NOAA FISHERIES

Standard Operating Procedure

Title: **Sonde Sensor Calibration (pH, RDO, Temperature/Conductivity, Chlorophyll-a)**	Version Number: 1	Effective Date: **01/01/24**	Page 1 of 12

Revision History		
Version No.	**Effective Date**	**Description**
1.0	*01/01/2024*	*Original composition by M.Kachmar*

Procedure Owners: **Date:**

KACHMAR.MARIAH.LYNN.1620667907 Digitally signed by KACHMAR.MARIAH.LYNN.1620667907 Date: 2024.01.25 11:50:49 -05'00'

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MILKE.LISA.MICHELLE.1365840276 Digitally signed by MILKE.LISA.MICHELLE.1365840276 Date: 2024.02.07 14:40:02 -05'00'

Lisa Milke, EAD Chief

Title: **Sonde Sensor Calibration (pH, RDO, Temperature/Conductivity, Chlorophyll-a)**	Version Number: 1	Effective Date: **01/01/24**	Page 2 of 12

1 Purpose

The purpose of this SOP is to provide concise guidance and methodology for calibrating the Aqua Troll 600 sonde to accurately collect temperature, salinity, pH, RDO, and chlorophyll-a data of quality.

2 Scope

This SOP is pertaining to the EPA Long Island Sound Study funded Oyster Health project that is incorporating water quality metrics to understand host-pathogen-environment relationships.

3 Definitions/Acronyms

none

4 Safety Precautions

All team members will wear appropriate clothing depending on weather conditions including but not limited to waders, rubber boots or protective footwear, gloves, hats, sunglasses, long sleeve shirts and pants. Thick protective gloves (e.g garden gloves) should be worn when handling fouling organisms. Team members will wash hands thoroughly after field trips end. A first aid kit will be present for any injury. Extra water will be provided to avoid dehydration or heat stroke. Team members will take regular breaks when needed.

Exercise weather-appropriate field safety measures by monitoring conditions before and during the trip. Do not perform fieldwork during dangerous conditions (e.g. lightning, extreme winds, extreme floods). Do not visit field sites alone (use buddy system). Inform PIs of dates and times of fieldwork. Confirm safe return to the lab. At intertidal sites, perform procedures during low tide. At subtidal sites, divers are to follow NOAA diving regulations according to the instructions of the lab diving coordinator (Barry Smith).

5 Reagents/Media

1. Bottle of distilled water (1 L) for rinsing sonde and probes.
2. In-Situ pH filling solution for replacing fluid inside pH sensor (SKU 0056900). In Garage.
3. In-Situ Quick Cal solution for calibrating pH sensor (SKU 0033250). Fridge, Rm 29. Refer to note under Section 8 (Quality Control).

6 Supplies/Equipment

Field Tablet (containing VuSitu app).
Robust zip-ties (e.g., 24 inch) for securing the sonde to the PVC housing at subtidal sites.
1516 inch socket wrench
1516 inch open-end wrench

Adjustable wrench
Sonde supply box
High vacuum grease
RDO sensor Calibration sponges
Extra pins and bolts for housing
Desiccant tube
Screw drivers
Hex driver
Allen key
Sonde wipers
Alcohol pads
D batteries (minimum of 2, Energizer or Duracell only).
Paper towels (for collecting discarded pH filling solution).
Towel / cloth for wiping things down.
Dish scrubbing pad
Scrub brush
Shucking knife
Q-tips or cotton buds
500 ml empty container with sealable top
First aid kit
Hand sanitizer
Wire cutters

7 Equipment

8 Quality Control

1. All team members will be trained to complete all field tasks, including training on data entry requirements for each specific task. Environmental monitoring data will be regularly checked for drift and sensors will be calibrated when necessary.
2. Reagent care:
    a. Quick Cal solution: Use a new (unopened) bottle of Quick Cal solution each field trip for calibrating the pH probe. Keep in a dark bottle (it is light sensitive). Quick Cal can be kept unopened in the fridge for ~6 months (check expiration date on bottle). Use a fresh, unopened bottle for each calibration (once opened it lasts ≤ 7 days in the fridge). Before opening and using the solution (e.g., while driving to the field site), let it come to ambient temperature. This helps the probe stabilize during the calibration process.
    b. pH filling solution: Store at room temperature (or fridge, but not frozen or at high temperatures; In-Situ tech support, 7/7/23). It lasts for ~2 years (check expiration date on bottle).

9 pH Sensor

1. *The pH sensor is recommended to be calibrated every 10-12 weeks. The reference filling solution inside the pH sensor should be replaced between deployments or when measurements begin to drift.* See section 9.5.a.
2. Unscrew the copper guard from the sonde, as well as the black cap that is screwed into the top end of the copper guard.
3. Rinse the probes, copper guard, and black end cap with distilled (or tap) water. Dry all pieces using a towel or cloth. *Don't wipe the sensor surfaces.* If necessary, debris can be gently removed following the 'Monthly Sonde Maintenance' SOP.
4. **Calibrate the pH sensor**
    a. Rinse the probes with Quick Cal solution (Figure 1):
        i. Screw the copper guard onto the sonde in the calibration/storage position.
        ii. Connect the data sonde with the VuSitu app on the Samsung tablet (see Monthly Sonde Maintenance' SOP for instructions), then select Calibration / Quick Cal Calibration. The calibration steps will require the probes to be rinsed with Quick Cal (rinse it twice) before refilling with Quick Cal for the actual calibration.
        iii. To rinse sensors with calibration solution:
            - Hold the sonde with the probes facing upwards.
            - Unscrew the black end cap from the top of the copper guard
            - Pour enough Quick Cal solution to fill the probe well (created by copper guard) about 2/3 of the way up.
            - Lightly screw on the black end cap of the copper guard and rinse the sensors and well by swirling the Quick Cal solution.
            - Remove the black end cap and pour the Quick Cal solution into a discard container.
            - Repeat the previous rinsing step, giving the probes a second rinse of Quick Cal solution (keeping enough Quick Cal for the calibration step).

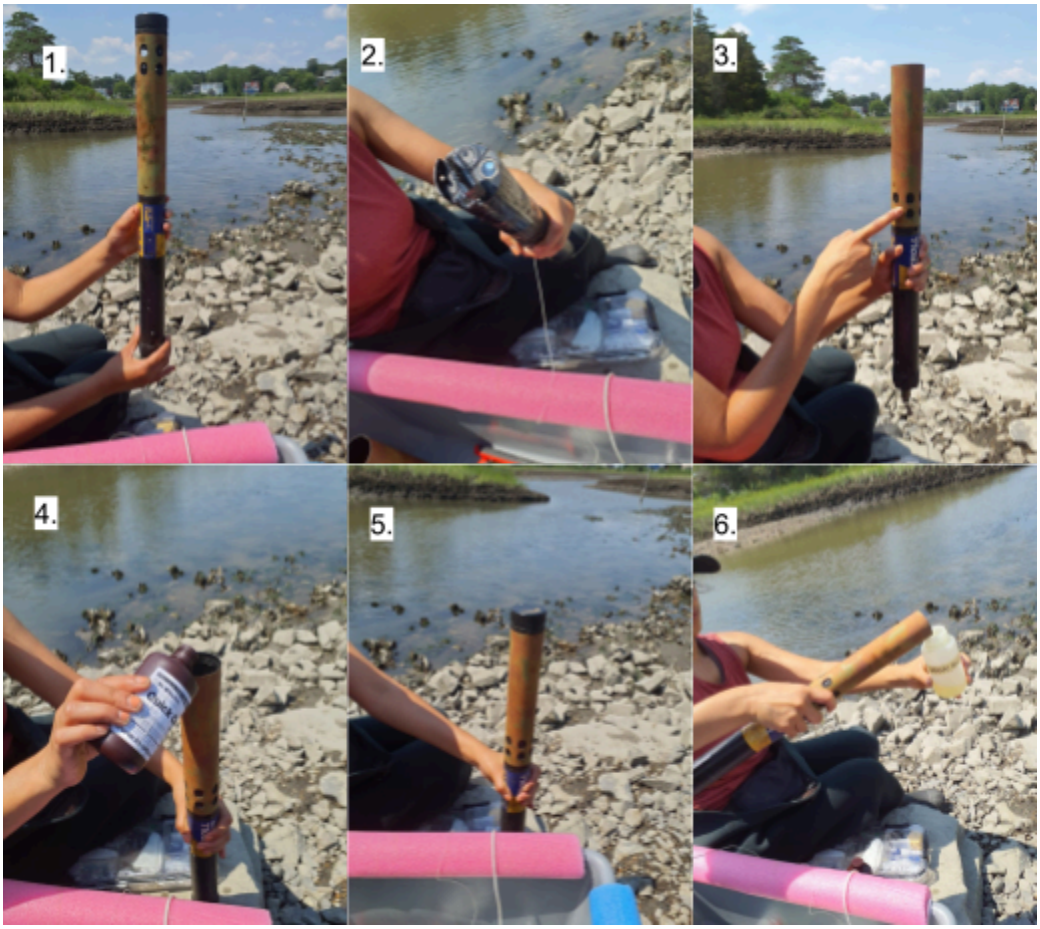
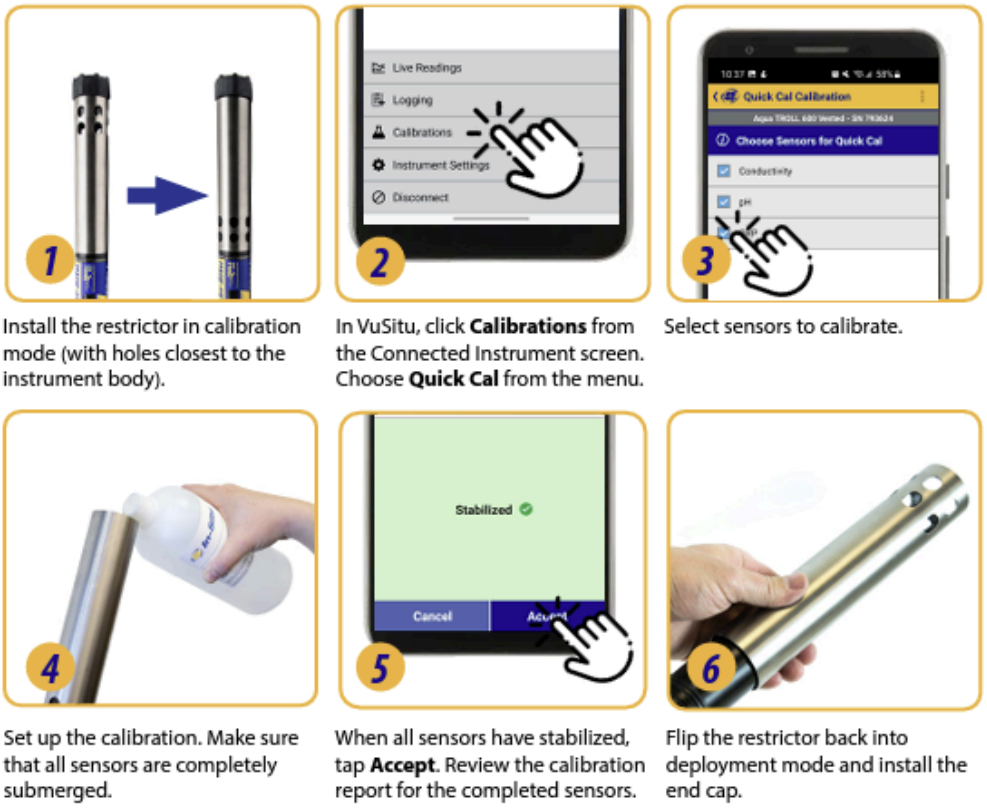


Figure 1. Rinsing the sensors with Quick Cal solution. 1. Holding sonde with probes facing upward to connect to bluetooth. 2. removing copper guard to expose sensors. 3. Placing copper guard in storage position. 4. Filling with Quick Cal solution. 5. Capping copper guard and swirling solution. 5. Discarding Quick Cal solution into a waste container.

b. After rinsing the probes, refill the probe well with fresh Quick Cal solution and calibrate the pH sensor by following the steps in the app (Figure 2):
    i. If the sensor has significant drift or fails to calibrate, try replacing the filling solution as described in Section 9.5. If the calibration continues to fail, consult with PI's on the best available option (i.e. switching sonde with a backup instrument, taking sonde back to the lab for maintenance, contacting In-Situ).



Install the restrictor in calibration mode (with holes closest to the instrument body).

In VuSitu, click **Calibrations** from the Connected Instrument screen. Choose **Quick Cal** from the menu.

Select sensors to calibrate.

Set up the calibration. Make sure that all sensors are completely submerged.

When all sensors have stabilized, tap **Accept**. Review the calibration report for the completed sensors.

Flip the restrictor back into deployment mode and install the end cap.

Figure 2. Steps for calibration of the pH sensor in the VuSitu app.

5. **Reference filling solution:**
    a. The sensor fill solution has a shelf life of 2 years. Replace the fill solution every 5 to 6 months or when:
        i. The sensor fails to calibrate within the acceptable slope and offset range.
        ii. Sensor readings vary.
        iii. Readings during calibration at pH 7 are greater than +30 mV or less than -30 mV
        iv. Sensor is slow to respond.
    b. Remove the pH sensor from the sonde:
        i. Rotate the wiper arm (the little brush that cleans the sensors) into a position so that it does not obstruct the top of the pH probe. (Note: each type of probe is labeled on its side with raised lettering).

ii. Use the longest of the three types of sonde screwdrivers (hex driver) to undo the screw that secures the pH probe to the sonde. The screw will unscrew from the sonde housing, but it will stay attached to the probe itself (i.e., it will not fall out and get lost). (Figure 3)

Figure 3: Location of the screw to remove the pH sensor from the sonde and the appropriate screwdriver needed to perform removal.

c. Once the screw is undone, insert the same screwdriver into a small hole at the base of the probe (at the junction between the base of the probe and the sonde; not at the sensor end). Gently push upwards until the pH probe releases from the sonde. Then lift the pH probe from the sonde, making sure that the electronic interfaces remain dry.
d. Replace the pH filling solution in the probe using the instructions below (Figure 4).

Note 1: Be careful not to lose the reference junction (little screw top on the probe's filling solution reservoir) - unscrew it over the gray tray. Keep it moist and clean.

Note 2: Before putting the reference junction back on, make sure the O-ring is cleaned and vacuum greased. Tap the probe lightly before resealing the filling solution reservoir to release any trapped air bubbles.

Figure 4: Steps to replace the reference filling fluid

e. Replacing the reference junction:

i. Replace the junction when the sensor fails to calibrate with a reasonable slope and offset, even after you have replaced the filling solution.
   1. Unscrew the reference junction and discard.
   2. Replace the filling solution and screw in a new reference junction.
   3. Soak for 15 minutes, then calibrate the sensor.

f. Reattach the pH probe onto the sonde:

i. Make sure the pH probe's O-ring is clean and dry.
ii. Apply a little vacuum grease (tubes in the red box) to the O-ring.
iii. Gently push the probe into its original sonde slot, making sure everything is aligned properly (check alignment at the sensor end too).
iv. Gently tighten the screw that secures the probe to the sonde using the longest of the three types of sonde screwdrivers (red box). It does not need to be especially tight (beware of cracking the plastic housing).

10 Conductivity sensor

1. *The conductivity sensor should be calibrated every 12 months, e.g. before the sondes are redeployed for a new field season.*

2. If there is reason to believe that the sensor needs calibrating, use the Quick Cal solution and follow the instructions for Quick Cal calibration of the pH sensor (Section 9.4).
    a. *In the VuSitu Quick Cal calibration steps, make sure that the conductivity sensor is selected for calibration.*
    b. If the calibration continues to fail, consult with PI's on the best available option (i.e. switching sonde with a backup instrument, taking sonde back to the lab for maintenance, contacting In-Situ).

11 Temperature sensor

1. The sonde manual (p. 45) recommends that the Temperature sensor should be calibrated "only when required by user protocol".
2. There is no user-calibration option for the temperature sensor. It is possible, however, to check the accuracy of the temperature sensor using the ice bath method also used for HOBO temperature loggers (In-Situ tech support, May 2023).
3. It is also possible to check the accuracy of the temperature sensor by taking side-by-side temperature readings to compare sonde data against another instrument (e.g., HOBO temperature logger) that was recently ice bath tested.
    a. The offset should be no more than +/- 0.5 degrees. If the sensor has significant drift, consult with PI's on the best available option (i.e. switching sonde with a backup instrument, taking sonde back to the lab for maintenance, contacting In-Situ).

12 RDO Sensor

1. *The RDO sensor should be calibrated every 12 months depending on site and storage conditions. Calibration should at minimum occur before the sondes are redeployed for a new field season.*
2. To calibrate the RDO sensor, use the 'RDO 100% saturation with water saturated air method'. (Figure 5)
    a. The 2 point offset should +/- 0.3 mg/L with a 2-point slope of 0.7 to 1.3. If the sensor has significant drift or fails to calibrate, consult with PI's on the best available option (i.e. switching sonde with a backup instrument, taking sonde back to the lab for maintenance, contacting In-Situ).
    b. Round sponges are in the sonde maintenance kit box which can be found in the LISS field gear station in the garage of the NEFSC.
    c. Remove the cap from the copper guard. Place the copper guard (aka restrictor) in the calibration/storage position (holes facing screen and centered on the instrument)

d. Lightly dry the RDO sensor before calibration using a cotton swab.
e. Saturate the small round sponge with water. Seawater or freshwater okay. The water only serves to saturate the air in the copper guard.
f. Place the sponge on the guard cap and *loosely install the cap allowing for airflow. If the cap is too tight it will disrupt the calibration*. Be sure to keep the face of the sensor dry.
g. Leave the sponge in the copper guard for 5 minutes.*Do not wait beyond 5 minutes before performing the calibration. Condensation may start to form on the RDO sensor, which interferes with calibration.*
h. Open the VuSitu app, click calibration, RDO sensor, and follow the instructions to complete the calibration. .

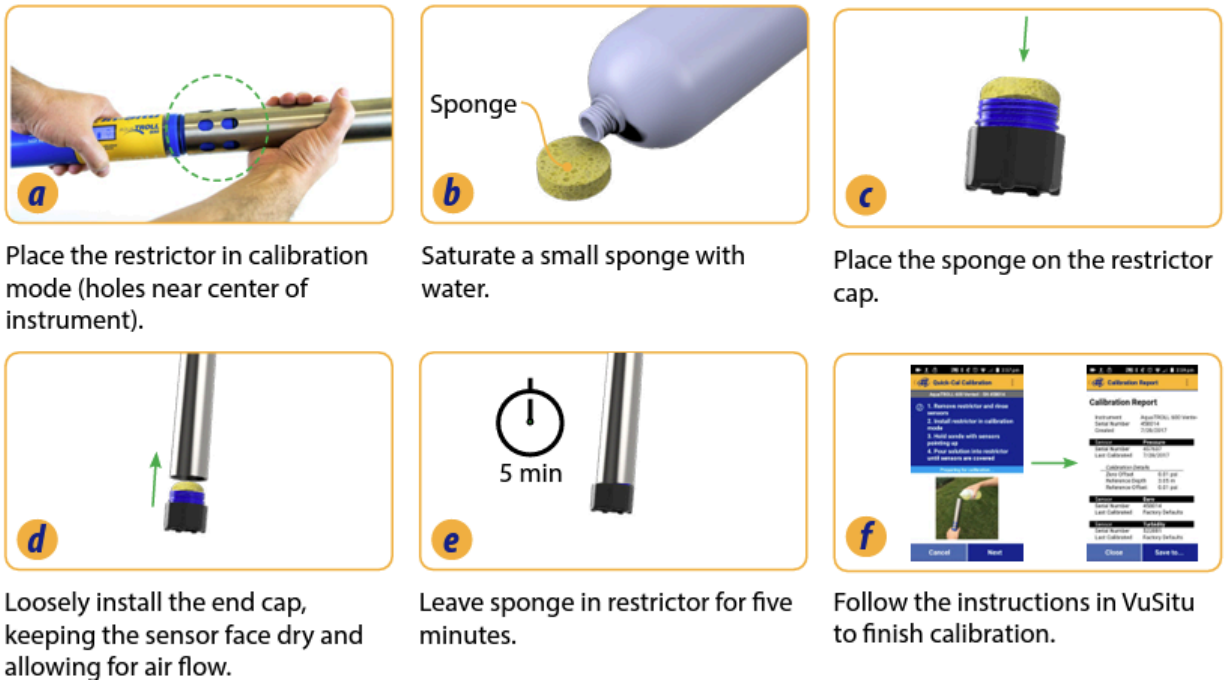


Figure 5 : Steps to calibrate the RDO sensor.

13 Chlorophyll-a sensor

1. *The chlorophyll-a sensor should be calibrated every 12 months. Calibration should occur before the sondes are redeployed for a new field season.*
2. If the sensor needs calibrating, reset the zero point by performing a calibration with deionized water:

a. Remove the copper guard of the sonde.
b. Rinse the guard and sensors with distilled water.
c. Screw the copper guard back on in calibration mode (screw it on by the threads at the vent hole end of the guard) and invert the sonde (sensors facing up).
d. Fill the sensor chamber with distilled water to the bottom of the cap end threads.
e. Screw the end cap onto the copper guard. *It is important that no light is able to enter the sensor chamber.*
f. Connect the sonde with the VuSitu app on the Samsung tablet.
g. Select Calibrations from the menu.
h. Choose the Chlorophyll option and follow the instructions.

14 Waste Disposal

1. Discarded pH filling solution should be poured onto a paper towel while in the field and disposed of in the trash on return to the lab (as per In-Situ instruction sheet).
2. In the field, discarded Quick Cal solution should be poured into a waste container and sealed. On return to the lab, it should be poured down the sink with running tap water (as per instructions from In-Situ tech support, 7/7/23).

15 Recording calibrations and data

1. Quality control and assurance (QAQC) data following the github repository.
2. Make sure all notes have been updated in the appropriate field notebooks, sonde lab notebook, and the calibration spreadsheet.

16 References

Data sonde, main unit

- Aqua TROLL 600 Multiparameter Sonde — Quick Start Guide — Manual

Data sonde, added on components

- Aqua TROLL Temperature-Conductivity Sensor
- Aqua TROLL RDO Sensor (Includes RDO X Cap)
- Aqua TROLL Chlorophyll a Sensor — Instruction Sheet
- Aqua TROLL pH / ORP Sensor — Instruction sheet
- Aqua TROLL Wiper — Manual
- Twist-Lock Backshell Hanger — Manual

Data sonde accessories

- Quick Cal solution — Safety Data Sheet
- Aqua TROLL Replacement Desiccant — Manual

- pH Filling Solution Manual

Spec Sheet for Data Sonde and Sensors

17 Appendices

A. Shelf life of QuickCal Solution
   a. Per In-Situ - Shelf life of unopened bottles, in cold dark places is only 4 months and after opening 7-21 days.

END OF DOCUMENT