Version of Record: https://www.sciencedirect.com/science/article/pii/S003991401930219X
Manuscript_32e40746d8826829f5b994d641506890

A Robust and Inexpensive Pump Profiler to Monitor Stratified Water Columns with high Vertical Resolution

Jeffrey M. Hudson[1*], Daniel J. MacDonald[2], Emily R. Estes[2], George W. Luther III[1,2]

[1] Department of Civil & Environmental Engineering, University of Delaware, Newark, Delaware 19716, USA [2]School of Marine Science and Policy, University of Delaware, Lewes, Delaware 19958, USA

*E-mail: hudsonjm@udel.edu

Abstract

A pump profiling system for real time sample collection has been constructed for a cost of <$1000 (USD) and mated with a ship's rosette that has conductivity, temperature, depth (CTD) and other sensors. The system permits the collection of ~ 15L of water in one minute without exposure to O_2 from air for discrete sampling of chemical, microbial and other constituents as well as for real time analyses using sensors. We also coupled a shipboard voltammetry system with solid-state microelectrodes to detect dissolved O_2 and H_2S. Electrode O_2 detection limits (DL) are ~ 3 μM and compare well with *in situ* Clark electrode O_2 data (DL ~ 2 μM) from the ship's CTD rosette system. H_2S measurements also were reliable, based on previously compared methods. Best resolution of the profiling system can be as small as its orifice of 2.54 cm (0.0254 m) in a quiet sea state, which is an

improvement over the maximum resolution achievable using 10L Niskin bottles that are 1 meter in length.

Keywords: Pump Profiler, Stratification, Redox, Voltammetry, Chesapeake Bay

Introduction

Stratification and subsequent redox speciation in marine environments can be complex and dynamic. Detection and quantification of redox active species and their gradients are vital for gaining a larger perspective of marine biogeochemical processes.[1] Pump profiling systems aboard research vessels and small boats can be utilized for *in situ* chemical analysis and collection of discrete samples for laboratory based analyses.[2-11] Many pump profiling systems in the literature report extensive and expensive pump and hose networks to transport water samples to the surface, where samples are collected and analyzed via traditional techniques in shipboard or home laboratories.[12-14] Pump profiling systems shorten the time between sampling events and laboratory analysis and provide a means to obtain environmental data rapidly and without contamination.

Voltammetric microelectrodes, in conjunction with a laboratory potentiostat and a pump profiler system, are an ideal analytical method to measure redox active species. This approach minimizes the cost of pressure housings, connectors and cables for *in situ* electronic equipment. Microelectrodes can be deployed as a cheaper, more durable

alternative to other techniques that compliment pumping systems.[2-8,15] Multiple redox species can be detected in a single voltammetric scan including O_2, hydrogen peroxide (H_2O_2), Mn(II), I^-, Fe(II), organically complexed Fe(III), FeS clusters, and reduced sulfur species.[15-20] Microelectrode surfaces are small (100 uM), so permit operations at low currents (nano-amps or nA) and in solutions with lower ionic strengths.[19] Additionally, small electrode surface areas increase sensitivity to redox-active species and allow for fast scans (1-2 V/s) to be made in a matter of seconds because small surface areas decrease the charging current relative to the Faradaic current.[19,21] Microelectrodes are easily transportable[19] and can reduce analytical errors from contamination and handling.[19] Finally, because electrodes allow for the rapid analysis of the water column, they can guide scientists in making decisions with respect to the collection of discrete samples for the determination of other chemical and microbial species by other methods.[4-7]

In this article, a pump profiling system for on-board use has been constructed for a cost of under $1000 USD and mated with voltammetric solid-state electrodes to detect dissolved O_2 and H_2S in an estuarine water column. The system was designed to be inexpensive and easy to clean so that it can be applied to many different field scenarios. In a quiet sea state, a vertical resolution of 2.54 cm (0.0254 m) can be obtained. Data collected during our cruises were provided with a resolution of 0.5 ± 0.1 meter (error is based on sea state). The profiling system resolution is an improvement over traditional 10L Niskin bottles, which are commonly found on ships and which have a resolution of 1 meter (the length of the Niskin bottle). The principle redox analytes measured are O_2 and H_2S as they represent oxic and anoxic boundaries in the water column, and can be used to delineate zones where other redox-active trace metals (i.e. Fe(II), Mn(III),Mn (II)) are present.[2,6-8,17,

[20,22] To demonstrate that the pump profiling system can collect samples without contamination due to O_2 in air, microelectrode O_2 data were compared to data from an *in situ* Clark oxygen sensor that was mounted on a ship's conductivity-temperature-depth (CTD) rosette system. H_2S concentration data are also plotted to obtain full concentration versus depth profiles. O_2 and H_2S profiles were measured from two Chesapeake Bay research cruises (2017, 2018) that demonstrate the system's robustness and enabled us to observe dynamic spatial and temporal changes in the bay. Finally, our pump system allows for collection of large water volumes to determine many other environmentally relevant chemical constituents including nutrients (PO_4^{3-}, NO_3^-, NO_2^-, and NH_4^+), trace metals, organic carbon for DOC analysis, proteins for analysis, and microbes for microbial work.[2,4,5] Nitrite data from bottled samples in 2017 are plotted with O_2 and H_2S data to show the versatility of our pump profiler.[6]

Methods

Electrode Fabrication

Au/Hg amalgam working electrodes were fabricated according to previous methods.[15,16] BNC cable (Newark Electronics) was stripped down to the copper conductor wire, which was then fixed and soldered to 100 μM gold wire (Alfa). The soldered wire was fixed within 0.125" PEEK™ tubing. With the wire fixed inside the PEEK™ tubing, 105 epoxy (West System) and 206 hardener (West System) were mixed at a 1 part to 4 parts ratio and injected via syringe into the tubing. After hardening overnight, electrode surfaces were

sanded and polished using sandpapers with increasingly finer grits (100-1000), and subsequently with diamond pastes (15, 6, 1, 0.25 μm, Buehler).

Following polishing, working, Ag/AgCl reference, and Pt counter electrodes were placed in a 0.1 M $Hg(NO_3)_2$ (dissolved in 0.1 M HNO_3 with a pH ~ 1.0) plating solution purged with N_2. Working electrode gold surfaces were plated by reducing Hg(II) for 4 minutes at a fixed potential of -0.1V. It is important to note that the reference electrode used for plating was encased within a glass frit to prevent Ag dissolution due to the low pH (1.0) of the plating solution. Following electrode plating, a polarization procedure was performed, where the working electrode and Pt counter electrode were transferred to a 1M NaOH solution and held at a potential at -9V for 90 seconds. Finally, working electrodes were run in seawater up to 50 times to ensure electrode functionality and to obtain O_2 signal reproducibility. Multiple electrodes were fabricated, tested, and calibrated aboard the ship before use in sampling.

Pump System

A photo collage of the pump system is shown in Figure 1. Water for sampling was pumped shipboard by way of a network of tubes (schedule 40 PVC, 1-inch outer diameter, OD), clear PVC tubing and fittings. Tubes and plumbing connectors reduced in size from 1 3/8'' outer diameter (1" inner diameter, ID) at the sampling end to 1/8'' outer diameter Teflon or polypropylene tubing at the voltammetric sampling cell aboard the ship. The connectors included schedule 40 PVC pieces, bushings (3/4 to ½ "), and NPT fittings, some of which are shown in Figure 1. A bilge pump (12V Model 27DA, West Marine / Rule

Industries) at the bottom of the pump system was fastened to the CTD rosette apparatus with hose clamps and/or zip ties (Figure 1). The pump orifice was within 5-10 cm of the orifices of other sensors on the CTD whereas the bottom of the Niskin bottles was 40 cm above the sensors. The pump was powered by a 12V marine deep cycle battery (West Marine AGM 105 Amp hr, #1231422). Here, a 30.5 m 16-gauge extension cord was mated to the electrical wires of the bilge pump by soldering the wires, covering them with heat shrink tubing then encapsulating them with underwater splicing tape (Scotch 223) to be waterproof and connected to the battery. Large 1" ID (1-3/8" OD) tubing (30.5 m of clear high-pressure PVC tubing with 3/16" wall thickness from McMAster Carr #5238K778) was fastened to the pump and secured to the CTD rosette with zip ties and hose clamps. The tubing could be extended with the CDT-rosette system to a maximum depth of 25 meters over which measurements were made. The dead volume of the tubing is approximately 14.7 L, and the pump is capable of flushing the tubing in one minute. Aboard the ship, the tubing was connected to a 3-way or T tube (3/4" NPT at all 3 ports) that had three ball valves (2;3/4" and 1; ½"), which were fastened with an assortment of fittings to the T tube and that could be reduced on the exit side to other sizes as necessary (Figure 1). A 3/4" black ball valve on the bottom of the T tube could be stepped down in size to ¼ or 3/8 inch (using a Swagelok ¾" NPT to ¼ or 3/8" compression fitting adapter), which allowed for sample flow to be diverted for the collection of discrete samples. The third part of the T tube utilizes a 1/2" ball valve that was attached to the voltammetry cell with a nylon Swagelok fitting adapter (from ½ inch NPT to ¼ compression) to reduce the tubing size. Furthermore, this ¼" tubing was reduced to 1/8" OD tubing above a sink in the wet lab, where flowing water could be diverted to a 50 ml falcon tube (Figure 1) attached to a ring

stand that served as our cell for electrochemical analysis. All tubing reported was either of Teflon or polypropylene grade. The entire pump ensemble cost less than $1000, and most supplies were readily obtainable at local hardware, marine and scientific supply stores. An oxygen sensor (Clark electrode, SBE Inc.) and fluorescence sensor (Eco-FL Fluorometer, WETLabs) were also part of the CTD Rosette to take measurements during sampling.

Experimental Procedure

Real time voltammetry was performed by pumping water from discrete depths to the electrochemical cell aboard ship with the pump system for 30-45 min before and after slack tides when water movement was at a minimum. Electrochemical measurements during CTD rosette casts were made at each sampling depth desired. To reduce costs of the electrochemical cell, the working, reference, and counter electrodes were placed in a 50 ml centrifuge tube attached to a ring stand that acted as an electrochemical cell to measure O_2 and H_2S. Water was pumped into the bottom of the tube so that the tube was filled to overflowing levels at least three times prior to any measurement to ensure that no oxygen from air would contaminate the waters (Figure 1; this approach is similar to the sampling of oxygen for Winkler titrations)[14,19]. Electrodes were attached to a potentiostat (DLK 60, Analytical Instrument Systems, Inc.). Oxygen calibrations were performed concurrently with experiments (see Calibrations). When only O_2 was present, linear sweep voltammetry (LSV) was performed at potentials ranging from -0.1 V to -1.8 V at scan rates of 1000 mV/s after application of a conditioning potential at -0.1 V for 2 sec. For sulfide measurements,

cyclic voltammetry (CV) was performed by scanning from -0.1 to -1.8 and back to -0.1 V. For sulfide scans, a 5 second conditioning potential was applied to the working electrode poised at a potential of -0.7 V before sulfide scans to ensure the removal of any reactive redox species that were previously deposited on the electrode surface. Likewise, a 2 second conditioning potential poised at -0.1 V was applied prior to the initiation of all scans. At each depth sampled, multiple scans were performed until scan peaks reached a repeatable current; this took at least one minute, which was the flushing time of the 30.5 m tubing. In addition to electrochemical measurements, samples at discrete depths from the pump system could be collected for lab-based analyses through the other port of the T tube apparatus. After each depth was sampled, the tubing was flushed for 1-2 minutes through the ¾ inch ball valve without the ¾" NPT to 3/8" sampling adapter prior to the next set of analyses and sample collections at a different depth. After a cast was completed, the entire system was flushed for 3 minutes with distilled water from the ship's evaporators and the clear PVC tubing containing distilled water was laid out on the deck in a figure eight configuration for storage.

Shipboard nitrite determination was performed using the method of Grasshoff (1983).[23]

Electrode Calibrations

Oxygen calibrations were performed during sampling events. To perform a calibration, anoxic samples with nondetectable oxygen concentrations (<3 uM) were first analyzed for sulfide. Upon analysis of the anoxic samples (typically 5 or more scans), the

anoxic sample was bubbled with air from an aquarium bubbler (Penn-Plax, Sea Pony) to reach 100% oxygen saturation and to purge H_2S. Upon saturation, LSV scans were then performed and corresponding current amplitudes were measured to obtain a two-point calibration curve (zero current for nondetectable O_2 and measured current for 100% O_2 saturation). Multiple calibration curves were built per rosette cast to account for changes of salinity and temperature with water column depth, as changes in salinity (i.e. electrolyte concentration in electrochemical cell) and temperature affect electrode response.[3,15]

Sulfide calibrations were performed based on previously described methods[8,15,16,18] Briefly, single $Na_2S\bullet 9H_2O$ crystals were weighed and prepared in sealed glass ampoules. Ampoules aboard the ship were kept in a refrigerator until use for calibrations. 3-10 mM calibration stock solutions were prepared by snapping an ampoule vial and dropping the sodium sulfide crystal into a volumetric flask with N_2 purged DI water.

Results and Discussion

Oxygen Calibrations; Effect of Salinity Changes on Electrode Response

The following calibration and field data were obtained from Chesapeake Bay during two of our sampling campaigns in August 2017 and 2018 at a location below the Chesapeake Bay Bridge ($38^0 58'$ N; $76^0 22'$ W). Deeper basins in the Chesapeake experience anoxia above the sediment water interface during summer months.[2,4-7] The sampling location responsible for these data, located in the upper bay, is deep (~25 m) and allows for entrainment of higher salinity water from ocean waters during tidal motion.[2,4-7,20]

Stratification of the upper bay water and influx of higher salinity water creates an increasing salinity gradient from the surface to bottom waters, as well as distinct anoxic, suboxic, and oxic zones.

Electrodes were calibrated in real time during rosette casts. Dissolved oxygen concentrations were lower at depths with higher salinity, therefore current response of the electrode was much lower. Figures 2a-e show three calibration scans (LSV) performed during cast 5 (August 4, 2017, 1:24 pm local time) to account for changing salinity. Figure 2a shows a scan from an anoxic water sample (23.3 m). After bubbling the sample with oxygen to 100% saturation, discrete O_2 and H_2O_2 peaks (-0.34 V, -1.23 V) were visible (2b). The O_2 peak amplitude was 55 nA and was used as a calibration point that represented 100% saturation. For each sample depth, the theoretical concentration of 100% O_2 saturation [C] was calculated using the method of Weiss[24], where T is absolute temperature and S is salinity (Eq. 1). A and B terms are volumetric stability constants for the calculation of solubilities from moist air at one atmosphere of pressure.[24]

(Eq 1) $$ln[C] = A1 + A2 * \left[\frac{100}{T}\right] + A3 * ln\left[\frac{T}{100}\right] + A4\left[\frac{T}{100}\right] + S‰[B1 + B2\left(\frac{T}{100}\right) + B3\left(\frac{T}{100}\right)^2]$$

The 100% O_2 peak height obtained was then plotted vs concentration determined from eq. 1 to obtain a two-point calibration curve, the other peak height being 0 nA for zero O_2. Figures 2c and 2e show scans before bubbling to 100% oxygen saturation, whereas 2d and 2f show scans after 100% oxygen saturation. At shallower depths (of decreasing salinity), O_2 currents were measured so 0 nA for zero μM O_2 was assumed based upon the deep samples where O_2 is not detected. Although temperature (T) is also a factor governing O_2

solubility, temperatures at all depths in the water column were relatively consistent (Figure 3b) making salinity a larger factor in electrode response.

Electrode Correlation with CTD for O_2 measurements

Microelectrode data from the pump profiling system were correlated with CTD O_2 data. At each sampling depth, an oxygen sensor at the bottom of the CTD rosette took readings of O_2 concentration (μM) and these are taken 1 minute prior to analysis by voltammetry so both measurements can be time correlated properly. Voltammetric microelectrode data are compared to CTD data from cast 5 in 2017 in figure 3a. Depths sampled ranged from 23.3 m to 11.8 m. The inset in figure 3a shows that values for electrode O_2 concentration are all consistent as a regression of electrode O_2 and CTD O_2 yields a slope of ~1 (1.016; $r^2 = 0.9972$) as expected. Slight deviation is due to the fact that voltammetry measures residual and Faradaic current, whereas the Clark sensor on the CTD does not give a measure of residual current. Salinity and temperature from cast 5 are also provided in figure 3b. Measurements were taken over a range of salinities (17.37ppt to 11.8 ppt) that required multiple calibrations. Temperatures were relatively consistent throughout the water column and varied only 0.3 ^{0}C from ~25.7°C to 25.4°C.

Chesapeake Profiles using the pump profiler

Full pump profiles of the Chesapeake Bay water column were obtained for both O_2 and H_2S using voltammetry, as well as for nitrite from samples collected with the pump

profiler.[23] O_2 and H_2S measurements were obtained rapidly and provided information for the collection of discrete samples for other dissolved constituents in the water column. As noted above, pump profiling O_2 data are consistent with CTD O_2 data, and H_2S data are also reliable based on previous intercomparisons[8,19]. Figure 4a shows a full profile from rosette Cast 10 (Aug 6, 2017, 7:10 am local time) for O_2 and H_2S (obtained by voltammetry), and figure 4b shows the salinity and temperature data from the same cast. The anoxic zone is clear at depths ranging from 22.3 meters to 18 meters. At these depths H_2S is present at concentrations up to 19.5 uM at 20 meters (Figure 4a). Above this region, a distinct suboxic zone void of O_2 and H_2S is present from sampling depths of 16 to 14 meters. At 13 meters, trace O_2 is present and increases towards the surface. Figure 4b shows salinity changes 6 units on descending from the lower part of the oxic zone at 11.5 m to the upper part of the anoxic zone at 18 m. Overall, salinity at 22.3 meters is 19.22‰ and decreased to 7.51‰ at 3 meters of depth. The decrease in salinity from bottom to top illustrates the density-driven stratification of the Chesapeake Bay, with fresher water on top of the saltier water. Temperature at all depths remains relatively consistent, ranging from 29.49 to 29.95 °C.

Temporal changes in Chesapeake Bay water chemistry were able to be monitored by the profiling system. Figures 4c and 4d show a profile from 2018 (Cast 6, July 30, 9:50 am). There is a notable decrease in H_2S concentrations when compared to 2017 (Figure 4a) as the maximum H_2S concentration was only 5.09 μM at 23.18 meters. Chesapeake data in 2018 consistently showed lower sulfide concentrations for all rosette pump casts. Additionally, in 2018, O_2 penetrated deeper into the water column, to ~19 m. The larger size of the oxic zone and smaller size of the anoxic zone could be attributed to a large influx

of freshwater (from many severe summer rain events) into northern Chesapeake Bay from the opening of the Conowingo Dam floodgates along the Susquehanna River, a large tributary located at the northern head of the bay.[25] Indeed, dam floodgates were opened immediately prior to the 2018 research cruise. Freshening of upper bay water was observed via salinity data (Figure 4d), which show the surface salinity at 3m in 2018 as 7.41 ppt, as opposed to 7.51 ppt in 2017 (4b). Earlier casts in 2018 had salinity as low as 2.61 ppt at 3m (data not shown). In 2018, the salinity gradient from 18 to 22 meters from the bottom of the oxic zone to the top of the anoxic zone was less than 2 salinity units, but the suboxic zone was again only 2 meters from 19 to 21 meters. Although pump profiler resolution can reach 2.54 cm, which allows for steep gradients to be observed, Figures 4a-d show that data collected only every 0.5 to 1.0 m is necessary to characterize the suboxic zone in both years. This is better than what can be achieved with Niskin bottles, where maximum resolution is constrained because of the length of the Niskin bottle (~1 m). A sharp salinity gradient in Figure 4d is not observed below a depth of 16.9 m but there is a salinity change of 9 units above this depth in the oxic zone. The salinity increase with depth shows that stratification had developed in 2018, but the penetration depth of O_2 from the freshwater river input was deeper and prevented the buildup of H_2S toward surface waters.

Voltammetric measurements provided rapid analysis of O_2 and H_2S while aiding scientists with sampling decisions for the measurement of other chemical species. Samples were collected in 2017 with the pump profiler to measure nitrite (NO_2^-), a nitrogen intermediate that forms under low O_2 conditions.[10,26,27] NO_2^- data are plotted with O_2 and H_2S (Figure 4a) and show that NO_2^- increases as O_2 concentration decreases at the top of

the suboxic zone, then decreases in the suboxic zone due to denitrification and in the anoxic zone, as it reacts with sulfide. This behavior has been shown previously in other low oxygen environments such as the Black Sea, which has a larger suboxic zone (as much as 40 m).[10] Fluorescence data in figure 4b and 4d indicate that the upper water column has a maximum fluorescence, which is an indicator of primary productivity.[28]

Conclusions

A robust and inexpensive pump profiler for measuring dissolved electroactive redox species in real time and for the collection of samples for other environmental purposes has been presented. The pump profiling system utilizes an inexpensive plastic bilge pump powered by a 12V battery to transport samples from discrete depths to deck surfaces in one minute. Voltammetric analysis of the pumped samples is performed in a 50 ml plastic centrifuge tube using Au/Hg solid-state microelectrodes. Our pump profiling method is accurate, as voltammetric O_2 data correlate well with CTD Clark sensor O_2 data. High resolution water samples can be collected for other species[2,4-7], including nutrients (NO_2^- in this work), dissolved organic carbon, dissolved inorganic carbon, pH, proteins, and microbes with better resolution than using Niskin bottles.

The pump profiler, equipped with a laboratory potentiostat, is portable enough to be used in other environments such as lakes (off docks and other platforms) and not constrained to use aboard research vessels. We have made a smaller and simpler version of the pump profiler to be used off small boats in up to a 6 meter water depth (Figure 5). The smaller system uses a pump that has the orifice outlet immediately stepped down from 1

inch to 3/8 inch for 3/8 inch Teflon or polypropylene tubing, but with no "T tube" (~0.14 L total tubing volume). A smaller 12 V battery is used for power as smaller volumes of water are pumped up for collection or to a cell for voltammetry and other sensors as desired. The tubing and power cord are attached with plastic zip ties to one inch OD schedule 40 PVC pipe, which is made into 1 or 2 meter sections that can be attached with screw couplings for easy (dis)assembly and storage. The PVC tubing has markings for every 5-10 cm and is lowered into the water. Hand held sensors (e.g., salinity and temperature) with long cables can also be attached to the schedule 40 PVC tubing with zip ties.

Finally, the pump profiling method is durable and extremely affordable. Including all components, the entire cost of the pump profiler system is under $1000 making the system extremely affordable compared to previous pump profiling methods. [10,11,29,30] The smaller profiler has a cost of around $250.

Acknowledgements

This work was supported by a grant from the Chemical Oceanography program (OCE-1558738) of the National Science Foundation to GWL. JMH acknowledges financial support from Dr. Yu-Ping Chin, University of Delaware Department of Civil and Environmental Engineering. We thank the crew of the R/V Hugh R. Sharp for help mounting the pump profiler to the ship's CTD-rosette system.

References

(1) Stumm, W., & Morgan, J. J. (1996). Aquatic Chemistry, John Wiley & Sons. *Inc., New York*.

(2) MacDonald, D. J., Findlay, A. J., McAllister, S. M., Barnett, J. M., Hredzak-Showalter, P., Krepski, S. T., ... & Emerson, D. (2014). Using in situ voltammetry as a tool to identify and characterize habitats of iron-oxidizing bacteria: from fresh water wetlands to hydrothermal vent sites. *Environmental Science: Processes & Impacts*, *16*(9), 2117-2126.

(3) Glazer, B. T., Marsh, A. G., Stierhoff, K., & Luther III, G. W. (2004). The dynamic response of optical oxygen sensors and voltammetric electrodes to temporal changes in dissolved oxygen concentrations. *Analytica chimica acta*, *518*(1-2), 93-100.

(4) Findlay, A. J., Di Toro, D. M., & Luther, G. W. (2017). A model of phototrophic sulfide oxidation in a stratified estuary. *Limnology and Oceanography*, *62*(5), 1853-1867.

(5) Findlay, A. J., Bennett, A. J., Hanson, T. E., & Luther, G. W. (2015). Light-dependent sulfide oxidation in the anoxic zone of the Chesapeake Bay can be explained by small populations of phototrophic bacteria. *Applied and environmental microbiology*, AEM-02062.

(6) Oldham, V. E., Jones, M. R., Tebo, B. M., & Luther III, G. W. (2017). Oxidative and reductive processes contributing to manganese cycling at oxic-anoxic interfaces. *Marine Chemistry*, *195*, 122-128.

(7) Field, E. K., Kato, S., Findlay, A. J., MacDonald, D. J., Chiu, B. K., Luther III, G. W., & Chan, C. S. (2016). Planktonic marine iron oxidizers drive iron mineralization under low-oxygen conditions. *Geobiology*, *14*(5), 499-508.

(8) Luther III, G. W., Catalano, G., De Lange, G. J., & Woittiez, J. R. W. (1990). Reduced sulfur in the hypersaline anoxic basins of the Mediterranean Sea. *Marine Chemistry*, *31*(1-3), 137-152.

(9) Herman, A. W., Mitchell, M. R., & Young, S. W. (1984). A continuous pump sampler for profiling copepods and chlorophyll in the upper oceanic layers. *Deep Sea Research Part A. Oceanographic Research Papers*, *31*(4), 439-450

(10) Codispoti, L. A., Friederich, G. E., Murray, J. W., & Sakamoto, C. M. (1991). Chemical variability in the Black Sea: implications of continuous vertical profiles that penetrated the oxic/anoxic interface. *Deep Sea Research Part A. Oceanographic Research Papers, Suppl. 2*, *38*, S691-S710.

(11) Friederich, G. E., & Codispoti, L. A. (1987). An analysis of continuous vertical nutrient profiles taken during a cold-anomaly off Peru. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(5-6), 1049-1065.

(12) Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography*, *14*(3), 454-458.

(13) Stookey, L. L. (1970). Ferrozine---a new spectrophotometric reagent for iron. *Analytical chemistry*, *42*(7), 779-781.

(14) Carpenter, J. H. (1965). The accuracy of the Winkler method for dissolved oxygen analysis. *Limnology and Oceanography*, *10*(1), 135-140.

(15) Brendel, P. J., & Luther, G. W. I. (1995). Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O2, and S (-II) in porewaters of marine and freshwater sediments. *Environmental Science & Technology*, *29*(3), 751-761.

(16) Luther, G. W., Glazer, B. T., Ma, S., Trouwborst, R. E., Moore, T. S., Metzger, E., ... & Taillefert, M. (2008). Use of voltammetric solid-state (micro) electrodes for studying biogeochemical processes: laboratory measurements to real time measurements with an in situ electrochemical analyzer (ISEA). *Marine Chemistry*, *108*(3), 221-235.

(17) McAdams, B. C., Adams, R. M., Arnold, W. A., & Chin, Y. P. (2016). Novel insights into the distribution of reduced sulfur species in prairie pothole wetland pore waters provided by bismuth film electrodes. *Environmental Science & Technology Letters*, *3*(3), 104-109.

(18) Luther III, G. W., Glazer, B. T., Hohmann, L., Popp, J. I., Taillefert, M., Rozan, T. F., ... & Nuzzio, D. B. (2001). Sulfur speciation monitored in situ with solid state gold amalgam voltammetric microelectrodes: polysulfides as a special case in sediments, microbial mats and hydrothermal vent waters Presented at the Whistler 2000 Speciation Symposium, Whistler Resort, BC, Canada, June 25–July 1, 2000. Electronic Supplementary Information available. See http://www. rsc. org/suppdata/em/b0/b006499h. *Journal of Environmental Monitoring*, *3*(1), 61-66.

(19) Luther, G. W., Bono, A. B., Taillefert, M., Cary, S. C., Taillefert, M., & Rozan, T. (2002). A Continuous Flow Electrochemical Cell for Analysis of Chemical Species and Ions at High Pressure: Laboratory, Shipboard, and Hydrothermal Vent Results. In *ACS SYMPOSIUM SERIES* (Vol. 811, pp. 54-72). Washington, DC; American Chemical Society; 1999.

(20) Luther III, G. W., Rozan, T. F., Taillefert, M., Nuzzio, D. B., Di Meo, C., Shank, T. M., ... & Cary, S. C. (2001). Chemical speciation drives hydrothermal vent ecology. *Nature*, *410*(6830), 813.

(21) Dayton, M. A., Brown, J. C., Stutts, K. J., & Wightman, R. M. (1980). Faradaic electrochemistry at microvoltammetric electrodes. *Analytical Chemistry*, *52*(6), 946-950.

(22) Oldham, V. E., Owings, S. M., Jones, M. R., Tebo, B. M., & Luther, G. W. (2015). Evidence for the presence of strong Mn (III)-bindidng ligands in the water column of the Chesapeake Bay. *Marine Chemistry*, *171*, 58-66.

(23) K. Grasshoff, "Determination of Nitrite, Nitrate, Oxygen, Thiosulphate," In: K. Grasshoff, M. Ehrhardt and K. Kremling, Eds., *Methods of Seawater Analysis*, Verlag Chemie Weinheim, New York, 1983, pp. 61-72, 81-84, 139-150.

(24) Weiss, R. F. (1970, August). The solubility of nitrogen, oxygen and argon in water and seawater. In *Deep Sea Research and Oceanographic Abstracts* (Vol. 17, No. 4, pp. 721-735). Elsevier.

(25) Bellmyer, J. (2018, July 23). Official: Dam gates opening no cause for concern. *Cecil Daily*. Retrieved December 13, 2018, from https://www.cecildaily.com/spotlight/official-dam-gates-opening-no-cause-for-concern/article_17c17a37-ef58-516d-bf10-a6afa0145ede.html

(26) Luther, G. W., & Popp, J. I. (2002). Kinetics of the abiotic reduction of polymeric manganese dioxide by nitrite: an anaerobic nitrification reaction. *Aquatic Geochemistry*, *8*(1), 15-36.

(27) Yücel, M., Beaton, A. D., Dengler, M., Mowlem, M. C., Sohl, F., & Sommer, S. (2015). Nitrate and nitrite variability at the seafloor of an oxygen minimum zone revealed by a novel microfluidic in-situ chemical sensor. *PloS one*, *10*(7), e0132785.

(28) Moore, T. S., Nuzzio, D. B., Di Toro, D. M., & Luther III, G. W. (2009). Oxygen dynamics in a well mixed estuary, the lower Delaware Bay, USA. *Marine Chemistry*, *117*(1-4), 11-20.

(29) Konovalov, S. K., Luther, G. I. W., Friederich, G. E., Nuzzio, D. B., Tebo, B. M., Murray, J. W., ... & Murray, K. J. (2003). Lateral injection of oxygen with the Bosporus plume—fingers of oxidizing potential in the Black Sea. *Limnology and Oceanography*, *48*(6), 2369-2376.

(30) Glazer, B. T., Luther III, G. W., Konovalov, S. K., Friederich, G. E., Nuzzio, D. B., Trouwborst, R. E., ... & Romanov, A. S. (2006). Documenting the suboxic zone of the Black Sea via high-resolution real-time redox profiling. *Deep Sea Research Part II: Topical Studies in Oceanography*, *53*(17-19), 1740-1755.

Figure 1 A) The pump system is zip tied to the CTD rosette, with the bilge pump on the bottom. Tubing connects the structure to the onboard flow cells. B) A closer view of the bilge pump zip-tied to the bottom of the CTD rosette near other sensors. C) Bilge pump fastened to rosette. D) 1 3/8" tubing at sampling end attached to bilge pump E) T tube

apparatus on deck of the vessel. Flow could be diverted for sample collection through the bottom valve (pointing left) or through the T tube to the electrochemical cell. F) ¾" to ¼" nylon fitting with ¼: tubing leading from the T tube to the voltammetry cell. G) An example of the nylon fitting used to reduce tubing diameter throughout the pump flow path. H) Electrochemical cell. A 50 ml falcon tube is attached to a ring stand. Working, reference, and counter electrodes are placed inside the cell. When pump flow is turned on, sample flows into the cell and continuous real time measurements are made.

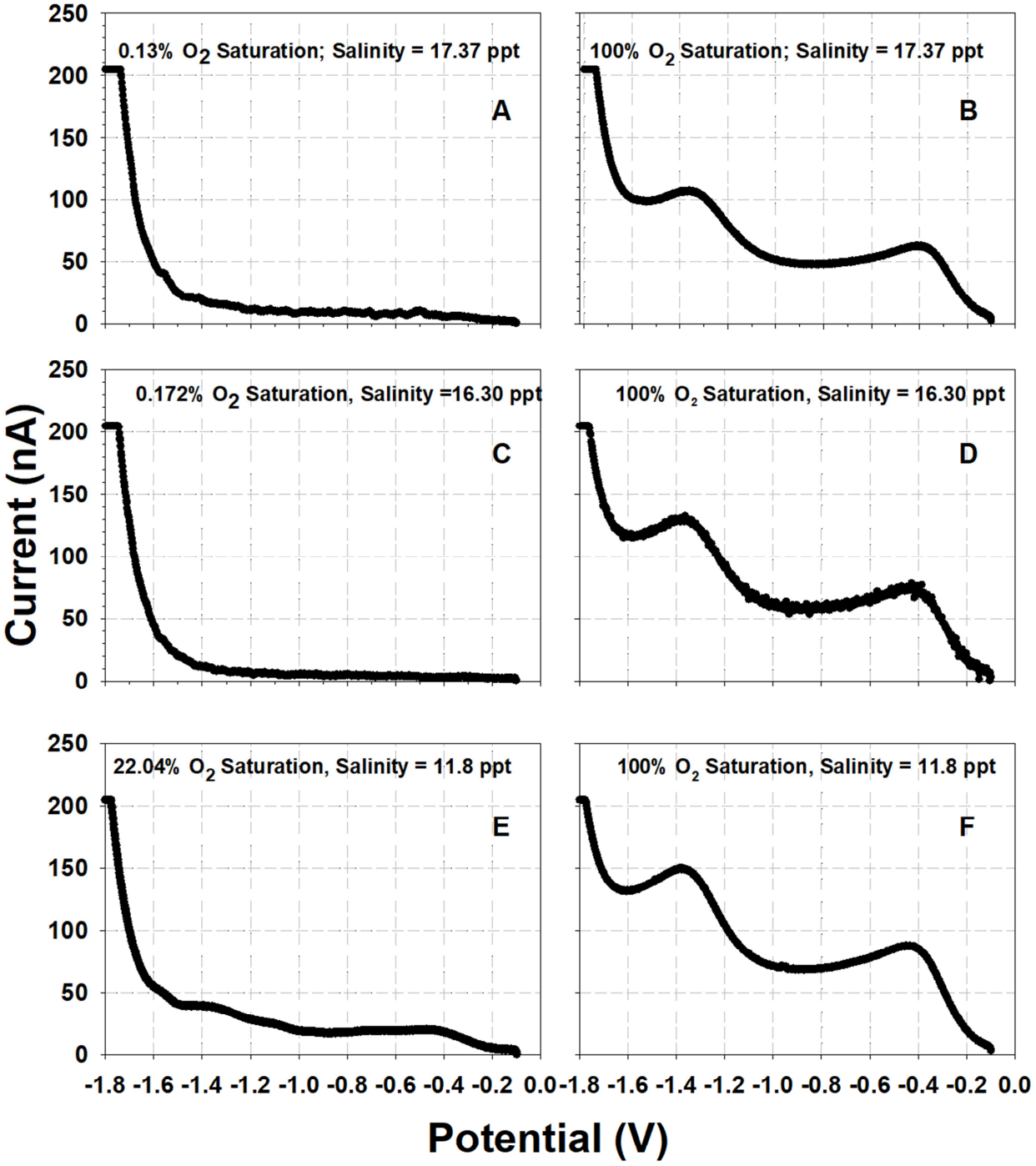

0.13% O2 Saturation; Salinity = 17.37 ppt
A
100% O2 Saturation; Salinity = 17.37 ppt
B
0.172% O2 Saturation, Salinity =16.30 ppt
C
100% O2 Saturation, Salinity = 16.30 ppt
D
22.04% O2 Saturation, Salinity = 11.8 ppt
E
100% O2 Saturation, Salinity = 11.8 ppt
F
Current (nA)
250
200
150
100
50
0
-1.8 -1.6 -1.4 -1.2 -1.0 -0.8 -0.6 -0.4 -0.2 0.0
Potential (V)

Figure 2 O_2 electrode Calibrations performed during sampling. Each row includes the original sample (left) and the sample following bubbling for 100% O_2 saturation (right). A) With decreasing salinity, O_2 concentrations increase, increasing the amplitude of O_2 waves. Scan A shows an anoxic sample (salinity of 17.37‰) B) bubbled to 100% O_2 saturation C) Suboxic sample (salinity of 16.3‰) D) 100% O_2 saturation of suboxic sample E) A sample from the lower-oxic zone salinity of (11.7‰) F) 100% O_2 saturation of sample. All calibration scans were performed using linear sweep voltammetry (LSV) at 1000 mV/s.

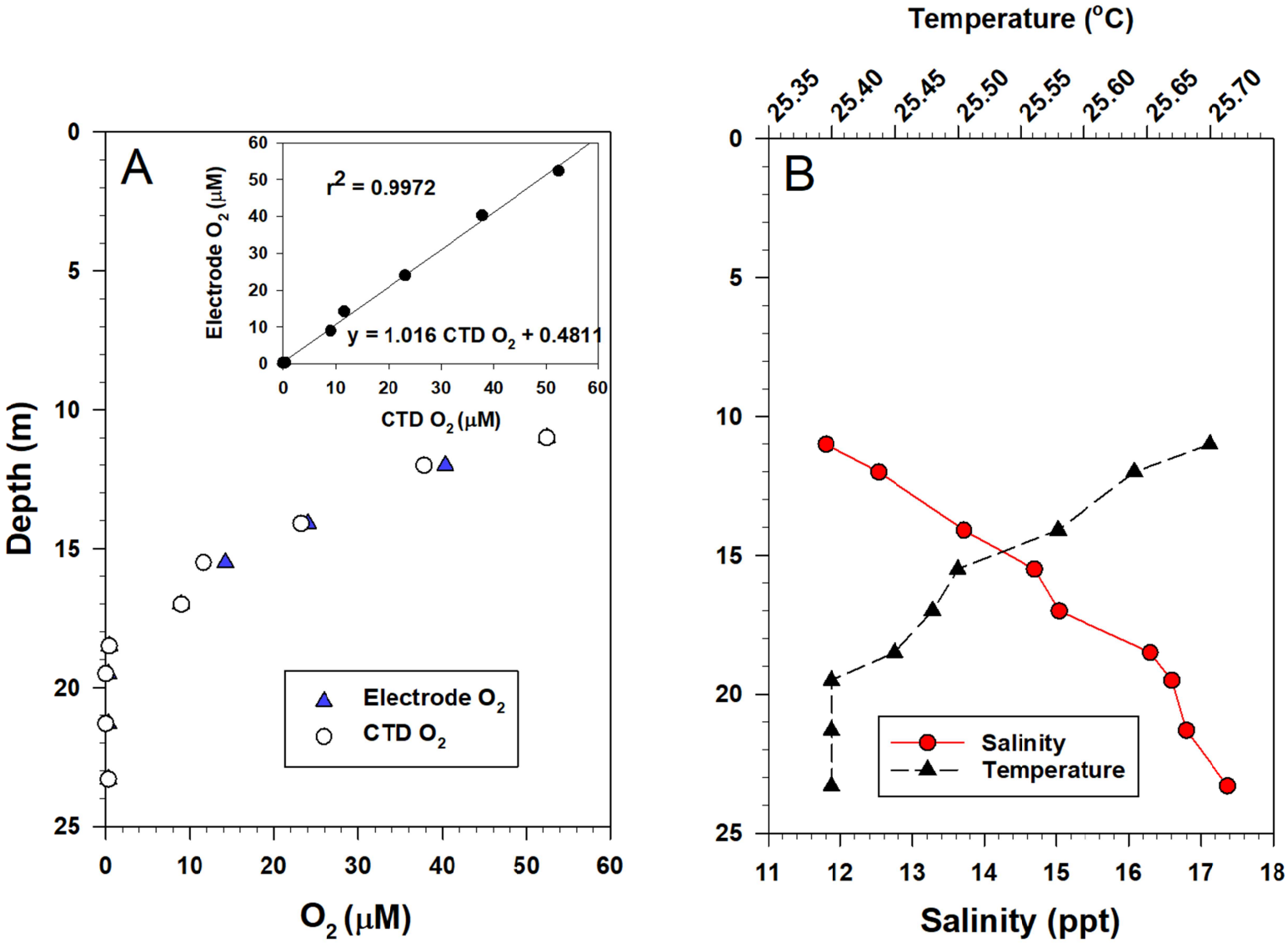

A
Depth (m)
O_2 (μM)
Electrode O_2
CTD O_2
r^2 = 0.9972
y = 1.016 CTD O_2 + 0.4811
Electrode O_2 (μM)
CTD O_2 (μM)
B
Temperature (°C)
Salinity
Temperature
Salinity (ppt)

Figure 3 A) CTD O_2 and electrode O_2 concentration vs depth is plotted from Cast 5 in 2017 (August 4, 1:24 pm). The inset is a regression of CTD O_2 vs electrode O_2 that shows a slope of 1.016, which indicates agreement between the two methods. B) Salinity and temperature are provided from the same cast. While temperature only varied 0.3 ^{0}C, salinity changed from 17.37 ppt to 11.80 ppt.

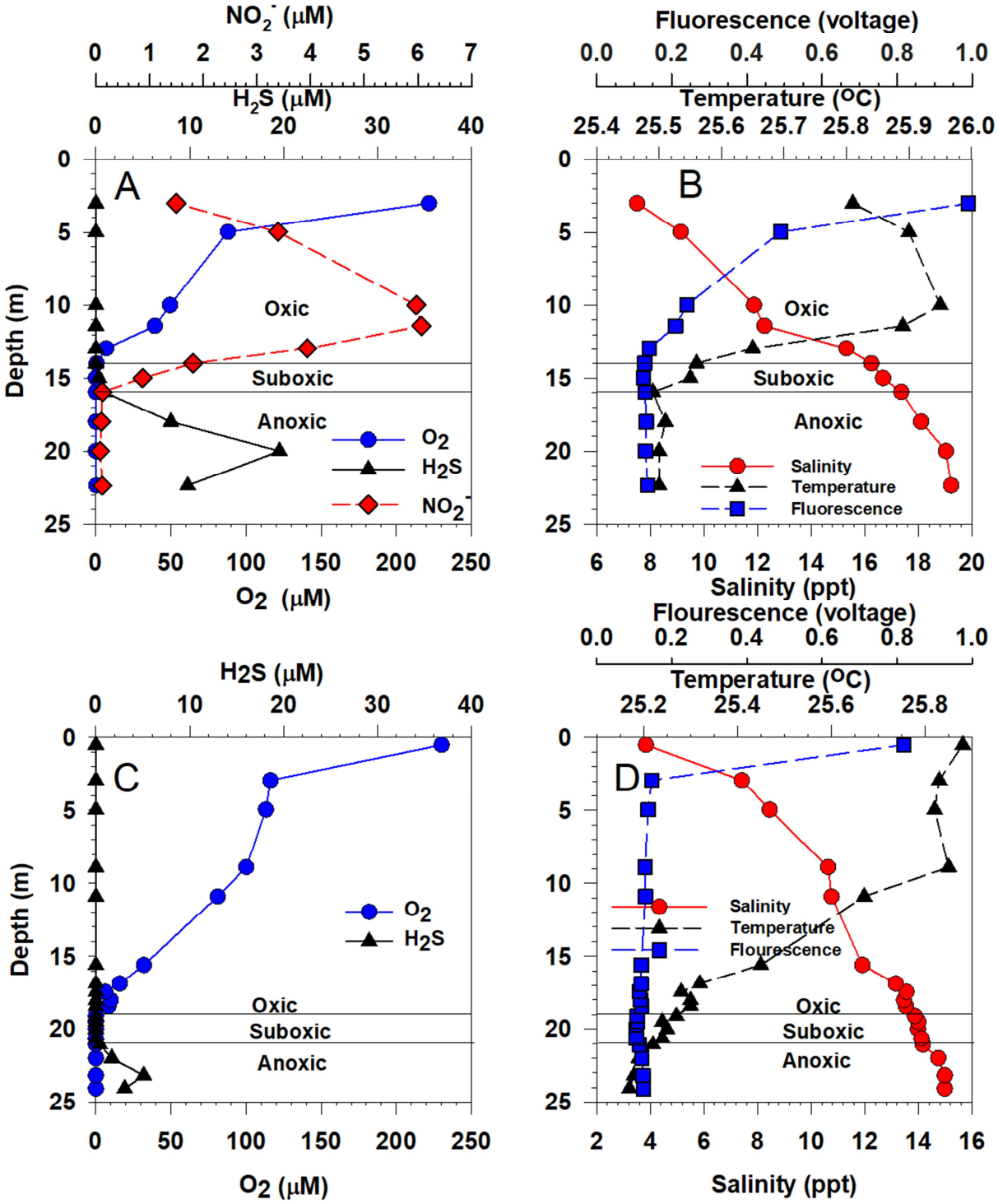

A
NO_2^- (μM)
H_2S (μM)
Depth (m)
O_2 (μM)
Oxic
Suboxic
Anoxic
O_2
H_2S
NO_2^-
B
Fluorescence (voltage)
Temperature (°C)
Salinity (ppt)
Salinity
Temperature
Fluorescence
C
H2S (μM)
D
Flourescence (voltage)
Flourescence

Figure 4 Full profiles of O_2, H_2S, NO_2^-, fluorescence, temperature, and salinity plotted vs. depth. A) Cast 10 (Aug 6, 2017, 7:10 am local time) plot of O_2, H_2S, and NO_2^-. B) Cast 10 plot of fluorescence (voltage), salinity (ppt) and temperature (°C). C) Cast 6 (July 30, 2018, 9:50 am local time) plot of O_2 and H_2S only. D) Cast 6 plot of fluorescence, salinity and temperature. A depth resolution of 0.5 meters was chosen to better characterize the anoxic to suboxic to oxic transition zone for figures 4c,d.

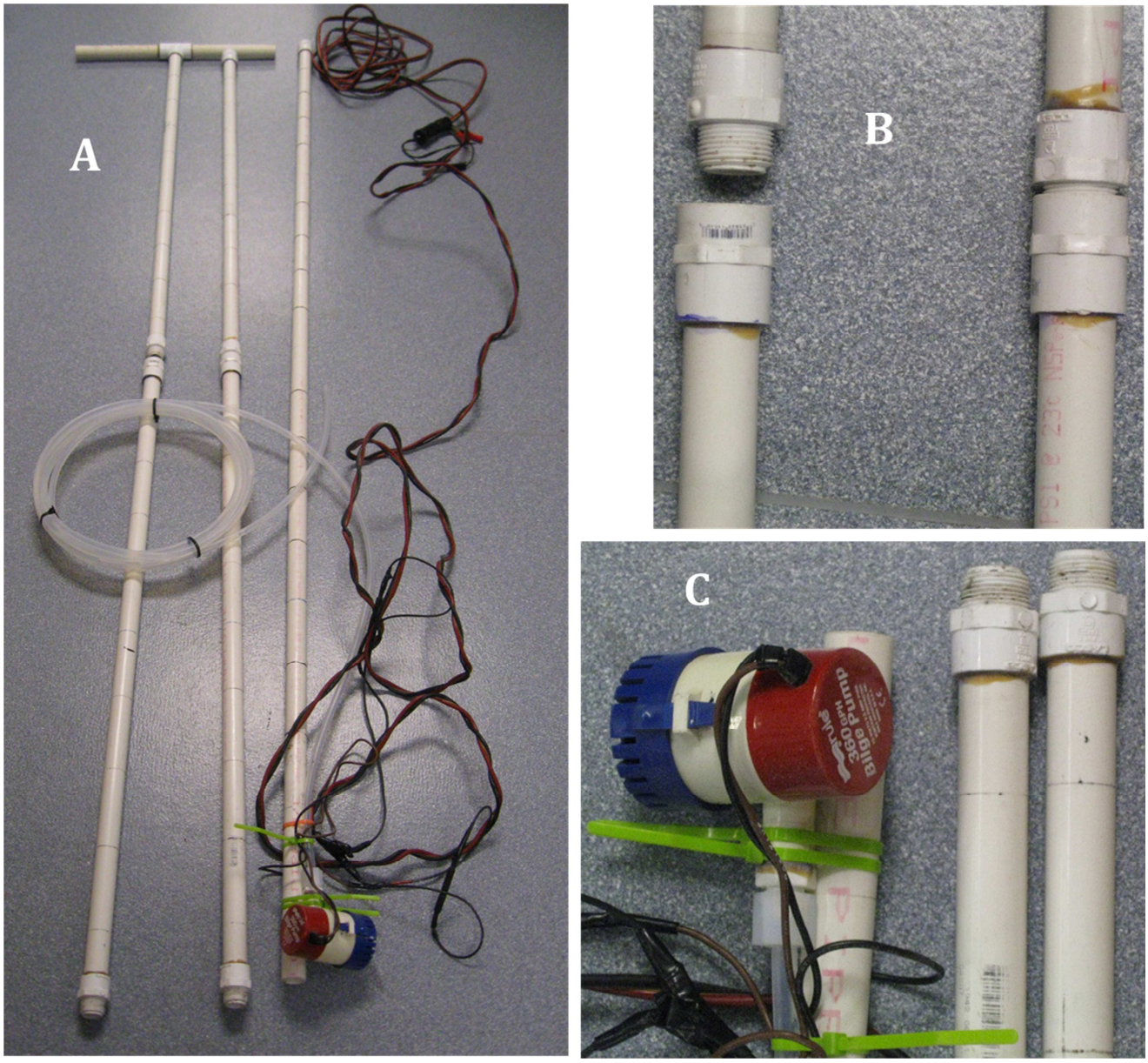
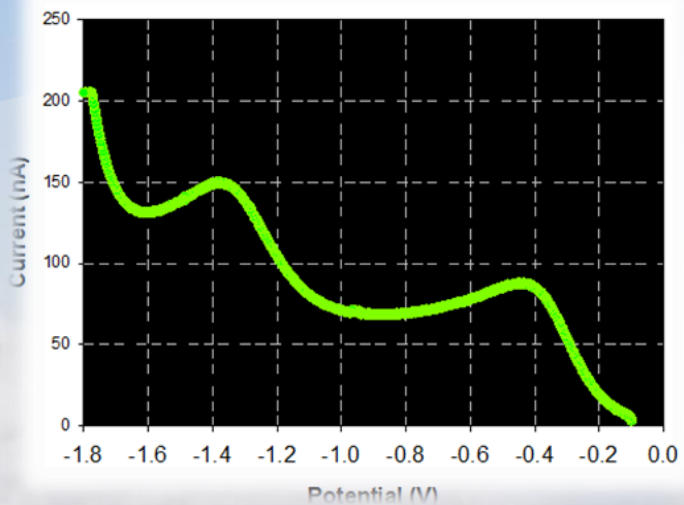
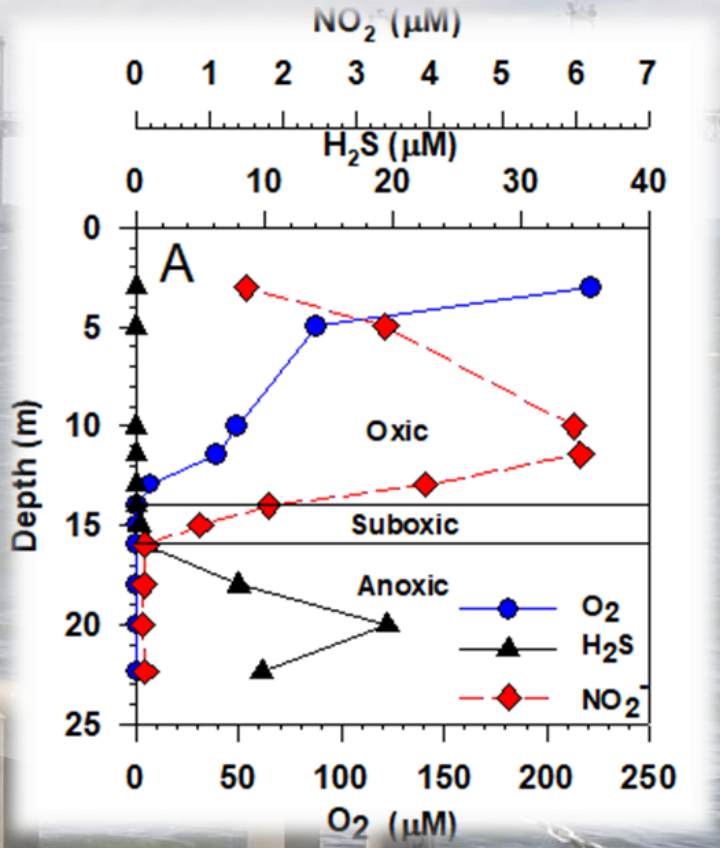


Figure 5 A) Small boat pump profiler with 2, 2 meter extensions and 2,1 meter extensions. B) Closeup of screw couplings. C) Closeup of the pump with 3/8 inch Teflon tubing attached to the extension.


250
200
150
100
50
0
-1.8
-1.6
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0


NO_2^- (μM)
0 1 2 3 4 5 6 7
H_2S (μM)
0 10 20 30 40
A
Depth (m)
0 5 10 15 20 25
Oxic
Suboxic
Anoxic
O_2
H_2S
NO_2^-
0 50 100 150 200 250
O_2 (μM)