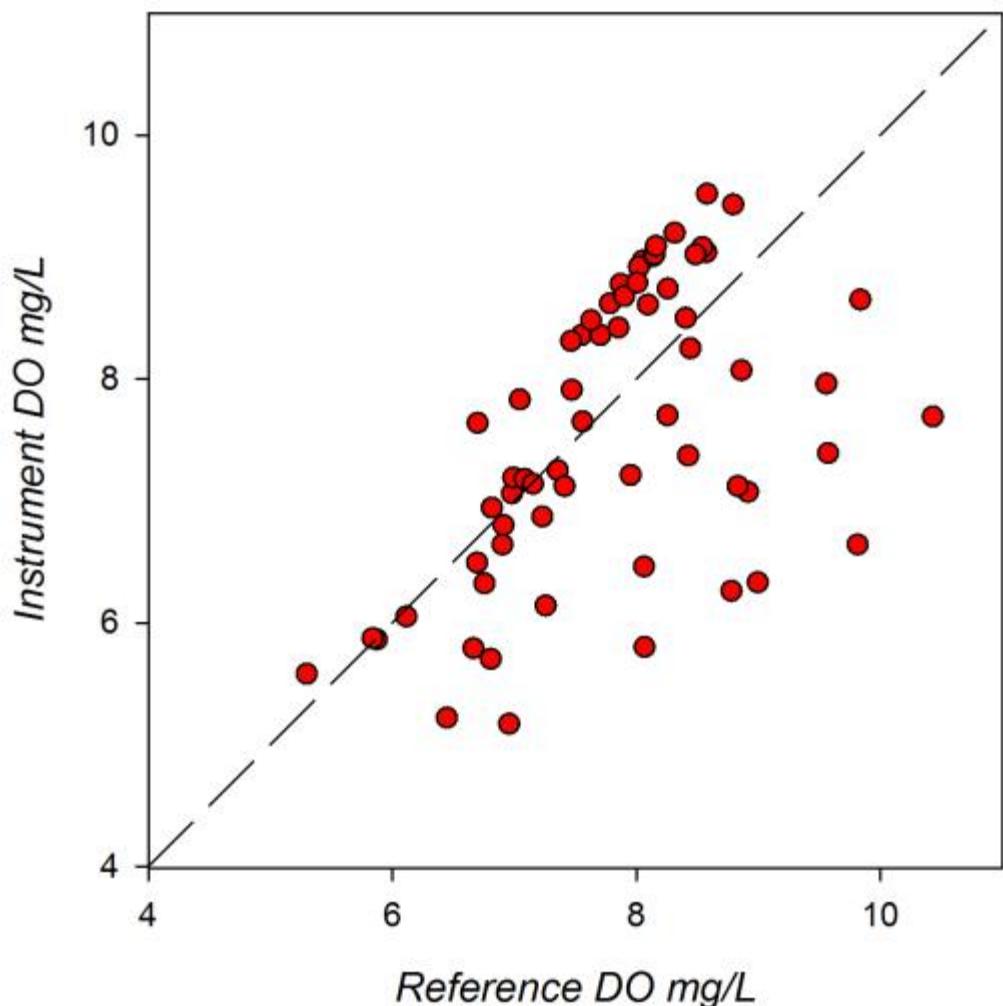


Figure 6. CBL field response plot for HOBO U26 compared to reference DO samples. The plotted line represents a 1:1 correspondence.

Photos of test instrument before and after the field deployment to indicate potential impact of biofouling (Photo 4).



Photo 4. The HOBO instrument before and following the 12 week CBL field trial.

Moored Deployment off Coconut Island in Kaneohe Bay, Hawaii

An 18 week long moored field test was conducted in Kaneohe Bay from September 22, 2015 to January 20, 2016. The deployment site was located at 21.43° N x 157.79° W, on the fringing reef flat surrounding Coconut Island (HIMB) in a depth of 3 meters (Photo 5). Kaneohe Bay, located on the eastern side of Oahu, Hawaii, is a complex estuarine system with a large barrier coral reef, numerous patch reefs, fringing reefs, and several riverine inputs. Tides in Kaneohe Bay are semi-diurnal with mean tidal amplitude of approximately 68 cm day.



Photo 5. Aerial view of HIMB deployment site (left) and instrument rack in-situ (right).

Time series results of ambient conditions for tidal height, temperature, and salinity are given in figure 7. Temperature at the sensor level ranged from 23.1 to 29.1°C and salinity from 27.3 to 34.7 over the duration of the field test. The bottom panel displays the maximum difference recorded between all reference thermistors mounted at the same depth but located across the mooring rack. The average temperature difference observed across the space of the mooring rack was $0.15 \pm 0.17^\circ\text{C}$, with a maximum of 1.23°C . Differences between instrument and reference readings resulting from this variability should be minimized as the sampling bottle integrates across the mooring space.

The HOBO U26 reported data throughout the entire deployment and generated 11296 observations based on its 15 minute sampling interval over the 17 week deployment. However, only 11179 of the measurements were considered acceptable based on excluded values that were more than 2 mg/L from reference samples over a similar timeframe. The accepted data resulted in a data completion rate for this deployment of 99%. Time series results of the HOBO and corresponding reference DO results are given in figure 8. Ambient DO measured by the HOBO ranged from 2.07 to 15.92 mg/L while the range captured by reference samples was 3.630 to 9.851 mg/L. The average and standard deviation of the differences between instrument and reference readings (limited to ± 2.0 mg/L DO; n=107 of 129 potential observations) were $0.090 \pm .576$ mg/L, with a total range in the differences of -1.195 to 1.808 mg/L. There was a small, but statistically significant, drift in instrument offset (slope = 0.009 mg/L/d; $r^2 = 0.18$; p=0.009) throughout the

deployment period but the scatter resulted in a very low goodness of fit in the regression. This rate would include any biofouling effects as well as any electronic or calibration drift.

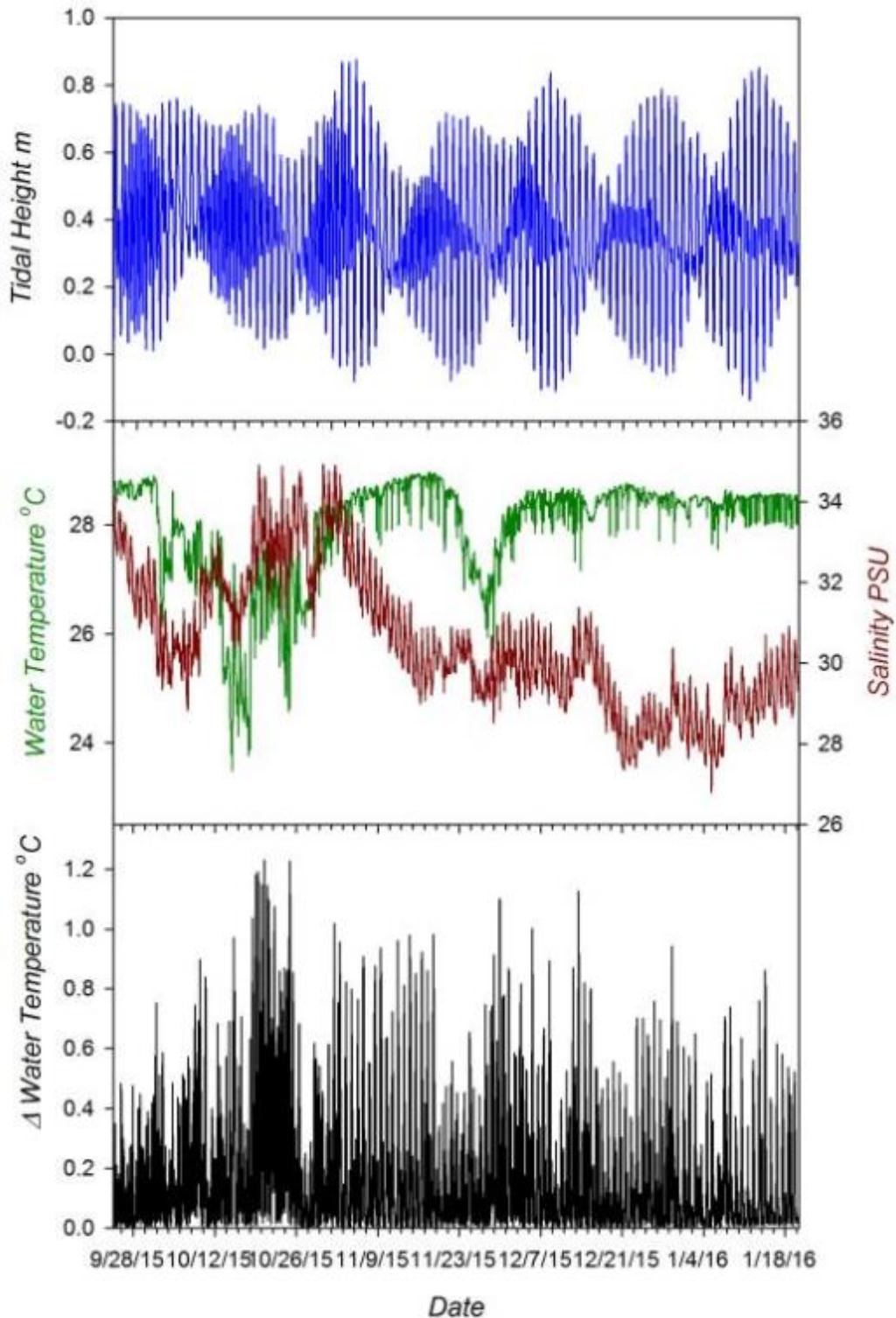


Figure 7. Environmental conditions encountered during the 4 month HIMB deployment on the fringing reef flat off Coconut Island Test sensor array deployed at 1 m fixed depth, variation in local tidal heights indicate active water flow around instrument (*Top Panel*). Variation in salinity (red) and temperature (green) at depth of instrument sensor detected by an SBE 26 and two RBR Solo thermistors (*Middle Panel*). Temperature range determined from max-min temperatures detected by RBR thermistors spanning instrument sensor array (*Bottom Panel*).

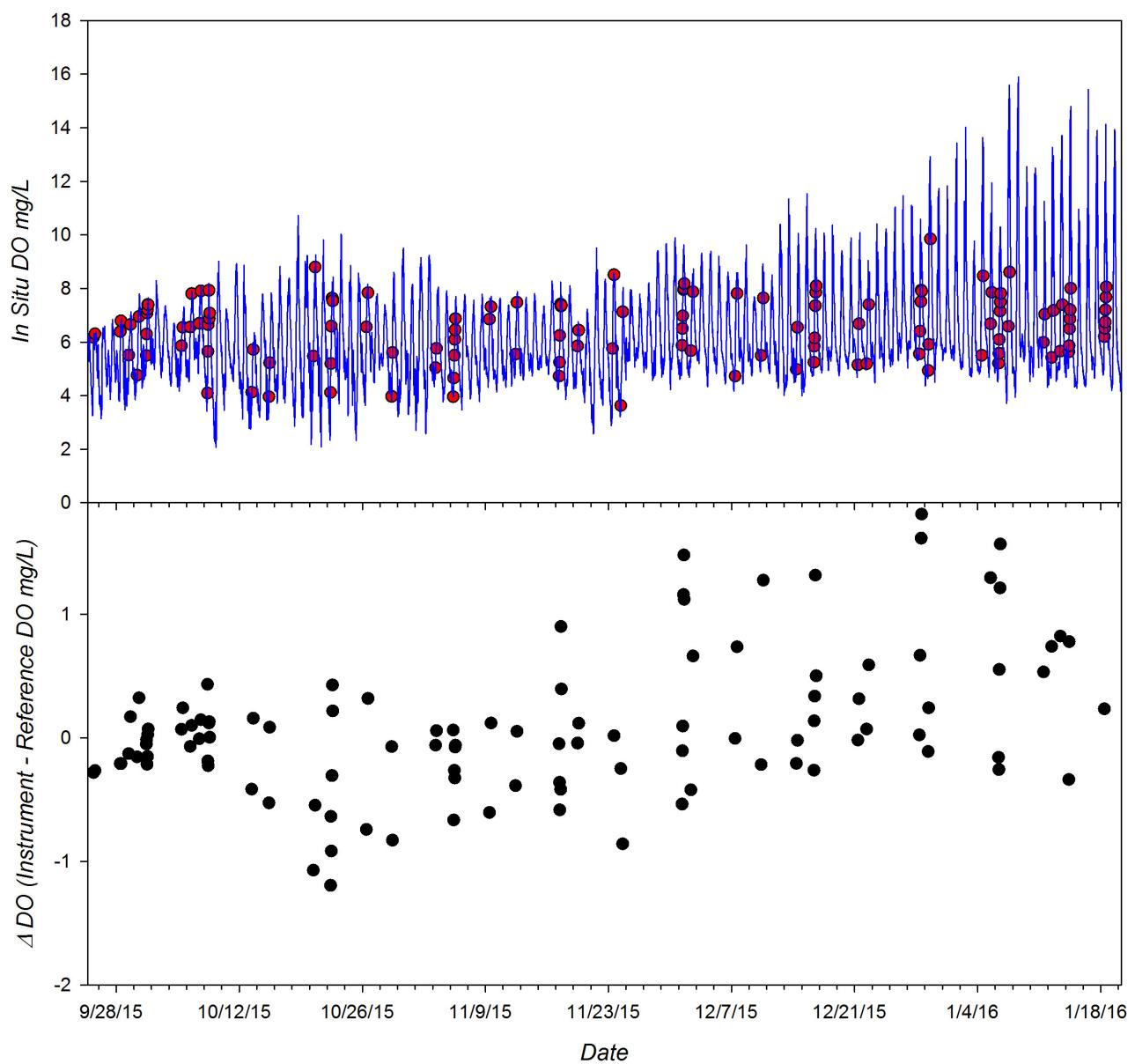


Figure 8. *Top panel:* Time series of DO measured by HOBO U26 deployed during the 18 week HIMB field trial. Continuous DO recordings from instrument (blue line) and DO of adjacent grab samples determined by Winkler Titrations (red circles.) *Bottom Panel:* Time series of the difference between the HOBO and reference measurements for each matched pair in the range of $\pm 2\text{mg/L}$ DO ($n=108$ of a total possible 129 observations).

A cross-plot of the matched observations for the first month of deployment is given in figure 9. Comparisons were limited to one month as a representation of a typical field deployment and to focus on initial instrument offset versus impacts of biofouling during an extended deployment. A linear regression of the instrument versus reference measurements ($r^2 = 0.550$; $p < 0.0001$) had a slope of 1.595 and intercept of -3.102.

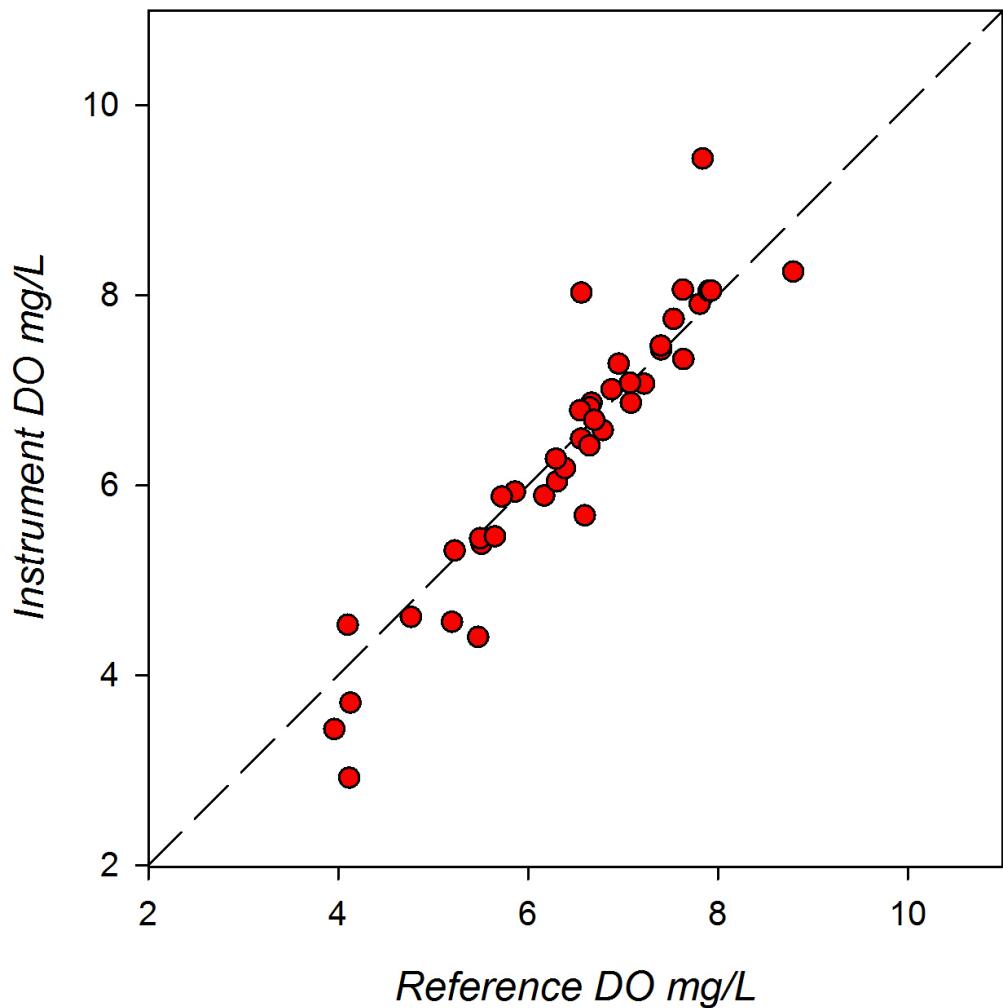


Figure 9. HIMB field response plot of HOBO compared to reference DO samples determined by Winkler Titration. The plotted line represents a 1:1 correspondence.

Photos of and example of the test instrument prior to deployment and the test instrument after the HIMB field deployment to indicate potential impact of biofouling (Photo 6).



Photo 6. The HOBO U26 prior to and following the 18 week HIMB field trial.

A global summary of instrument versus reference readings for all three field deployment sites are plotted in figure 10. Although there was significant scatter at CBL (likely due to high biofouling), the HOBO U26 response was fairly linear over all three salinity ranges including freshwater, brackish water, and oceanic water. The response curves were generally consistent across the concentration range within a given test site as well as over the wide range of DO conditions (4 - 14 mg/L) across sites regardless of temperature or salinity. A linear regression of the subset data ($r^2 = 0.94$; $p < 0.0001$) had a slope of 1.106 and intercept of -0.895.

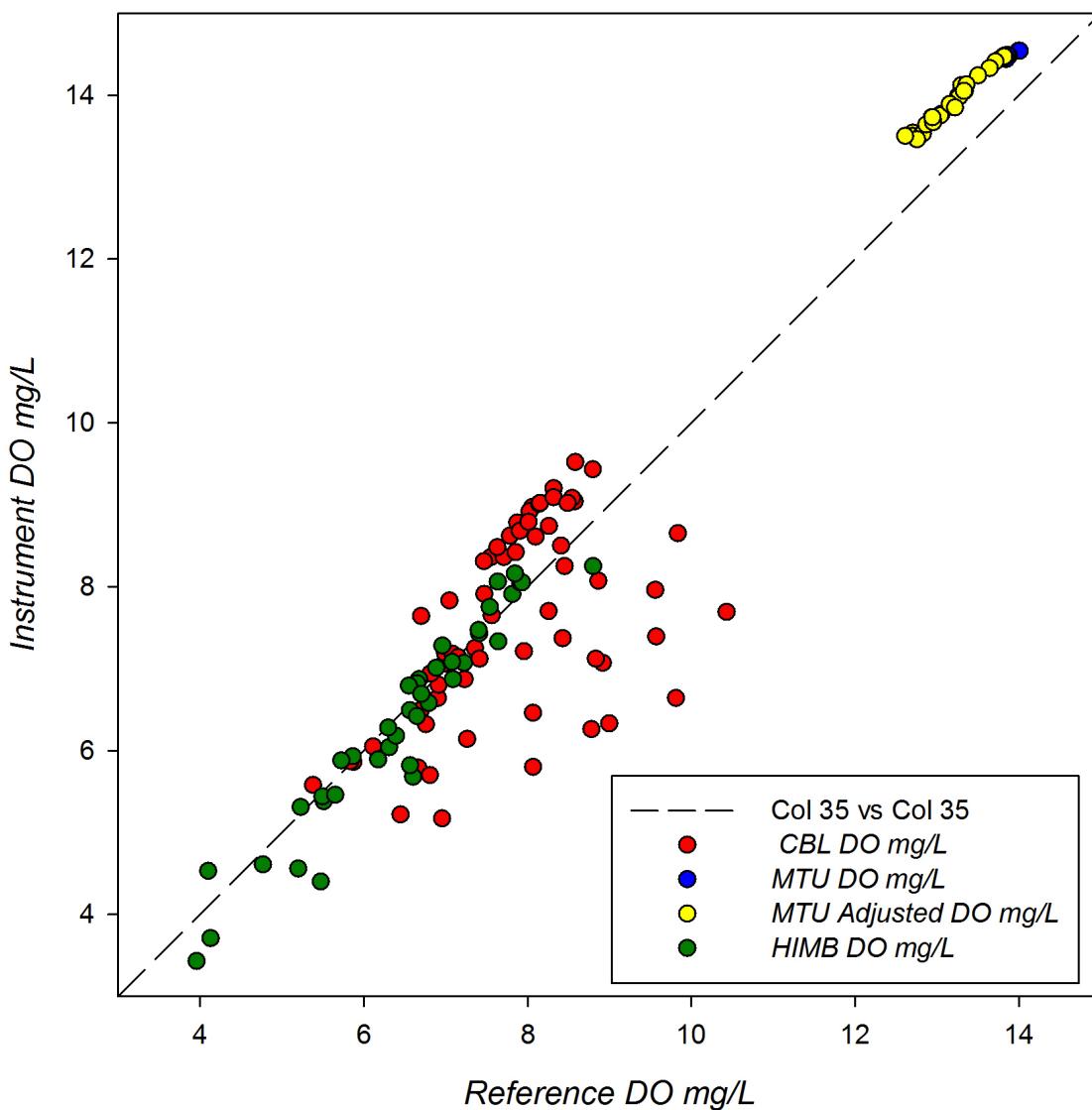


Figure 10. Global response plot for the HOBO U26 instruments observed during the three ACT field trials. Black dotted line represents a 1:1 correspondence.

Great Lakes Profiling

Great Lakes profiling tests were performed aboard the R/V Laurentian at two separate locations in order to experience both normoxic and hypoxic hypolimnion (Photo 7). The normoxic site was located in Lake Michigan (43.184°N , 86.456°W) within a 64m deep water column, while the hypoxic site was in Muskegon Lake at 43.22°N , 86.30°W with a 24m deep water column.

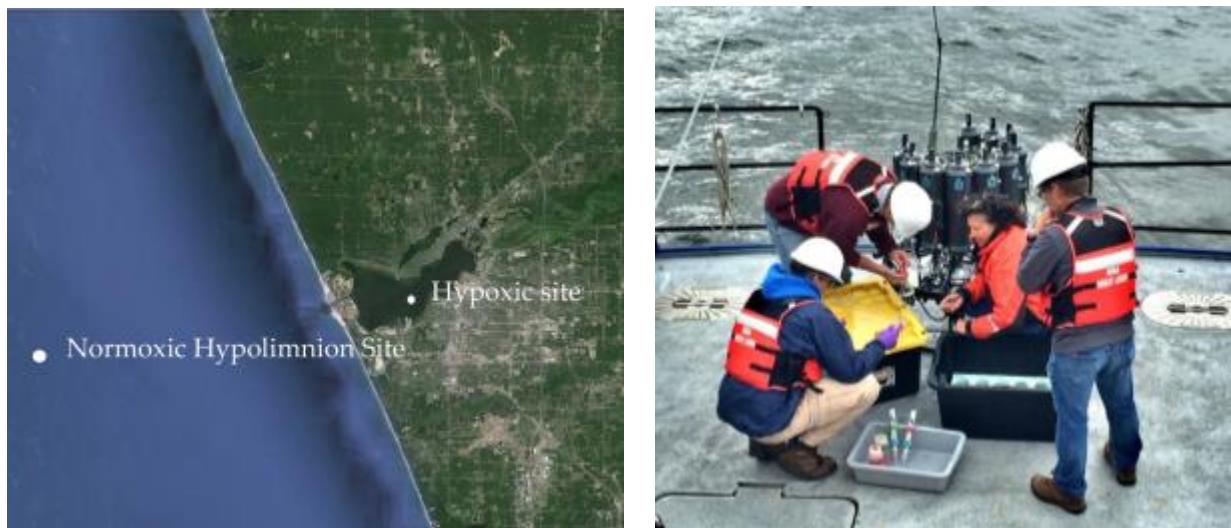


Photo 7. Aerial view of Lake Michigan and Muskegon Lake (left) and profile rig used for GL profiling

Two profiling trials were conducted at each location. The first trial involved equilibrating test instruments at the surface (3m) for ten minutes and then collecting three Niskin bottle samples at one minute intervals. Following the third sample, the rosette was quickly profiled into the hypolimnion where samples were collected immediately upon arrival and then each minute for the next 6 minutes (figure 11 and 13). The second trial was performed in the reverse direction where instruments were equilibrated at depth, three samples collected, and then profiled into the surface and sampled over the next 7 minutes. Note for Muskegon Lake cast 1 was discarded due to bottle misfires, so a third cast was performed to repeat the original trial 1 pattern (Fig. 11). It should be emphasized these tests were not intended to demonstrate profiling capability but rather ability and time response to equilibrate to large changes in temperature and DO over minute time scales.

Temperature and DO concentration profiles for Muskegon Lake and Lake Michigan are given in figures 12 and 14, respectively. In Muskegon Lake, the temperature ranged from 21.0°C at the surface to 13.5°C in the hypolimnion, with corresponding DO concentrations of 7.8 and 2.8 mg/L, respectively. In Lake Michigan, the temperature ranged from 21.0°C at the surface to 4.1°C in the hypolimnion, with corresponding DO concentrations of 8.6 and 12.6 mg/L, respectively.

Results for the HOBO U26 for the two Muskegon Lake trials are shown in figure 15. For cast 2, the HOBO was well matched during equilibration in the hypolimnion but then showed a strong positive bias when rapidly transitioned to the warmer, normoxic surface. The sensor did not fully equilibrate after 7 minutes and remained around 0.7 mg/L higher than reference values. For cast 3, the HOBO exhibited an initial 0.5 mg/L offset during equilibration at the surface and then showed an initial negative offset of -0.9 mg/L when rapidly transitioned to the colder, low DO

hypolimnion. The HOBO appeared to reach equilibration during the 7 minutes and ended with a final offset of around -0.2 mg/L. The range in measurement differences between instrument and reference was -0.15 to 1.78 mg/L for cast 2 and -0.92 to -0.17 mg/L for cast 3.

Results for the HOBO U26 for the two Lake Michigan trials are shown in figure 16. The HOBO had a slight positive offset of 0.3 mg/L during surface equilibration and then exhibited a strong negative bias (-3.0 mg/L) when rapidly transitioned to the cold high DO hypolimnion. The sensor did not fully equilibrate after 7 minutes and ended at -1 mg/L against the reference after seven minutes. For cast 2, there was an initial negative offset of -0.5 mg/L when equilibrated in the hypolimnion and a positive bias (3.1 mg/L) when rapidly transitioned into the warm, normoxic surface water. The sensor did not fully equilibrate after 7 minutes and ended at -1.1 mg/L against the reference after seven minutes. The range in measurement differences between instrument and reference was -3.03 to 0.40 mg/L for cast 1 and -0.81 to 3.05 mg/L for cast 2.

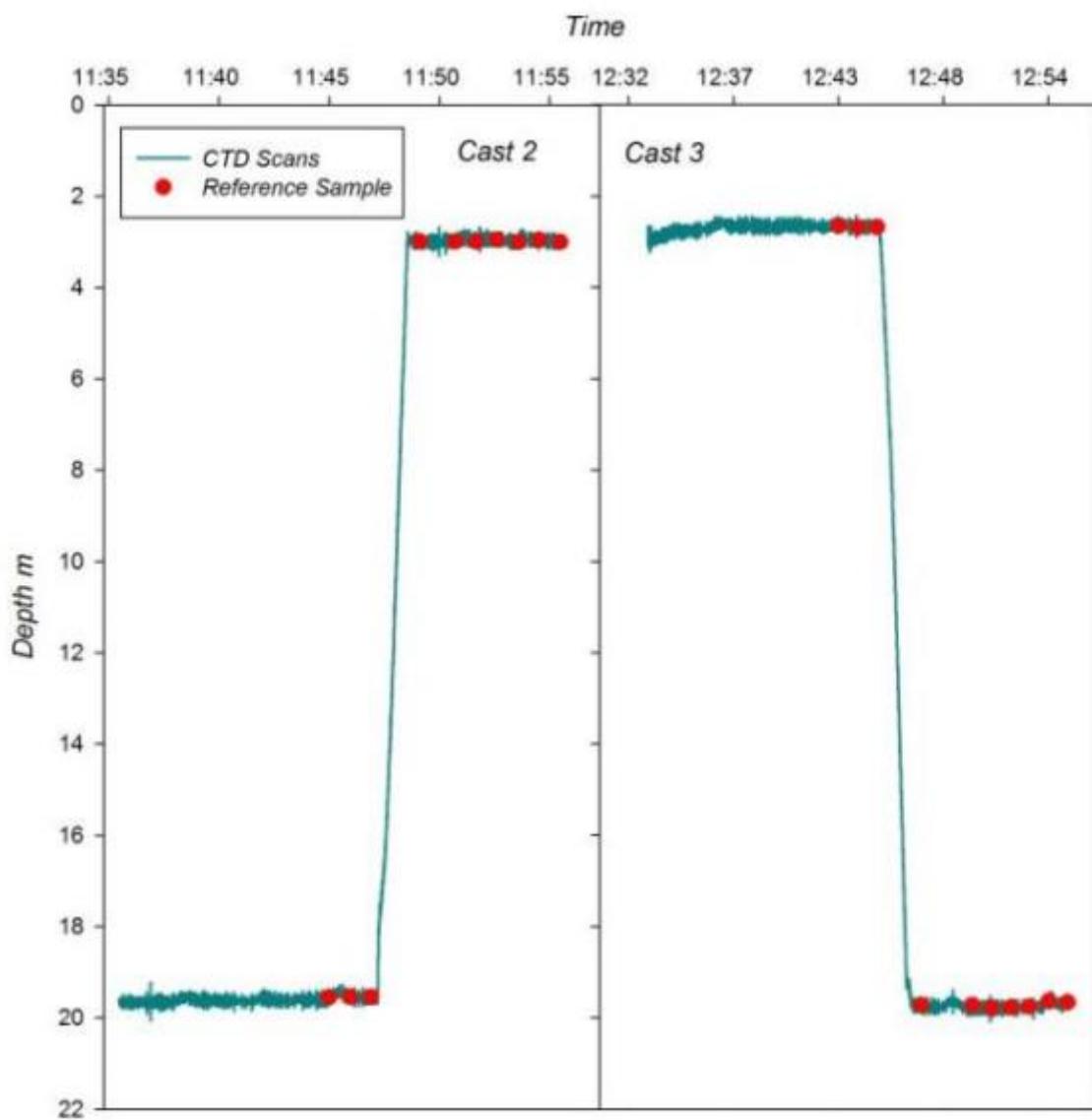


Figure 11. Time series of the rosette profiling and Niskin bottle sampling for the two profiling trials in Muskegon Lake.

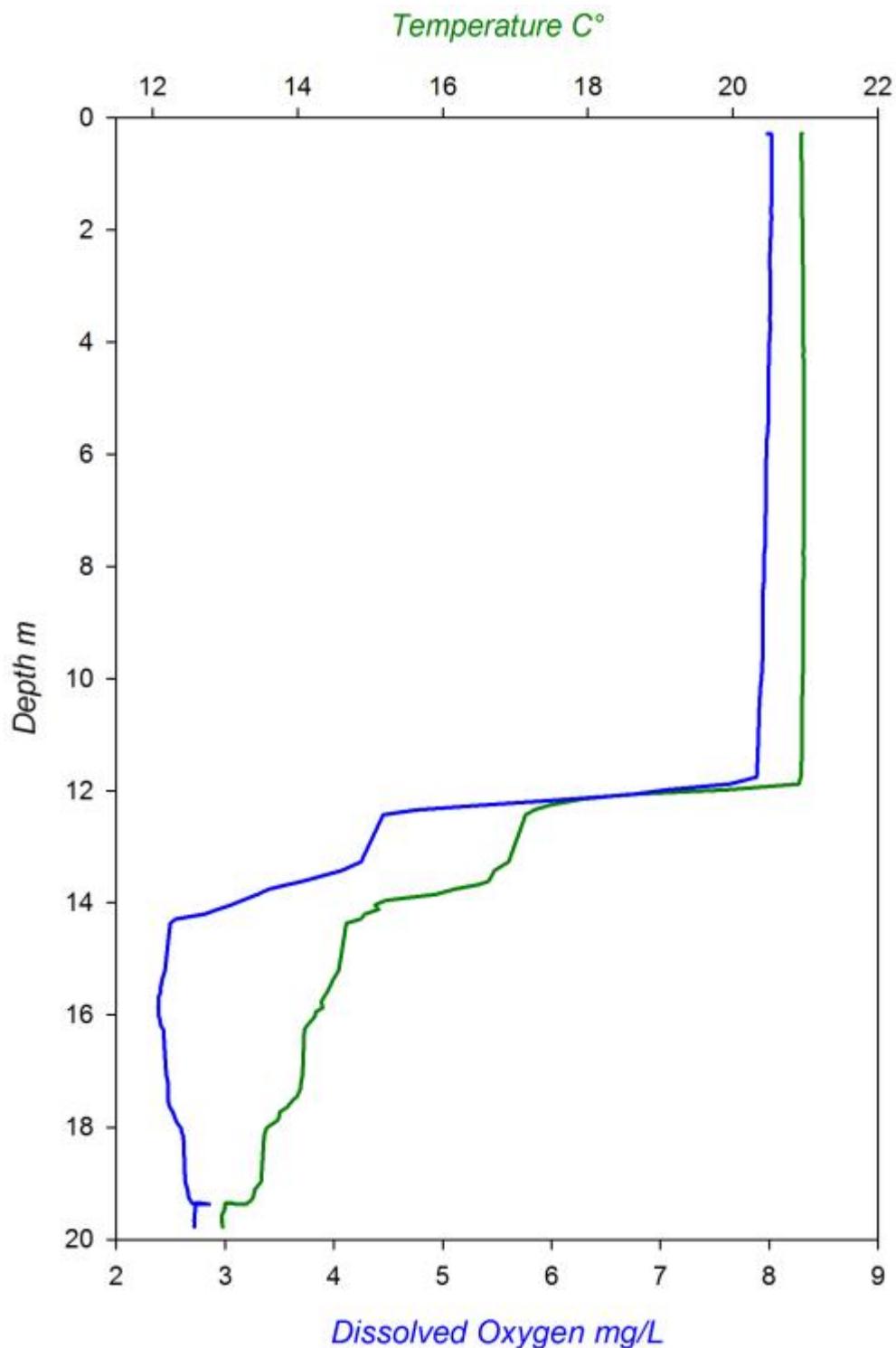


Figure 12. Temperature and DO concentration profiles for Muskegon Lake. Values were obtained from the average of multiple instruments sampling at 1s frequency. The hypolimnion was approximately 8m thick and contained depressed DO levels of less than 3 mg/L.

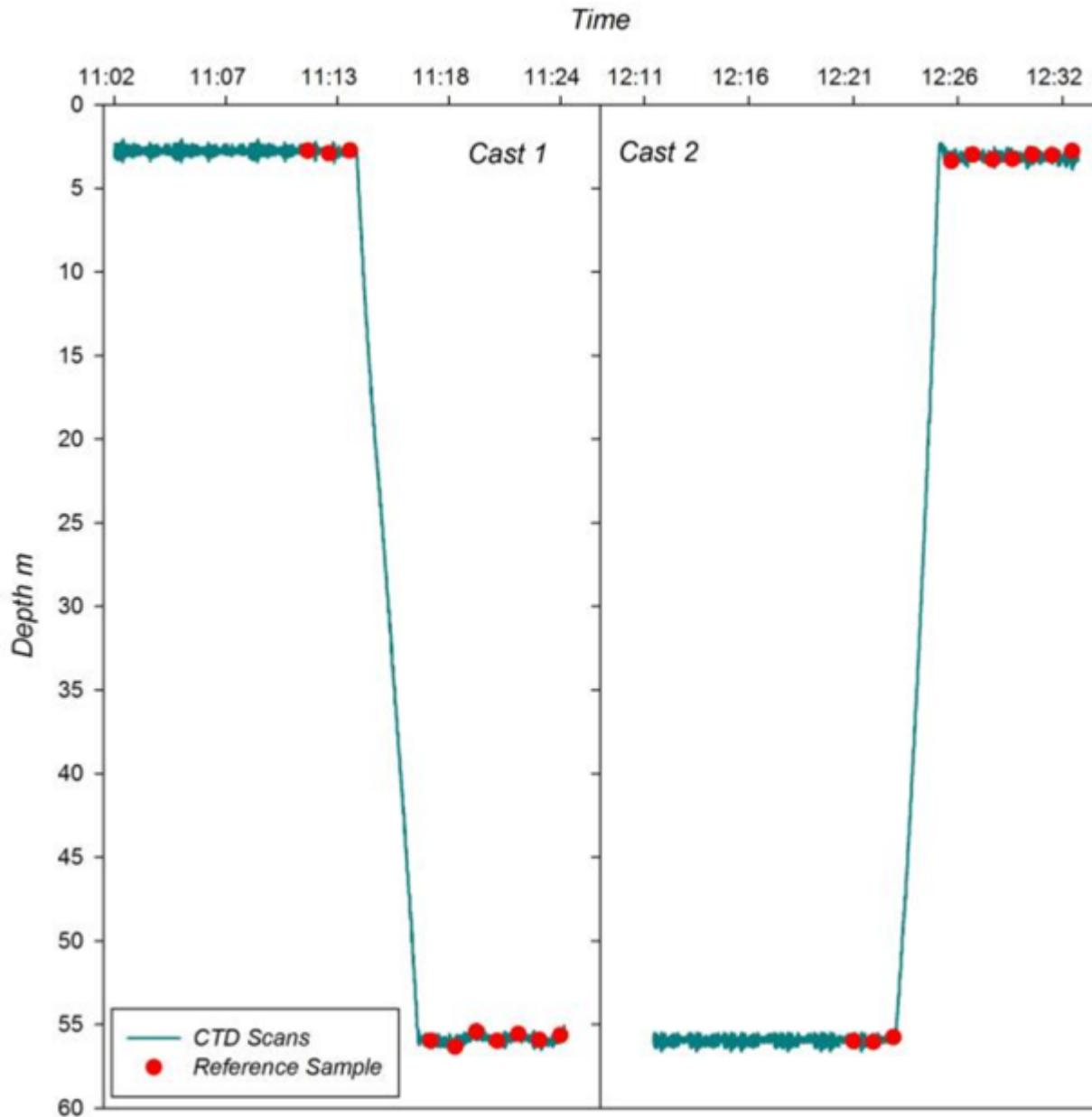


Figure 13. Time series of the rosette profiling and Niskin bottle sampling for the two profiling trials in Lake Michigan.

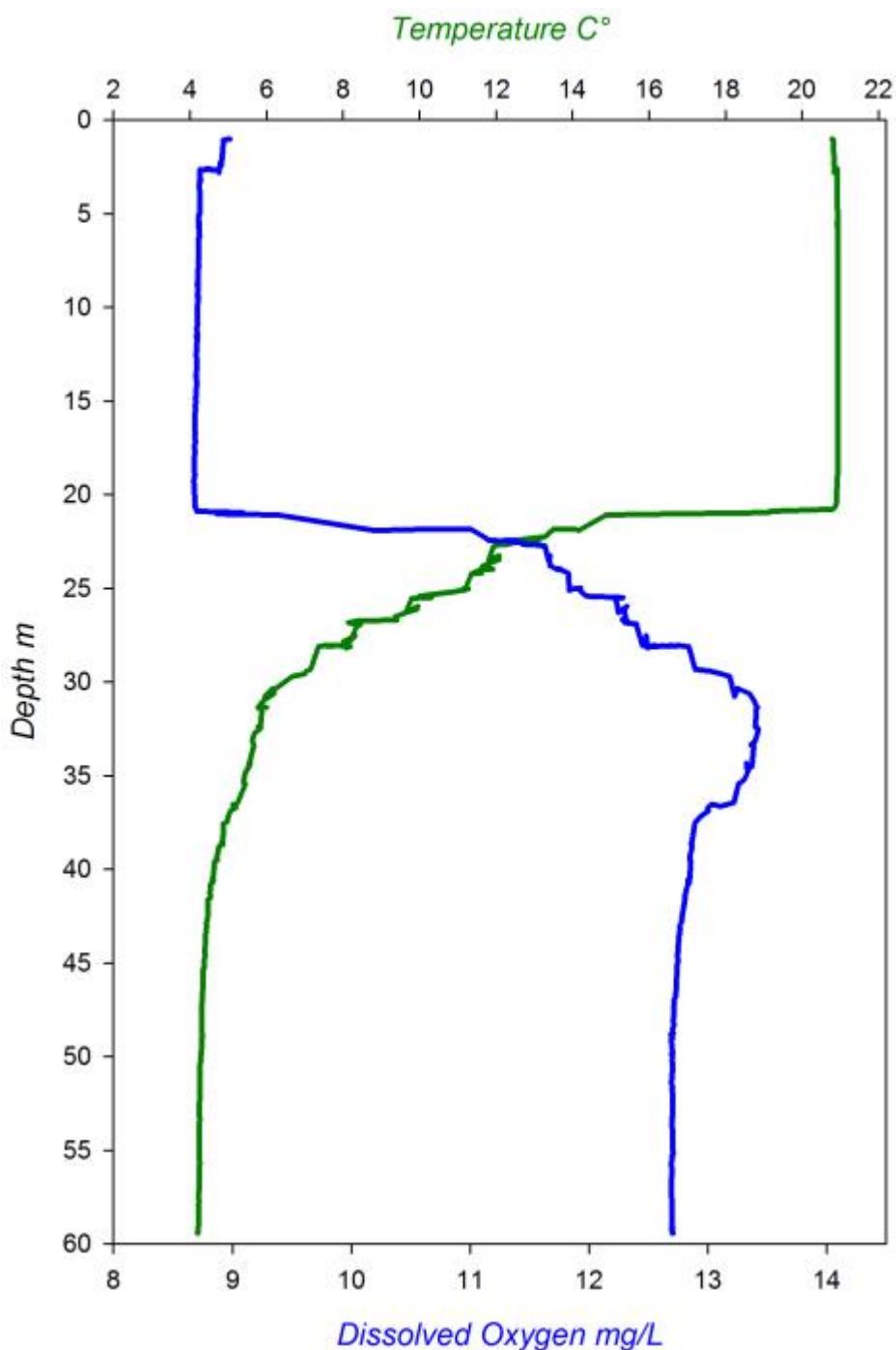


Figure 14. Temperature and DO concentration profiles for Lake Michigan. Values were obtained from the average of multiple instruments sampling at 1s frequency. The hypolimnion was approximately 40m thick (the rosette was stopped at 60m about 30m below the thermocline) and contained elevated DO levels of more than 12.6 mg/L.

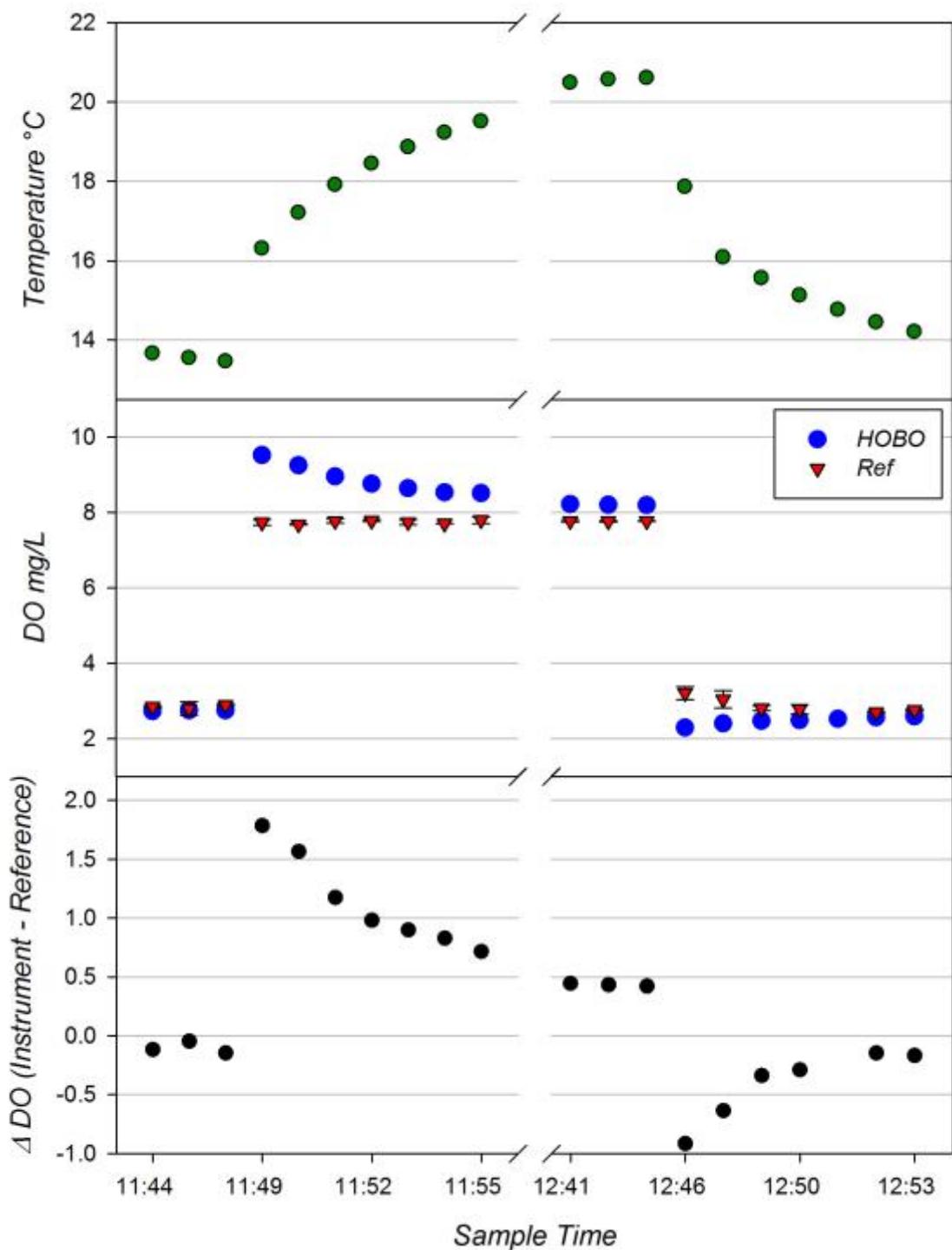


Figure 15. Results of the HOBO U26 for the Muskegon Lake trials. *Top panel:* HOBO temperature data at sample snap. *Middle panel:* DO recordings from instrument (blue dot) and DO of corresponding reference samples determined by Winkler Titrations (red triangles.) *Bottom panel:* Time series of the difference between the HOBO and reference measurements for each matched pair. Cast 2 and 3 are plotted on the same graph and separated by the axis break. Bottle 8 on cast 3 did not close fully and was discarded.

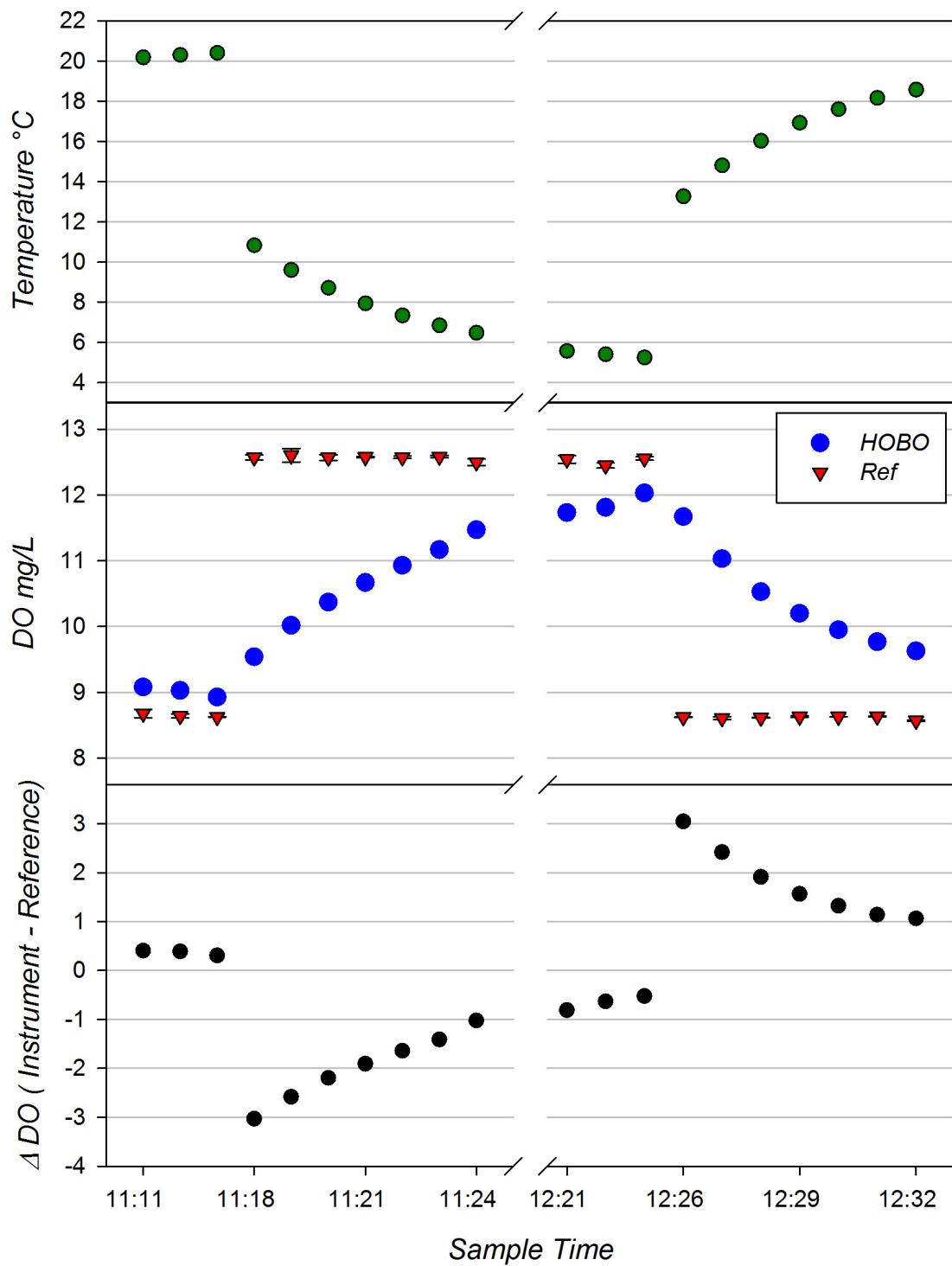


Figure 16. Results of the HOBO U26 for the Lake Michigan trials. *Top panel:* HOBO temperature data at sample snap. *Middle panel:* DO recordings from instrument (blue dot) and DO of adjacent sample snaps determined by Winkler Titrations (red triangles.) *Bottom panel:* Time series of the difference between the HOBO and reference measurements for each matched pair. Cast 1 and 2 are plotted on the same graph and separated by the axis break.

QUALITY ASSURANCE AND QUALITY CONTROL

All technical activities conducted by ACT comply with ACT's Quality Management System (QMS), which includes the policies, objectives, procedures, authority, and accountability needed to ensure quality in ACT's work processes, products, and services. The QMS provides the framework for quality assurance (QA) functions, which cover planning, implementation, and review of data collection activities and the use of data in decision making, and quality control. The QMS also ensures that all ACT data collection and processing activities are carried out in a consistent manner, to produce data of known and documented quality that can be used with a high degree of certainty by the intended user to support specific decisions or actions regarding technology performance. ACT's QMS meets the requirements of ISO/IEC 17025:2005(E), *General requirements for the competence of testing and calibration laboratories*; the American National Standards Institute (ANSI)/American Society for Quality (ASQ) E4-2004 *Quality Systems for Environmental Data and Technology Programs*; and U.S. Environmental Protection Agency, quality standards for environmental data collection, production, and use. An effective assessment program is an integral part of ACT's quality system. The ACT Quality Assurance (QA) Manager independently conducted two Technical Systems Audits (TSA) and data quality assessments of the reference sample data for the DO verification.

Quality Control Samples

Each site conducted weekly field duplicates which are presented below in Tables 6 – 8. The global average of the standard deviation among field duplicates for all field test sites was 0.03 ± 0.07 ($n=27$), with 11 values exceeding our expected quality threshold of better than 0.013 mg/L DO. The average of the standard deviation among MTU field duplicates was $.011 \pm .014$ ($n=12$), the average for CBL was $.074 \pm .006$ ($n=9$) and the average for HIMB was $.011 \pm .012$ ($n=6$). The higher variability at CBL likely reflected fine-scale heterogeneity in the water mass as was also noted by the greater variation in temperature across the mooring rack. In general, results attest to the representativeness of our sampling to water mass being analyzed by the test instruments and to consistent sample handling.

Table 6. Results of Field Duplicates (FD) for the Keweenaw Waterway, MI mooring test.

Date/Time	Rep	Temp	Spec Cond	DO	Std Dev	Mean	Std Dev	ABS Diff
1-14-15 10:30	FD1	.06	94	13.819	.005	13.819	0.00	0.000
	FD2			13.819	.002			
1-22-15 12:30	FD1	.31	99	12.981	.013	12.986	.007	.010
	FD2			12.991	.005			
1-29-15 16:00	FD1	.24	103	12.958	.041	12.947	.015	.021
	FD2			12.937	.013			
2-5-15 15:30	FD1	.21	106	12.671	.004	12.667	.006	.009
	FD2			12.662	.007			

2-19-15 15:30	FD1	.35	108	11.973	.008	11.974	0.000	.001
	FD2			11.974	.011			
2-26-15 15:30	FD1	.26	112	11.721	.011	11.755	.049	.069
	FD2			11.790	.076			
3-5-15 15:000	FD1	.34	112	11.615	.013	11.613	.002	.002
	FD2			11.612	.012			
3-12-15 10:15	FD1	.27	123	11.491	.028	11.477	.021	.029
	FD2			11.462	.007			
3-25-15 15:15	FD1	.72	118	11.474	.051	11.464	.015	.021
	FD2			11.453	.012			
4-3-15 10:00	FD1	.59	137	11.199	.012	11.201	.003	.004
	FD2			11.203	.001			
4-9-15 10:00	FD1	1.26	106	11.435	.008	11.430	.007	.010
	FD2			11.425	.009			
4-16-15 9:30	FD1	2.98	97	11.040	.005	11.042	.003	.004
	FD2			11.044	.006			

Table 7. Results of Field Duplicates (FD) for the Chesapeake Bay, MD mooring test.

Date/Time	Rep	Temp	Salinity	DO	Std Dev	Mean	Std Dev	ABS Diff
5-26-15 16:00	FD1	21.6	12.8	8.314	.011	8.269	.064	.090
	FD2			8.224	.004			
6-3-15 11:30	FD1	21.6	13.1	5.378	.003	5.297	.115	.163
	FD2			5.215	.015			
6-9-15 13:30	FD1	22.4	12.8	6.663	.0165	6.404	.366	.518
	FD2			6.145	.008			
6-17-15 9:30	FD1	26.6	12.1	8.827	.004	8.831	.006	.008
	FD2			8.835	.002			
6-24-15 11:30	FD1	27.5	11.0	7.051	.002	7.053	.003	.004
	FD2			7.055	.005			
7-7-15 14:00	FD1	26.9	9.9	6.157	.003	6.141	.023	.032
	FD2			6.125	.003			

7-15-15 9:30	FD1	27.3	8.7	5.781	.003	5.784	.005	.007
	FD2			5.788	.005			
7-22-15 9:30	FD1	28.0	9.2	7.151	.009	7.200	.069	.098
	FD2			7.25	.002			
7-28-15 13:30	FD1	29.13	8.3	8.336	.005	8.349	.019	.026
	FD2			8.362	.003			

Table 8. Results of Field Duplicates (FD) for the Kaneohe Bay, HI mooring test

Date/Time	Rep	Temp	Salinity	DO	Std Dev	Mean	Std Dev	ABS Diff
9-29-15 10:00	FD1	26.63	34.3	5.493	.006	5.501	.000	.016
	FD2			5.510	.007			
10-6-15 14:00	FD1	26.10	33.3	7.949	.007	7.880	.025	.139
	FD2			7.811	.014			
10-13-15 10:00	FD1	26.85	29.8	4.100	.016	4.114	.007	.027
	FD2			4.127	.006			
11-12-15 14:00	FD1	26.46	34.5	7.464	.029	7.472	.008	.016
	FD2			7.480	.017			
12-7-15 10:00	FD1	25.09	33.7	4.773	.011	4.750	.001	.046
	FD2			4.727	.012			
12-22-15 15:00	FD1	24.22	34.4	7.397	.025	7.399	.005	.005
	FD2			7.401	.032			

Technical System Audits

A Technical Systems Audit (TSA) is a thorough, systematic, on-site qualitative audit of ACT's sampling and measurement processes and procedures associated with a specific technology verification. The objective of a TSA is to assess and document the conformance of on-site testing procedures with the requirements of the Test Protocols (*DO Sensor Verification Protocols*, PV14-01 21 October, 2014) and associated Standard Operating Procedures (SOPs). The ACT QA Manager conducted two TSAs over the course of the verification:

- The field tests at the CBL during July 29 – 30, 2015;

- The field tests at HIMB during September 28, 2015 – October 1, 2015.

The audits were conducted in accordance with the procedures described in EPA's *Guidance on Technical Audits and Related Assessments for Environmental Data Operations (EPA QA/G-7)*. The audits included a review of staff, test procedures (sample collection, sample analysis, data processing, etc.), facilities, and documentation.

The TSA's' findings for the two field tests were positive and indicated that these components of the DO sensor verification were being implemented in a manner consistent with the Test Protocols and SOPs. Minor deviations, such as schedule changes, were documented in laboratory records. None of the deviations in the Test Protocols had any effect on data quality for the verification and no corrective action was required. All phases of the implementation of the test reviewed during the TSA were acceptable and performed in a manner consistent with ACT data quality goals. The overall quality assurance objectives of the test were met.

ACT personnel are well-qualified to implement the verification, and demonstrated expertise in pertinent procedures. Communication and coordination among all personnel was frequent and effective. ACT's internal record keeping and document control is well organized. The ACT staff understands the need for QC, as shown in the conscientious development and implementation of a variety of QC procedures.

All samples and instrument measurements were collected, analyzed and cataloged as described in the Test Protocols and SOPs. Examination of maintenance and calibration logs provided evidence of recent and suitable calibration of sampling and analytical equipment.

Data Assessments

Data review is conducted to ensure that only sound data that are of known and documented quality and meet ACT technology verification quality objectives are used in making decisions about technology performance. ACT's data review processes are based in part on two EPA guidance documents: *Guidance on Environmental Data Verification and Data Validation (QA/G-8)* [EPA, 2002] and *Guidance on Technical Audits and Related Assessments for Environmental Data Operations (QA/G-7)* [EPA, 2000].

At the outset of the assessment phase, the data were verified and validated to evaluate whether the data have been generated according to the Test Protocols, satisfy acceptance criteria, and are appropriate and consistent with their intended use of evaluating the performance of the test sensors. Data verification evaluates the completeness, correctness, and consistency of the data sets against the requirements specified in the Test Protocols, measurement quality objectives (MQOs) in the ACT Quality Assurance Project Plan (QAPP), and any other analytical process requirements contained in SOPs. The ACT QA Manager reviewed the reference sample data sets from all field sites and verified that the sampling and analysis protocols specified in the Test Protocols were followed, and that the ACT measurement and analytical systems performed in accordance with approved methods, based on:

- The raw data records were complete, understandable, well-labeled, and traceable;
- All data identified in the Test Protocols were collected;
- QC criteria were achieved; and
- Data calculations were accurate.

Data validation uses the outputs from data verification and included inspection of the verified field and laboratory data to determine the analytical quality of the data set. A representative set of approximately 10% of the data on core parameters was traced in detail from raw data from field and laboratory logs and instrument readouts through data transcription or transference through data manipulation through data reduction to summary data, data calculations, and final reported data. Data validation established:

- Required sampling methods were used;
- Sampling procedures and field measurements met performance criteria;
- Required analytical methods were used;

Data validation confirmed that ACT's sample measurement system performed in accordance with the performance goals specified in the ACT QAPP and the DO Test Protocols and that the data were accumulated, transferred, reduced, calculated, summarized, and reported correctly. There is sufficient documentation of all procedures used in the data collection and analysis to validate that the data were collected in accordance with the verification's quality objectives.

A Data Quality Assessment (DQA), the third and final process of the overall data assessment, is a scientific and statistical evaluation of validated data to determine if the data are of the right type, quality, and quantity to support conclusions on the performance of the DO sensors. The DQA determined that ACT's data quality objectives, described in Section 3.4 of the ACT QAPP, were achieved. This evidence supports conclusions that:

- The sampling design performed very well and was very robust with respect to changing conditions.
- Sufficient samples were taken to enable the reviewer to see an effect if it were present.
- Data on the performance of the DO sensors are unambiguous, and the vendors and buyers can make informed choices about the performance of a sensor with a high level of certainty.

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September 15, 2016

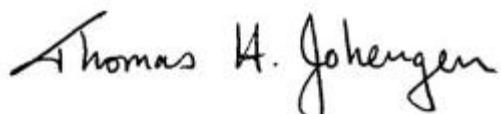
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Approved By: Dr. Mario Tamburri
ACT Executive Director

September 15, 2016

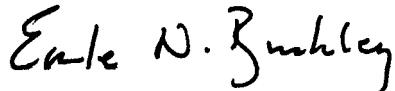
Date



Approved By: Dr. Tom Johengen
ACT Chief Scientist

September 15, 2016

Date



Approved By: Dr. Earle Buckley
Quality Assurance Supervisor

ONSET

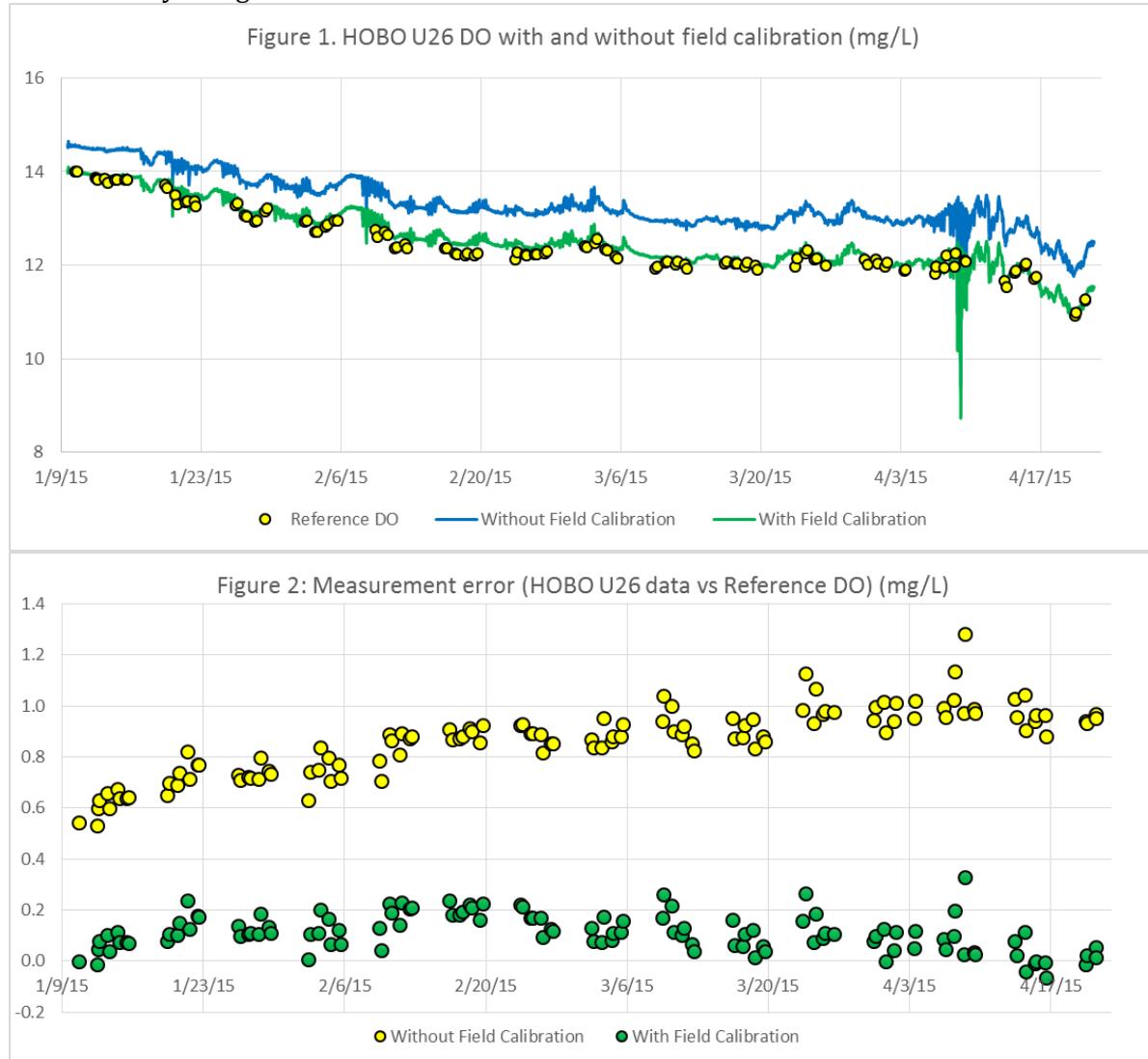
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Our thanks to all ACT personnel for all their excellent work testing, analyzing and sharing this valuable information with users. We are very pleased to see that the HOBO U26 Dissolved Oxygen Logger performed accurately and reliably in the lab tests. It is also good to see the results and challenges in field deployments, which helps to show the importance of following good field protocols to obtain the best possible results.

To our current and future users, we would like to share the following suggestions on ways you can improve your results when you deploy HOBO U26 Dissolved Oxygen Loggers.

Field calibration can be used to improve accuracy

In the MTU deployment there was a slight calibration error that resulted in an offset in the recorded data. This offset can be mostly eliminated by using the field calibration feature of Onset's HOBOware software along with precision measurements taken at the beginning and end of the deployment. If we use the first and last Winkler Titration reference values for the MTU deployment with HOBOware's field calibration, you can see how this improves the accuracy in Figures 1 and 2 below.



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Using 100% oxygen-saturated water can result in a better calibration in some cases

The HOBO loggers used in these tests were calibrated prior to deployment using 100% saturated air in the calibration boot provided with HOBO loggers. In some cases, such as the MTU deployment, the site water can cause the sensor to respond differently and require a different calibration gain. This can be especially apparent at high DO levels where a small gain error can get multiplied to a significant offset. To obtain the best possible calibration for these sites, you can do a lab calibration using water from the site and a [bubbler](#) to create 100% Oxygen saturated water. This can be used with HOBOware's Lab Calibration utility, the same as if the logger was in the calibration boot. Note that using a bubbler to create 100% saturated water only works for freshwater. If the logger will be deployed in brackish or salt water, it should be calibrated in either the calibration boot or freshwater with a bubbler, and HOBOware should be used to do the salinity adjustment.

Keep ahead of fouling

In the long-term MBL and HIMB tests you can clearly see the measurement degradation over time due to fouling. These tests were run with no sensor cleaning so the effects of fouling could be seen. In some cases the fouling can prevent water from reaching the sensor face which results in lower measurements such as seen in the MBL test (Figure 5 in the report). In other cases, biofouling can create a microclimate around the sensor that results in more extreme variations in the DO around the sensor, as seen in the HIMB test (Figure 8 in the report).

To reduce the rate of fouling, we recommend using the HOBO's optional antifouling cap (which was used in these tests), and cleaning the sensor face and the antifouling cap coils as much as needed to prevent the buildup of fouling. The frequency of cleaning depends on the environment. As seen in these tests, there is a wide variation in the rate of fouling. In some environments you may need to clean the sensor every week, while in other environments you may be able to go several months between cleaning.

Use field calibration readings to adjust for fouling

We recommend using field calibration readings taken at the beginning and end of a deployment to adjust for drift in DO measurements due to fouling. Onset's HOBOware software includes the ability to enter these readings to adjust the data for this measurement drift. The software calculates a linearly-changing correction factor between the starting calibration and the ending calibration points. This works well for cases where the fouling has resulted in moderate changes in the sensor sensitivity, such as the MTU deployment (Figure 2 in the report) or the first two weeks of the MBL deployment (Figure 5 in the report). Field calibration won't help if there is a large drop in the sensitivity such as seen in the second month of the MBL deployment. To determine if field calibration is appropriate, we recommend looking at the raw DO data to see if there were any dramatic changes in the data that could indicate excessive fouling.

The HOBO Logger is intended for fixed locations

The HOBO logger has a fast response to changes in DO as shown in the lab DO response time tests (Figure L in the report). On the other hand, its response time for large temperature changes is not fast, which reduces the accuracy of its temperature compensation of DO measurements when the temperature readings lag behind. This explains the error in the profiling tests (Figures 15 and 16 in the report) when the logger was rapidly transitioned through the boundary layer. This is why we do not recommend the HOBO DO logger for profiling applications where there can be sudden dramatic changes in the water temperature.

For more information on the HOBO U26 DO loggers and deployment advice, please visit the Onset web site at:
www.onsetcomp.com