Q-Box AQUA

Aquatic Respirometry Package Manual

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Overview of the Q-Box AQUA Aquatic Respirometry Package

The **Q-Box AQUA** is used to measure metabolic rate in resting aquatic animals using **intermittent closed respirometry**, in which measurements are made sequentially without removing the animal from the sample chamber. The system is used for measuring the decrease in dissolved oxygen (DO) due to respiration by fish or other aquatic animals. The oxygen consumption of the animal (VO2), is calculated from the rate of DO decline. The chamber is flushed between measurements with fresh water from the bath in which the chamber is submersed. This intermittent method overcomes the disadvantage of continuous closed respirometry in which the DO level in the chamber continually decreases. This decrease may stress the animal physiologically during extended experiments.

The diagram below shows the set up of a Q-Box AQUA system. Tubing for water flow is shown in blue (solid lines indicate flow; broken lines indicate no flow), whereas black lines indicate electrical connections. The components include two respirometer chambers (small and large), a 3-way valve controlled by the digital control unit (DCU), a DO probe held in a flow through vessel, and a liquid pump. All components are connected with tubing. During an experiment, the animal is placed in the chamber which is immersed in a water bath. In the "flush" mode, the 3-way valve is in the de-energized state where the loop is open. The pump then draws water from the bath to flush the chamber with fully oxygenated water under ambient conditions. The water returns to the bath from the chamber via the open portion of the T-piece between the chamber and the 3-way valve. Temperature, dissolved oxygen, pH etc. can also be controlled in the water bath with optional components. Contact Qubit Systems for these options.

During the measurement or "circulation" phase, the 3-way valve is energized to close the loop so that the pump circulates water through the closed system as shown by the solid blue lines. In this circulation phase, DO declines in the closed loop as the animal consumes O_2. From the rate of decline (i.e. the slope of the DO trace) the respiratory O_2 consumption (VO2) can be calculated. The flush-circulation cycle is repeated until the experiment is terminated. A VO2 value is automatically calculated from each circulation period.

With short periods of intermittent closed loop respirometry, build-up of waste products and severe depletion of dissolved oxygen are avoided. The intermittent method facilitates dynamic studies of VO2 with a time resolution of a cycle time (i.e. flush plus circulation time) which is selected by the user in the software. The flush time should be sufficient to restore the DO level to that of the external water bath, whereas the circulation time should be long enough to obtain an accurate slope of the DO decline. The depletion of O_2 during the measurement period should be kept to a minimum (< 10%) but will depend on the tolerance of the animal to hypoxia. **The ratio of chamber volume to animal volume should be 10 - 20 for resting studies**. Therefore, the standard Q-Box AQUA system will work well with animals that are minimum of 7 (small respirometer chamber) or 30 g (larger respirometer chamber) in size. Optional custom size respirometer chambers for larger animals are also available.

The dissolved oxygen is measured with an optical dissolved oxygen probe (S122). This probe is internally compensated for changes in both temperature (with a thermistor) and pressure (with a pressure sensor). These two parameters affect oxygen solubility in water as well as oxygen diffusion through the probe membrane. Therefore, the sensor may be used at different temperatures and pressures from those at the time of the original factory calibration or recalibration by the user.

Three additional sensors included in this package are a salinity probe (S130) for measuring dissolved salts (which affect oxygen solubility), a stainless-steel temperature probe (S132) for monitoring water temperature and an absolute pressure sensor for measuring atmospheric pressure. The optical dissolved oxygen probe maintains its calibration for long periods. However, if calibration becomes necessary, the measured temperature and pressure are required for determining the absolute air-saturated DO level in units of mg/ml. Correction for salinity is calculated in the software using the measured salinity data. An additional analog signal comes from the liquid pump PL1775. This signal is corelated with the flow rate of water through the system but is different for every setup. The user may monitor this voltage output at a specific flow rate during an experiment and for future experimental setups.

The five analog signals from the sensors are digitized with 2 LabQuest Mini interfaces (3-channel, 12-bit). Data is recorded, displayed, and manipulated on a PC or Macintosh computer using Logger Pro software.

Q-Box Aqua Setup

Figure A: Open (Flush) State

Note: solid blue lines indicate water flow, broken blue lines indicate no flow, black lines indicate electrical connections

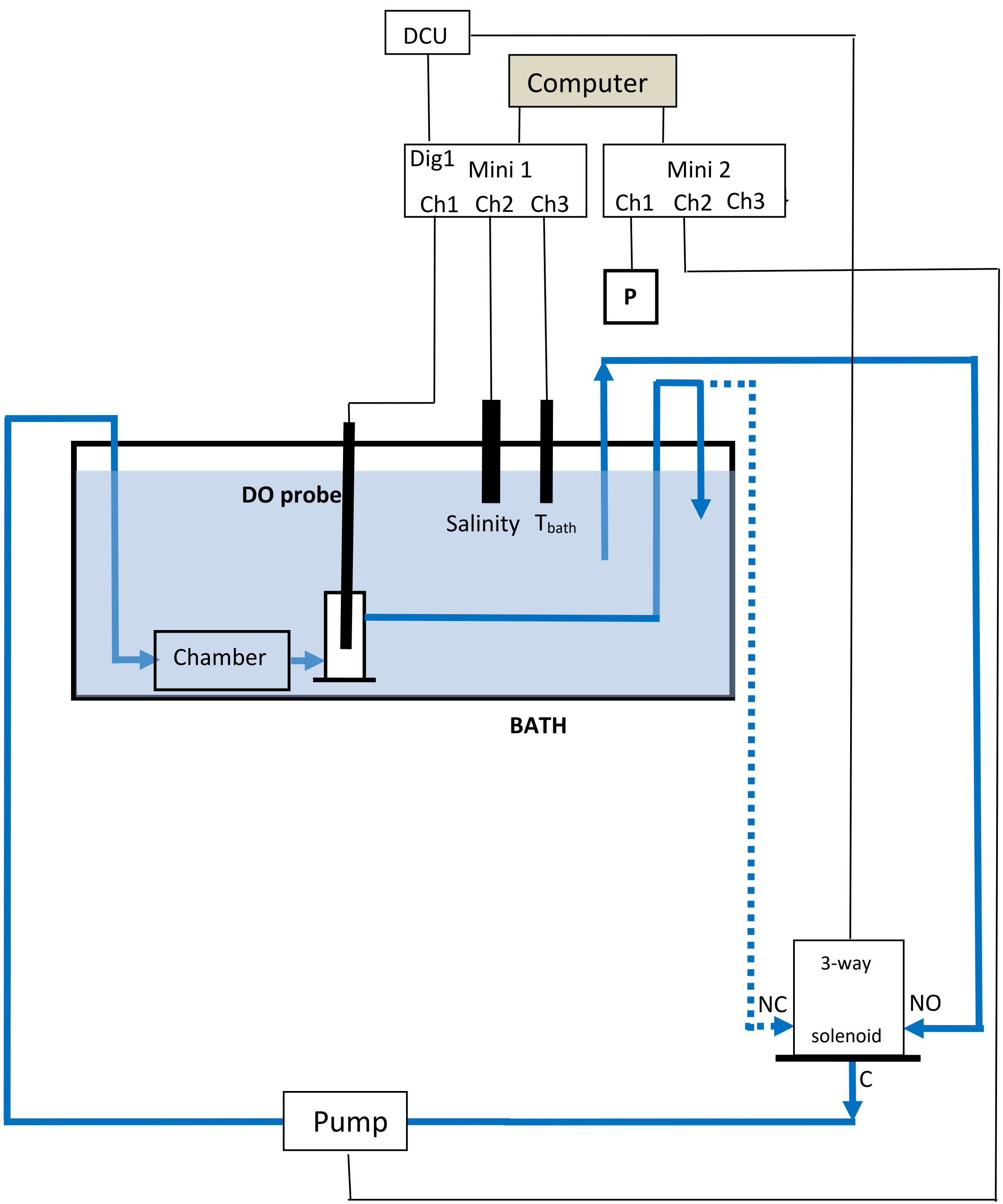
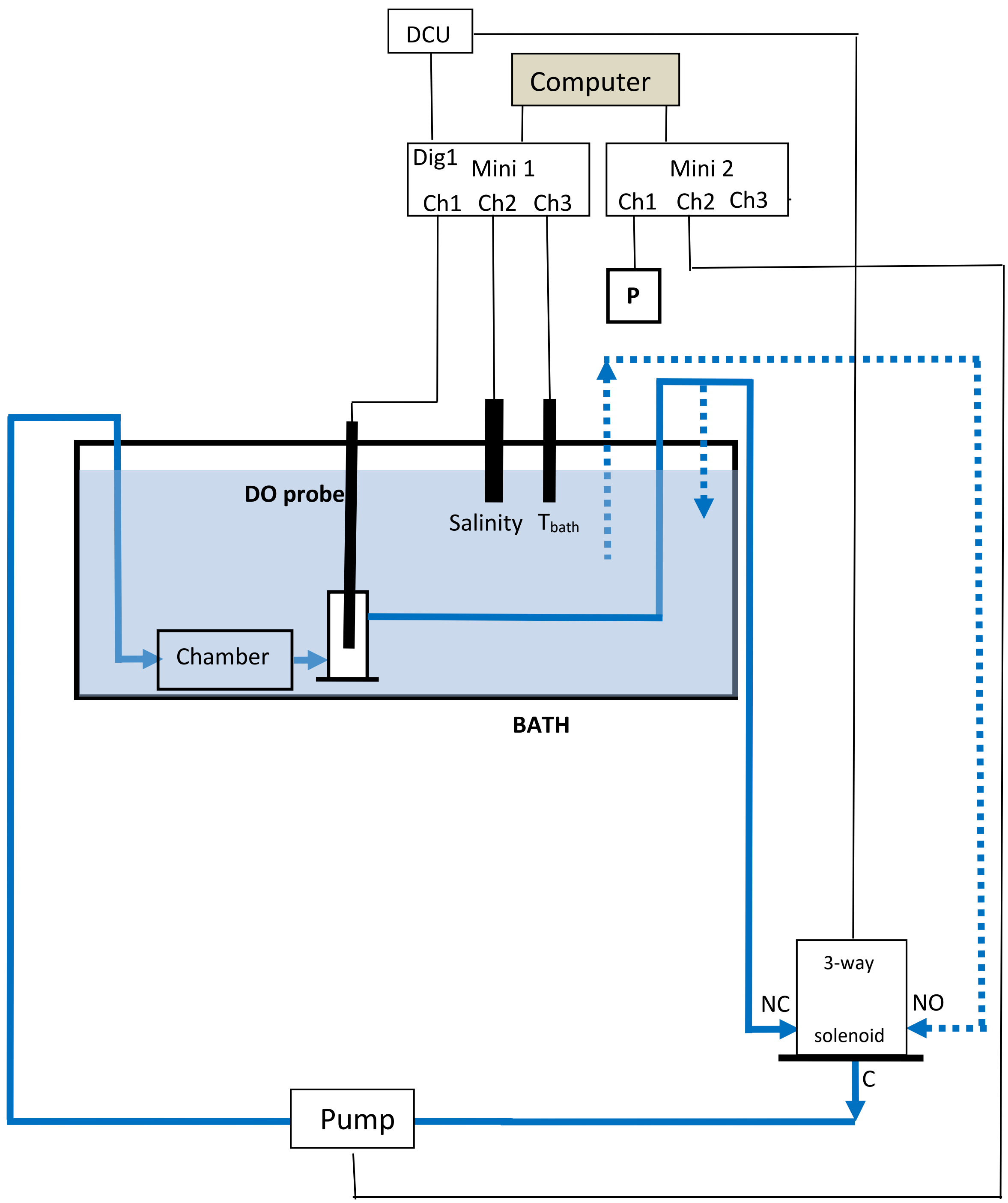
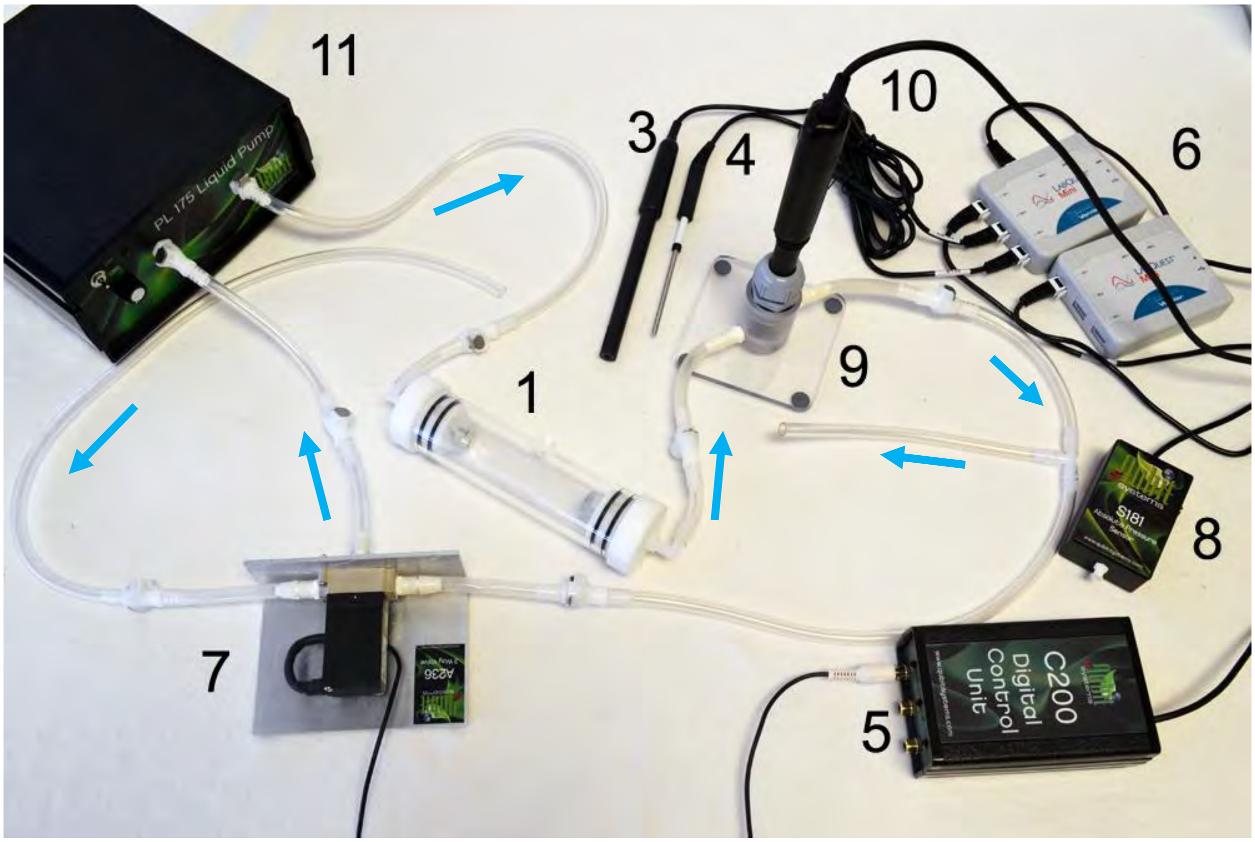


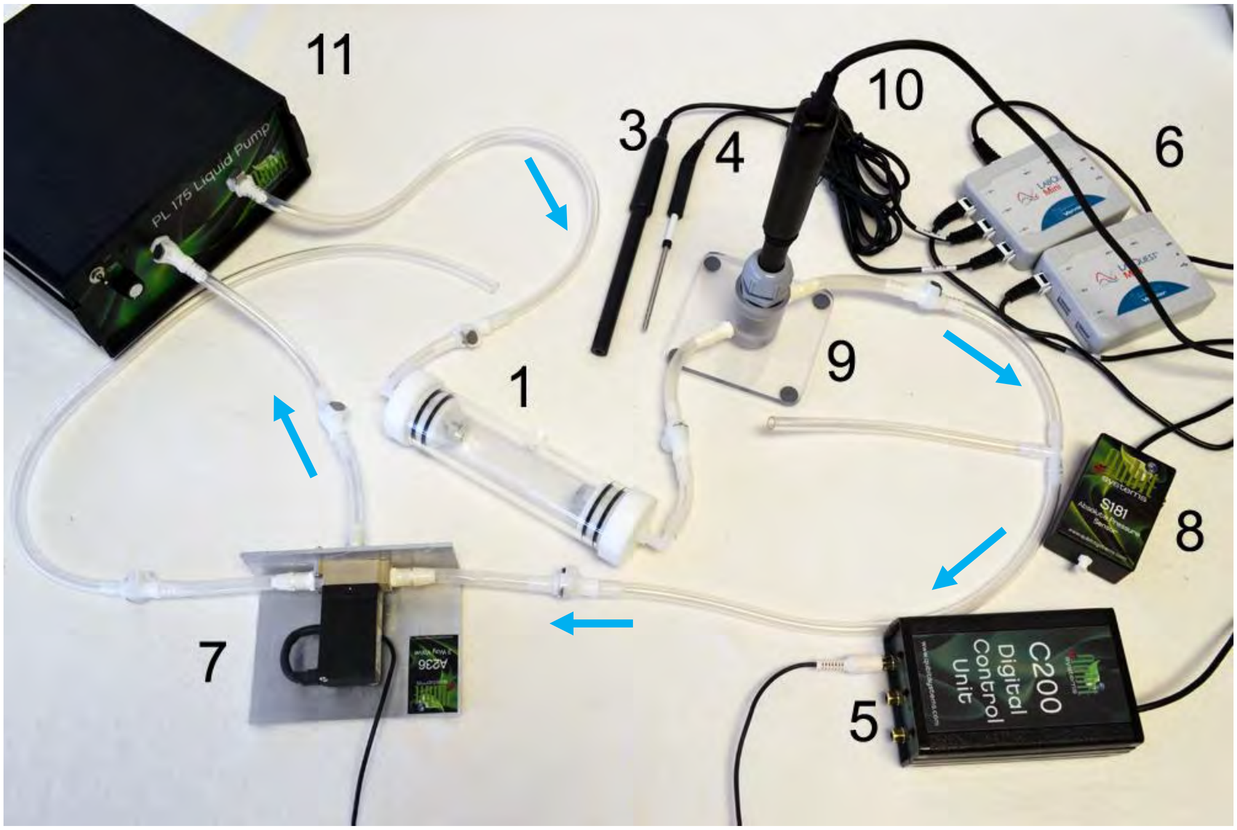
Figure B: Closed (Circulation) State



During the **flush** mode, intake of fresh water from the water bath, and output of water leaving the respirometer chamber and returning to the bath, is shown by blue arrows in the photo below.



In the photograph below, the direction of water flow during **circulation**, is shown by **blue** arrows.



Components of the Q-Box AQUA:

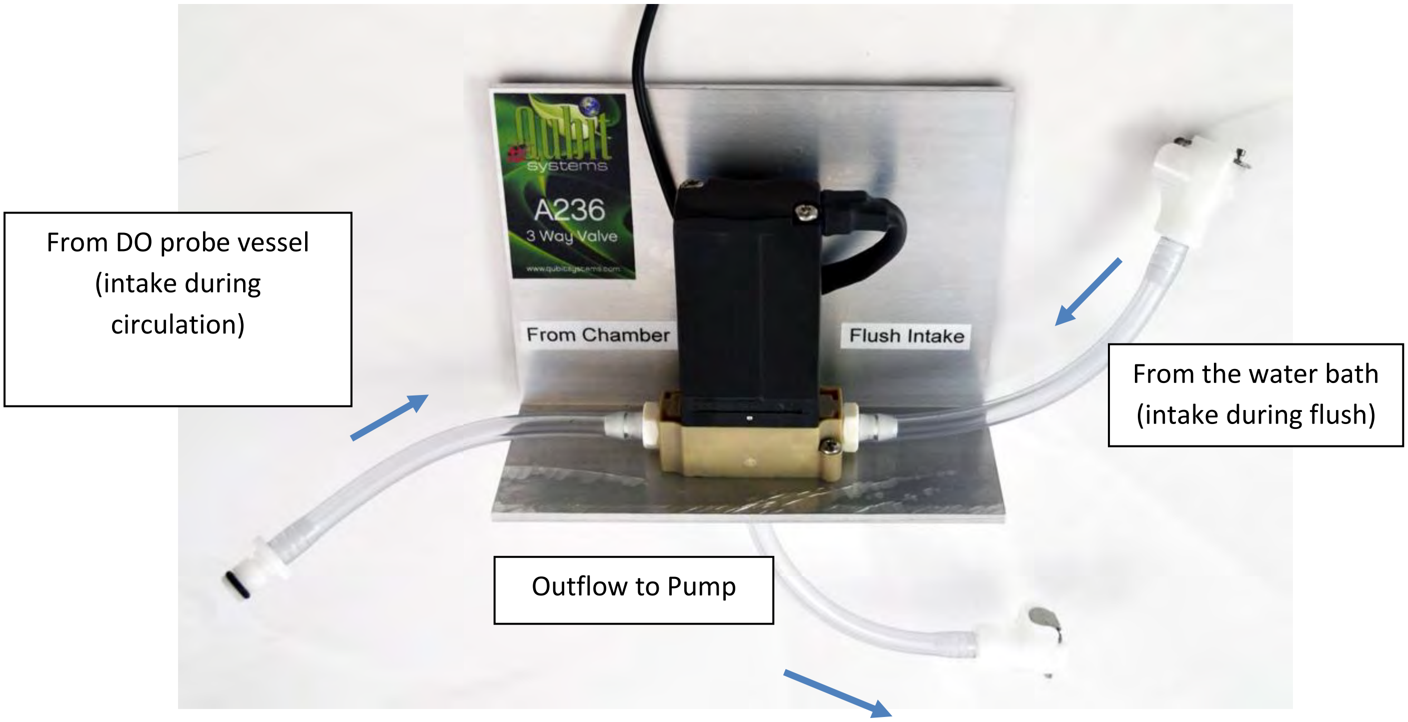
1. Small Respirometer chamber (G216: 3.84 cm ID, 15.3 cm long, vol. with end caps, 140 ml)
2. Large Respirometer chamber (G217: 8.2 cm ID, 16.7 cm long, vol. with end caps, 660 ml)
3. Salinity Probe for the water bath (S130)
4. Temperature probe for the water bath (S132)
5. Digital Control Unit (DCU) for control of 3-way valve (C200)
6. Data Acquisition Interface (C610 LabQuest Mini) x2
7. Three-way valve to switch between flush and circulation (A236)
8. Absolute Pressure Sensor (S181)
9. Flow-through vessel for DO Probe (A260)
10. Optical DO Probe (S122)
11. Liquid Pump (1 L/min max) (PL175) with analog output
12. Water filter
13. Q-Box AQUA Kit (tubing 1/4” ID, quick release connectors, extra filters for the chambers)
14. Logger Pro Data acquisition software (C901) and custom experimental files (C404)
15. Water Bath
16. Manual
17. Rugged Q-Box case for storage and transport of all components.

The Q-Box AQUA components are supplied in a rugged Q-Box case for easy storage and transport. Two layers of foam hold the components**. It is important to dry all wet components after each use before placing them back in the case.**

Start Up Steps:

1. The system is provided partially assembled with pre-cut tubing (1/4" ID) in place. Assemble the system as shown in the photo and diagram above (pages 5-8). If a different set up is required, cut and connect tubing between the components as shown in the above system diagram or as in the photograph. Keep in mind that the longer the tubing, the larger the volume of the system (and hence lower sensitivity) so ensure the tubing between the components is as short as the set up will allow. As supplied, the volume of the system **external to the chamber** (i.e. tubing, pump, 3-way valve and flow through vessel) is approximately 75 ml but it should be measured for each system for accurate number. When filling the water bath with water it is important to ensure that the temperature of the water is maintained as constant as possible and if it is significantly different from air temperature then any tubing external of the water bath that carries water between the pump, valve and the chamber is insulated to prevent heat transfer between the water and the atmosphere. Small changes in water temperature will result in significant changes in DO concentrations. Insulation of the water bath is also recommended. Maintaining the DO probe and the flow through vessel in the water bath during measurements ensure that the temperature of the water next to DO probe is maintained constant and the same as that in the respirometer.

The photo of the 3-way valve is shown below. Ensure that the tubing is connected as indicated. The intake from the water bath during flush mode is shown with blue arrows.



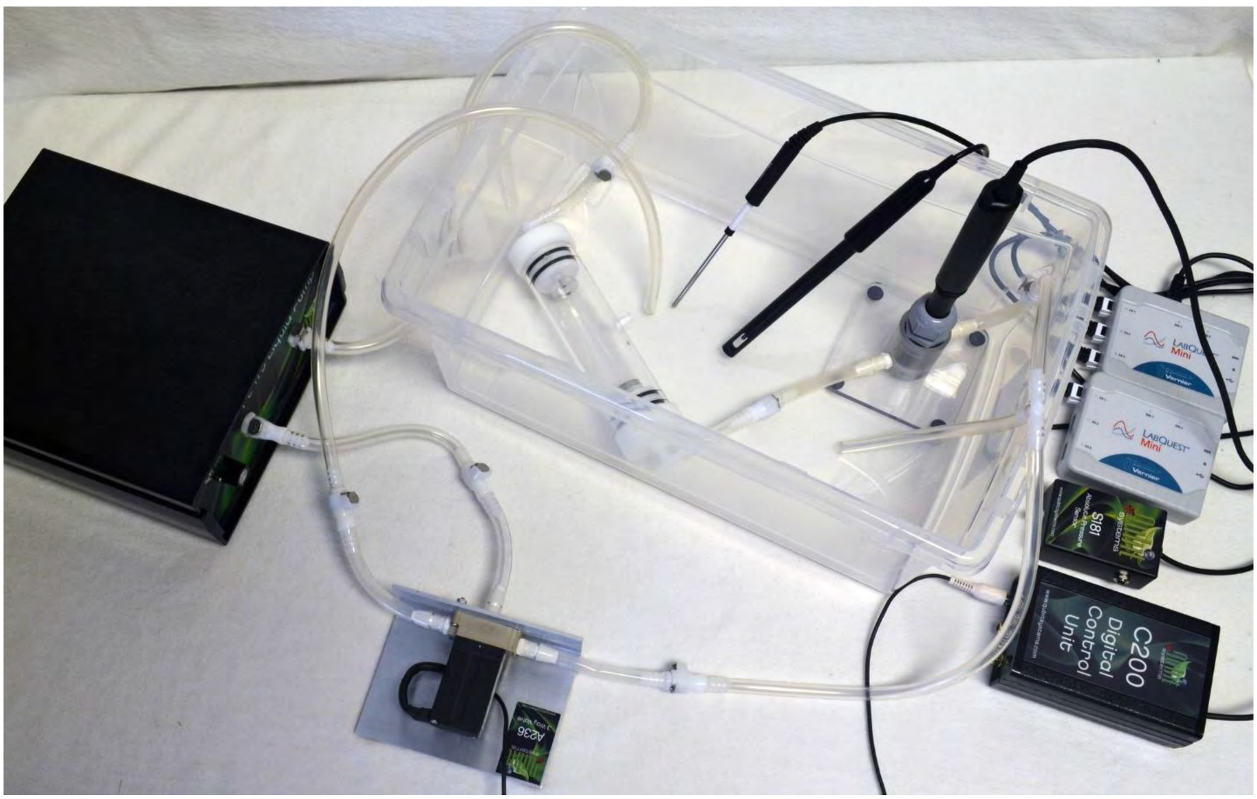
2. Connect the LabQuest Mini interfaces to the computer with the supplied USB cables.
3. Connect the DCU unit to the Dig 1 port on the LabQuest Mini 1 interface via the white BTA connector. Connect the 3-way valve (A236) cable to the DCU unit via the D1 input. Power the DCU unit via the power supply provided.

4. Place the DO probe in the flow-through vessel and connect the output cable to channel 1 of the LabQuest Mini 1 interface.

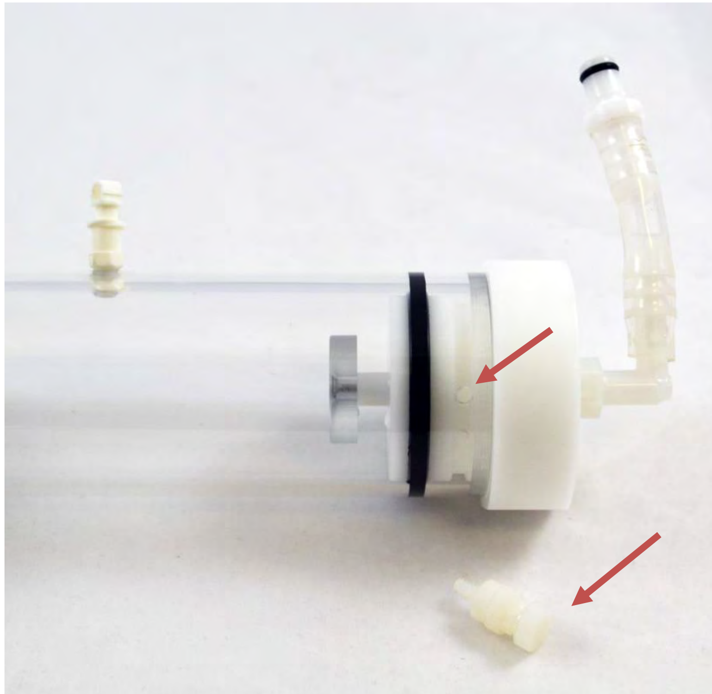
5. Ensure that the DO probe is placed in the flow through vessel deep enough so that the thermistor used for temperature compensation (silver dot), is submerged in the water flowing through the vessel (see photo below). The inlet flow past the bottom of the probe should prevent bubbles from collecting on the bottom, which would affect the DO readings. Any bubbles that collect at the top of the vessel may be voided by tipping the vessel so that the bubbles escape through the outlet at the top with stopper removed. **Ensure that the locking nut at the top of the vessel is tightly closed around the probe to prevent leaks.** Place the flow through vessel in the water bath so temperature of DO probe is the same as that of the chamber with animal.

6. Connect the Salinity probe (S130) to channel 2 of LabQuest Mini 1. The salinity probe is placed in the water bath to measure water salinity which is used in Logger Pro software to correct the measured DO for salt content. **Do not completely submerge** the sensor but ensure the electrodes are submerged.
7. Connect the Temperature Sensor (S132) to channel 3 of the LabQuest Mini 1 to measure water temperature of the bath. This is required for calibration. Place the sensor in the bath but **do not completely submerge** the sensor.
8. Connect the Pressure sensor (S181) to channel 1 of the LabQuest Mini 2 to measure atmospheric pressure and connect it to its power supply. Atmospheric pressure is required for calibration of the DO probe. **Do not place this sensor near water and do not submerge the sensor in water.**

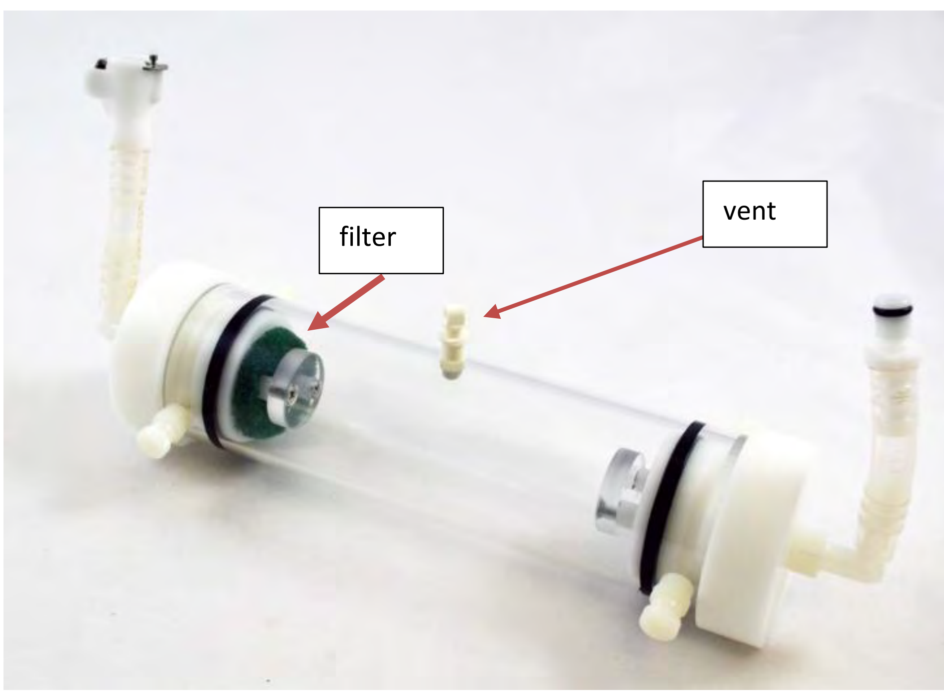
9. Connect the voltage output cable from the PL175 pump to channel 2 of LabQuest Mini 2
10. Once the system is set up, place the respirometer chamber and the DO probe inside the flow through vessel in the water bath. Fill the bath with air-saturated water to a depth at which the chambers are fully submerged. There should be enough water in the bath so that when the chamber is being manipulated during opening and closing of the end caps, air will not be introduced into the chamber. Air equilibrated water can be obtained by bubbling with an air stone or by leaving the water exposed to air for several hours. If necessary when using large fish with high O_2 consumption, full oxygen saturation of the water in the bath may need to be maintained by bubbling the water in the bath with air. The volume of water in the bath should be large enough to buffer any changes in water temperature. And to completely cover the aquatic chamber and the DO vessel. Fill the system with water by opening the chamber (pull off the inlet cap and disconnect the inlet tubing to avoid pulling a vacuum inside the system). The photo of the system with the water bath in place is shown below.



11. In both chambers, note the locking screws on either side of the end caps. These should be connected via small holes in the chamber tubing and lined up with the outer groove in the end cap and tightened to prevent the end caps from disengaging under pressure of water flow. **Ensure the screws fit into the grooves and do not press against the end cap outside of the groove as this will distort the chamber and result in a leak.**



12. Also note the presence of a circular filter at the outlet of the chamber. This is to prevent any debris from the animal entering the system and possibly plugging the 3-way valve or the liquid pump. Check this filter at the end of each experiment and clean it well. This filter may be replaced by unscrewing the end baffle first and removing the old filter. New filter (included in the accessories kit) may be attached.



13. Attach the liquid pump output to the animal chamber. Turn on the pump and set the flow of water through the respirometer during flush using the speed control on the pump. The flow can be set by timing the collection of a measured volume via the flush outlet (on the T-piece after the chamber). The maximum speed of the pump when connected to the system is approximately 1 L/min. When setting up the large respirometer chamber, start with low flow while getting rid of the air bubbles and filling the system with water. Then lock the end caps in place with the locking screws and increase the flow to the desired rate. A flow of about **300-400 ml/min** is optimal when using the large chamber. When using the small chamber, a flow of about **200-250ml/min** is optimal. The flow determines the "washout" time (and thereby time response) for the system. Normally an exchange of five system volumes is considered sufficient for washout or exchange of water during flushing. The liquid pump comes with analog output cable. This cable should be connected to channel 2 on LabQuest Mini 2 and the flow rate measured may be correlated to voltage output. Please note that the voltage output will differ with each setup.

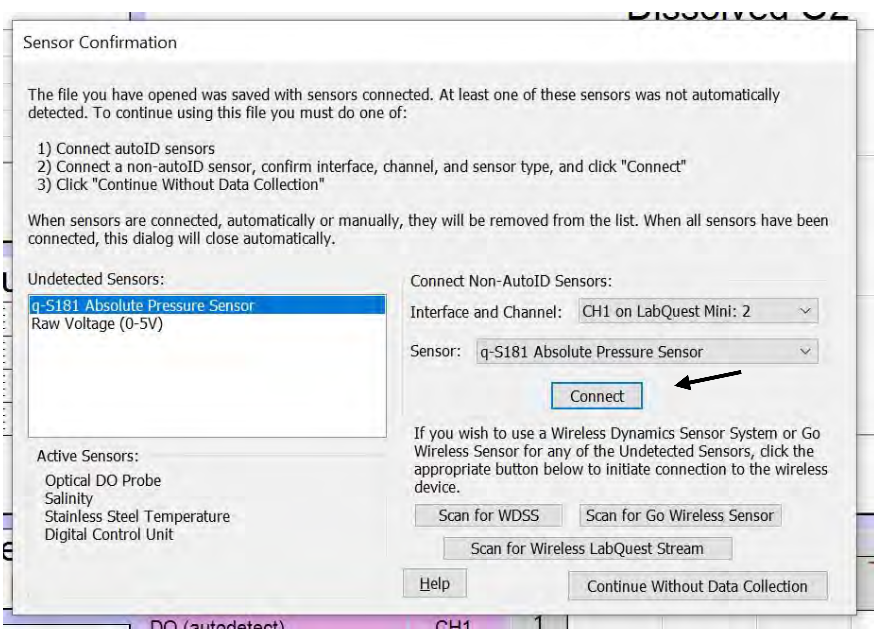
14. During set up, bubbles of air may need to be released from the closed system. First with the pump running, get rid of bubbles in the probe vessel by tipping the probe so the bubbles escape through the outlet. These bubbles will then flow through the system and collect in the chamber. Next remove the stopper from the vent on the top of the chamber (see photos above on pg. 13) and tip the chamber to release the bubbles. Ensure there are no bubbles trapped in the chamber, the DO probe vessel or tubing before starting experiments. Tipping the liquid pump upside down briefly and tapping it will also release any bubbles that may get trapped. Let the system run through a few Flush/Circulation cycles while collecting data and monitoring the DO levels. This data with an empty chamber will indicate DO stability and background levels of DO in the water.
15. After collecting data with an empty chamber, the software may be left running as a specimen is loaded into the chamber. **Ensure the stopper in the vent on top of the**

chamber is removed when opening and closing the chamber to avoid pressurizing the chamber and damaging the animal. Close the vent before collecting data with the animal.

16. The "active" volume of the respirometer during closed circulation is required for the VO2 calculation on Page 2 in Logger Pro. This volume is measured by filling the entire system with water during circulation. Then using the pump, this volume is emptied into a graduated cylinder. To completely drain the pump, tubing, and valves, disconnect the tubing and shake or blow out the water. The volume of water in the flush inlet and outlet tubes (see system diagram on page 5) should be subtracted, since the water in these tubes is not available to the animal during circulation. To calculate the volume of the 1/4" tubing use the 0.334ml/cm conversion. The total active volume of the respirometer is used in the calculations of VO2 on Page. 2 of the Experiment file and is entered on page 3 in the user parameters as "**RespirVol**".

17. **Load Logger Pro 3 software on the computer** and Load C404 customized files as per the instructions on pages 36 and 37 before DO data can be monitored.

18. Open the "**Q-Box AQUA Setup**" file to start Logger Pro software. The sensor confirmation window will appear.



19. Three sensors are auto-detected: Optical DO probe, salinity and temperature probes in analog input channels 1, 2 and 3, respectively of LabQuest Mini 1. Select "Qubit Systems q-S181 Absolute Pressure Sensor for channel 1 of LabQuest Mini 2 and raw voltage for Flow voltage output in channel 2 of LabQuest Mini2. A text box indicating the sensor connections to LabQuest Minis is included on Page 1 of the" Q-Box AQUA Setup" file.

Sensor Connections

Mini 1:

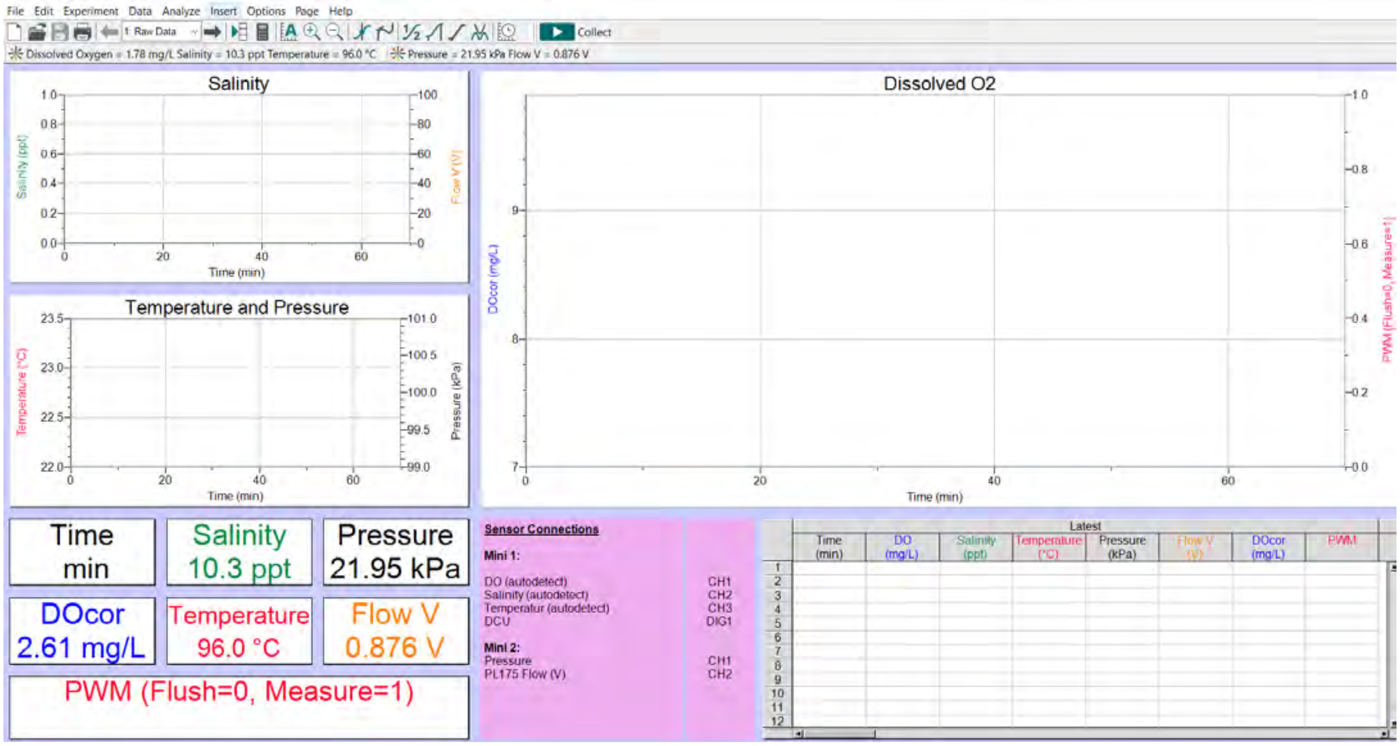
DO (autodetect)	CH1
Salinity (autodetect)	CH2
Temperatur (autodetect)	CH3
DCU	DIG1

Mini 2:

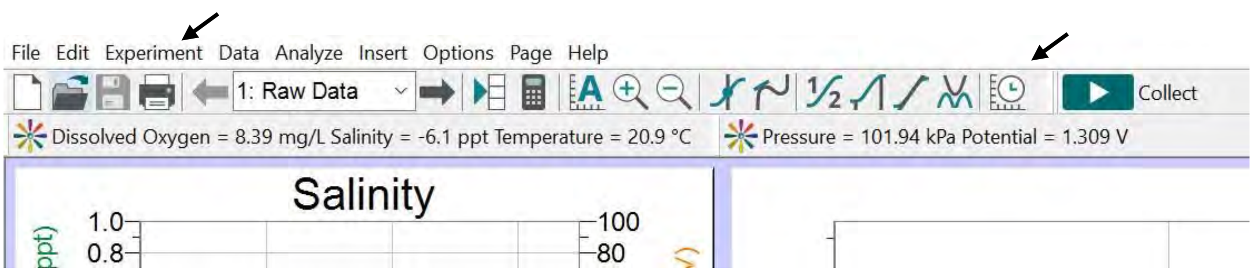
Pressure	CH1
PL175 Flow (V)	CH2

20. The optical DO probe is both temperature and atmospheric pressure compensated. It is supplied with factory calibration and it normally does not require re-calibration. However, if DO values do not appear to be correct or stable, a recalibration of the optical DO probe should be done. The calibration procedure is provided on page 29.

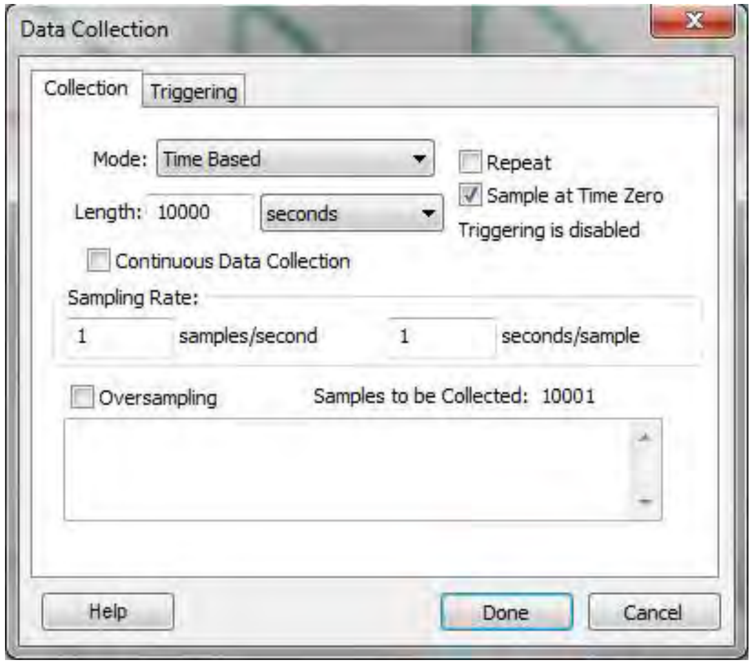
21. Once all sensors are connected the following initial screen will appear:



22. Before starting to collect data, set up the experiment length and sample rate by selecting ***Experiment>Data Collection*** in the main menu, or by clicking on the clock icon of the top menu.

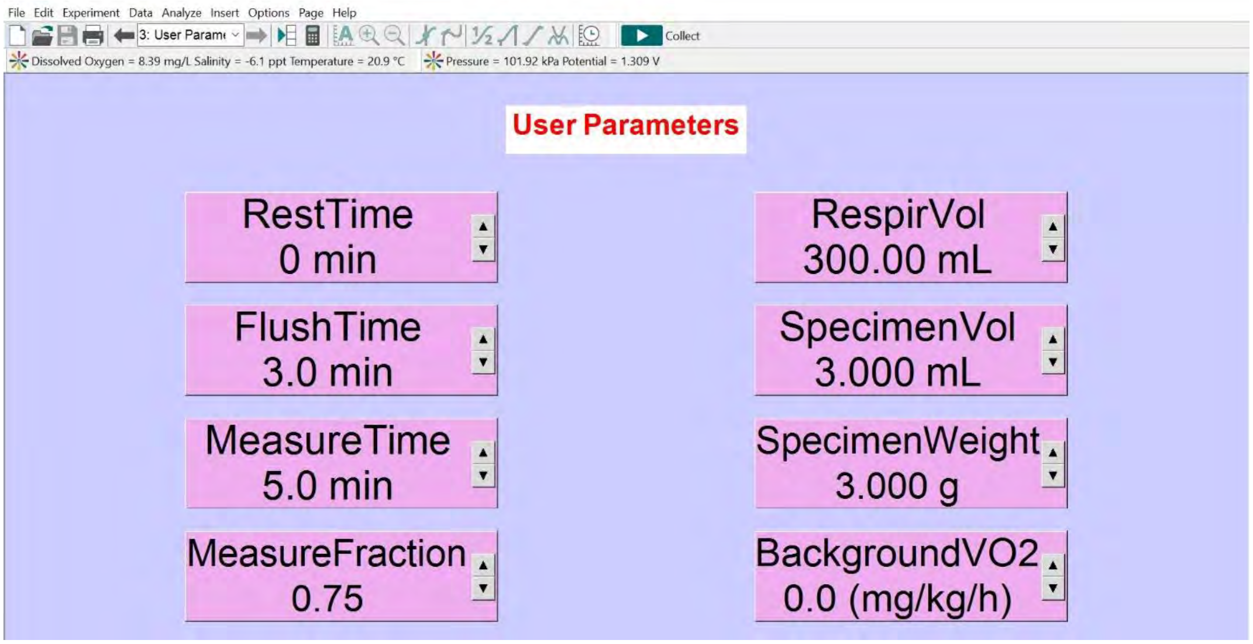


The following dialog box will appear:

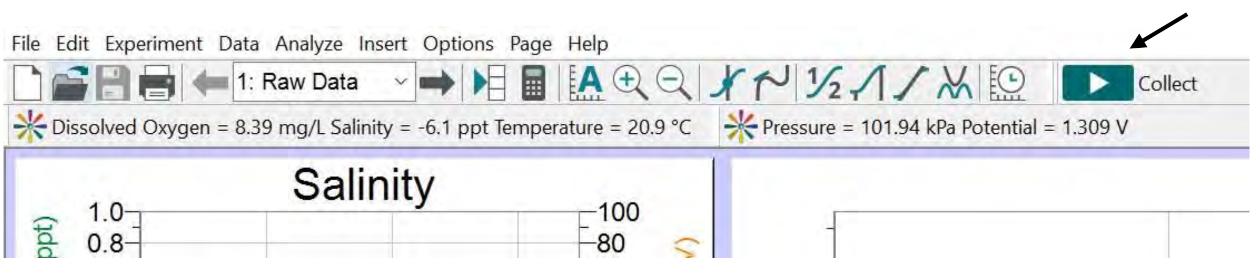


Input experiment length and data sampling rate as required. The default is 1 sample per second. Click "Done".

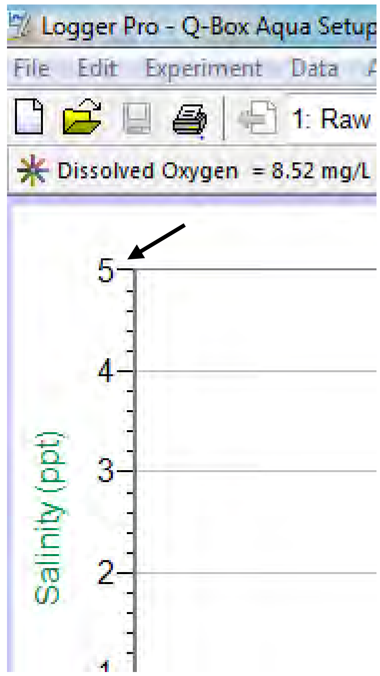
23. Set the length of the rest (initial acclimatisation), flush and circulation times on Page 3 of the "**Q-Box AQUA Setup**" file – in "User Parameters" as described below on page 24. The circulation time should be long enough to obtain sufficient decline in DO for an accurate slope determination. However, the decline in DO should not reach levels that can stress the animal. The flush time needs only to be long enough to restore the DO concentration in the chamber to the fully oxygenated level in the bath. The default is 5 min for circulation and 3 min for flush. The **MeasureFraction** user parameter sets the fraction of **MeasureTime** for calculations of the VO2 rate. This is set by default to 0.75 and should not be changed unless the data obtained during the **MeasureTime** (circulation) is in the latter portion of the period. **RespirVol, SpecimenVol** and **SpecimenWeight** user parameters also need to be set and these depend on the system that is used and the size of the animal. The user parameter **BackgroundVO2** should be set to 0 when initially collecting the data. This parameter allows for correction of the VO2 rates once data collection is completed if background decline in DO level was measured with an empty chamber before the animal was placed in it. Background slopes in DO are likely due to respiration from contaminants – bacteria, fungi, sample slime etc. Keeping the chamber and all the tubing clean is therefore important to minimize any contamination. The screen shot of page 3 – User Parameters of the experimental file is shown below.



24. Start data collection by clicking the green button "Collect" and stop data collection by clicking the red "Stop" button (green turns red during data collection).



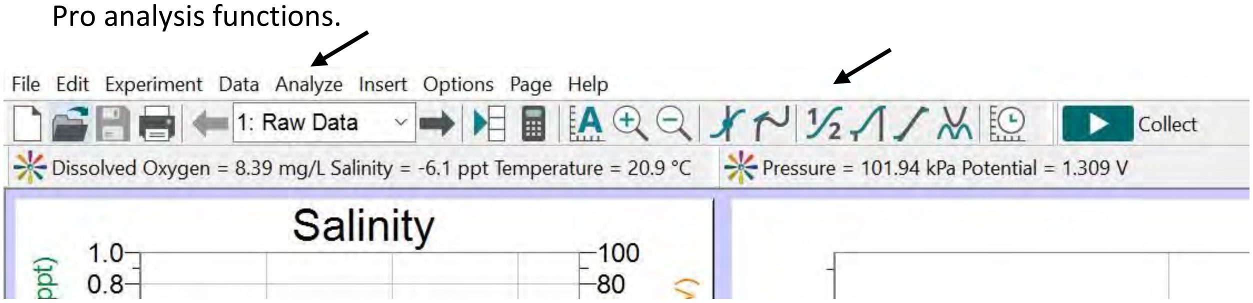
25. During an experiment, the range of x and y axes on graphs can be adjusted by clicking on the lowest or highest number and typing in the desired new value. Other graph options (auto scaling, strip charting, selection of variables plotted etc.) can be controlled by selecting the graph (double clicking on the graph) and selecting "Axes Options" menu.



26. Save the file under a new name using the commands ***File>Save As*** so as not overwrite the original "Q-Box Aqua Setup" file.

Logger Pro Data Analysis:

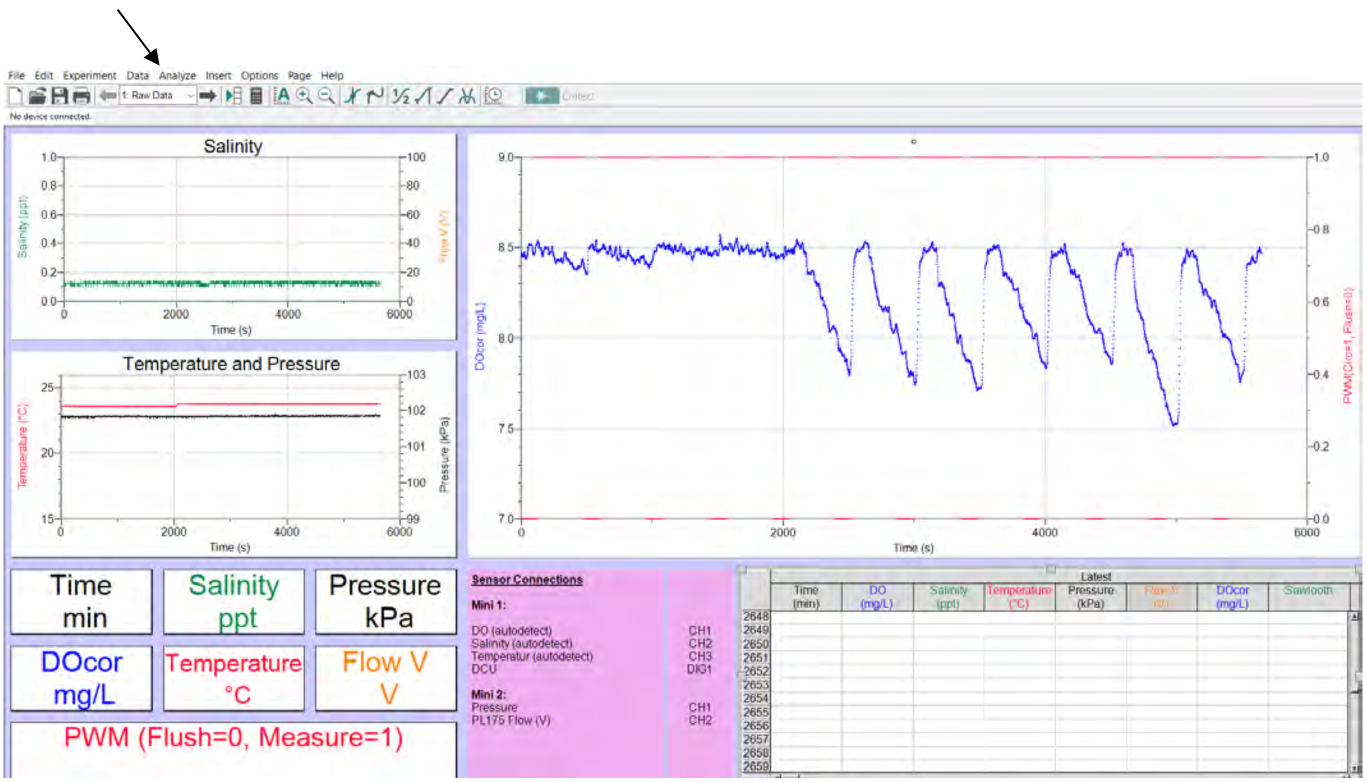
Upon completion of the experiment, the data can be analyzed in Logger Pro using various analysis tools (e.g. examine, slope, statistics, integral etc.) in the "Analyze" menu, or by clicking on the analysis icons in the top menu bar. To select the raw data for analysis, depress the left mouse button and drag the cursor across the data of interest in the graph. Select the analysis tool required. The Help menu provides extensive instructions for Logger Pro analysis functions.



The rate of respiration in mgO_2/L/sec for every circulation cycle is automatically calculated directly from the slope of the DO decline as recorded during circulation cycles and displayed in the sample data graph. The data can also be directly exported (***File>Export As>CSV***), and can then be opened in Excel for further analysis and calculations.

Sample Data:

Sample data from an experiment with a large goldfish (in a large respirometer chamber) are shown below.

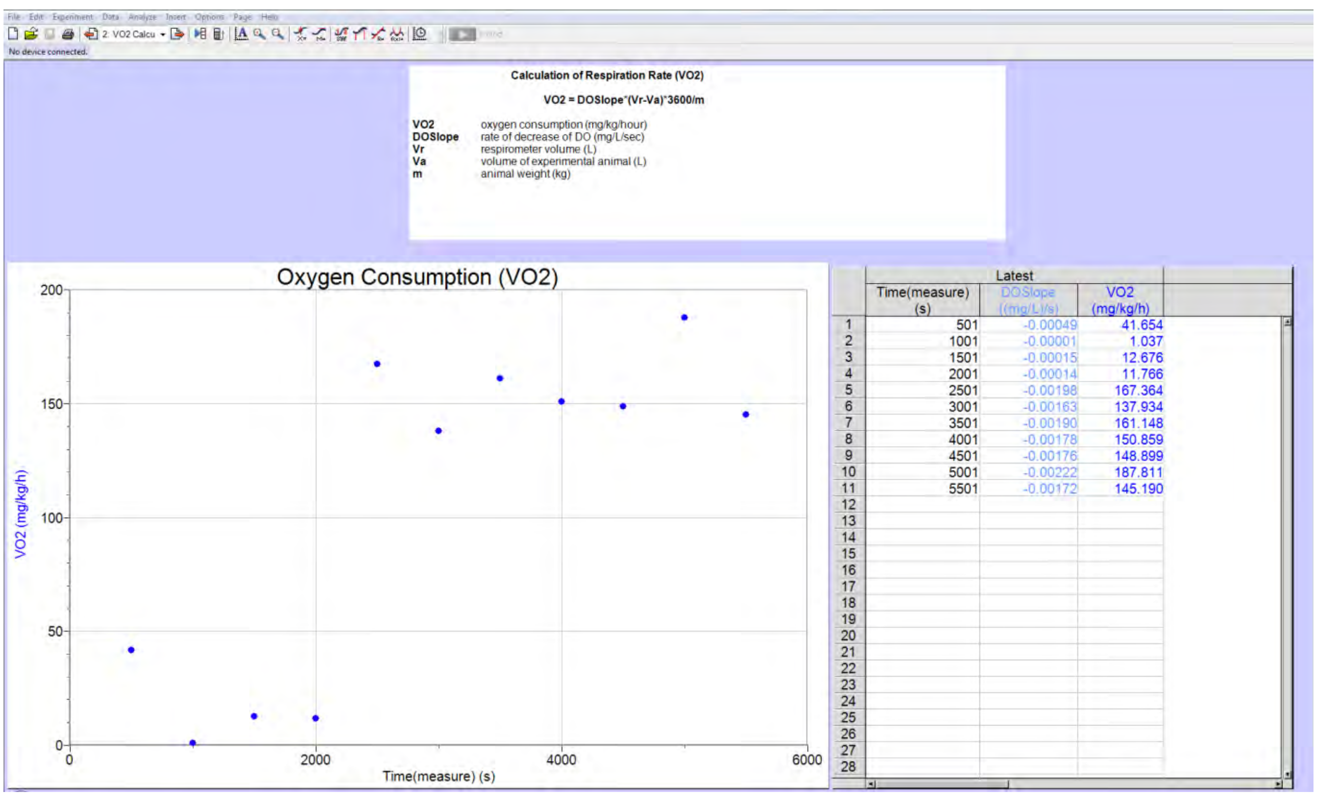


The Logger Pro "Q-Box AQUA Setup" file consists of 3 pages: 'Page 1 - Raw Data', 'Page 2 - VO2 Calculations', 'Page 3 - User Parameters'. A specific page selected by the "Page" command from the main menu or from the drop-down menu in the tool bar, as shown in the window above.

a. **Page 1** as shown above with the sample data is the 'Raw Data' page. These data are presented in a table, in graphs and in meters. Logger Pro is a very powerful and user friendly software, so the user can easily customize the graphs, meters or column calculations by double clicking on them and manipulating the available options or calculation equations. **It is highly recommended that any modifications to the Experiment File be saved under a new name to preserve the original unaltered file.** Select: ***File>Save As*** to save the experiment settings under a new file name.

The sample data from a large goldfish shown above was collected during successive recirculation and flush cycles. The time course of the flush and circulation phases is indicated by the red PWM (pulse width modulated) trace which has **a value of "0" during flush and a value of "1" during circulation.**

b. **Page 2** is the 'VO2 Calculations' page and it includes a table with calculations of oxygen consumption (VO_2) in mgO_2/kg/hr made automatically at the end of each closed circulation (i.e. measuring phase). The text box at the top of Page 2 explains the calculation of VO2 from the DOSlope. The slope for each circulation is calculated only during the latter 75% of the circulation phase (i.e. constant slope) has been reached after switching from flush (open) to circulation (closed). This fraction of the measurement time used in the slope calculation can be adjusted by the user via the "**MeasureFraction**" user parameter on Page 3 (see print screen of page 3 from the experimental file above on pg. 17 of the manual). This is only recommended if shorter period is required due to unstable or noisy data in the first part of the measurement time. Otherwise, the **MeasureFraction** parameter should be left at its default setting of 0.75.

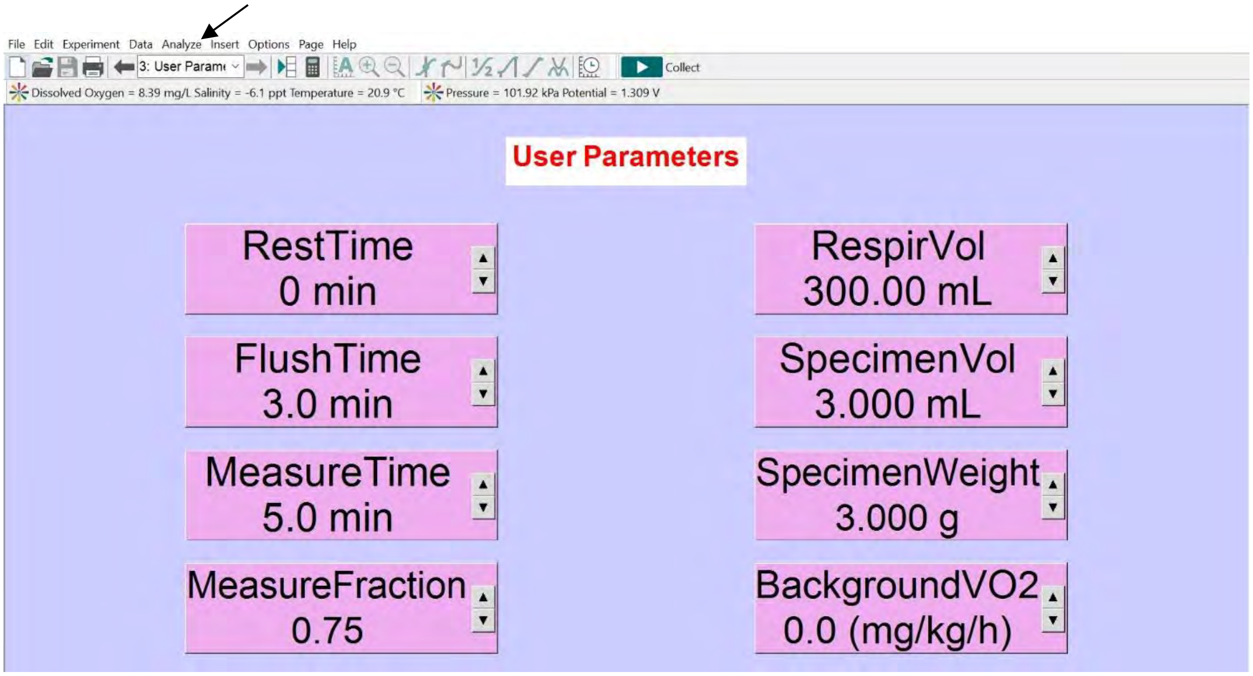


In addition to the DOSlope, the calculation needs the volume, **Vr,** (L) of the respirometer system which includes the chamber plus all of the other components such as tubing, flow-through vessel holding the DO probe, 3-way valve and liquid pump (see step #12 in **Start up Steps** on how to obtain the Respirometer volume). The Specimen volume, Va, (L) and specimen weight, m, (kg) are also needed. The volume of the specimen can be measured by placing the fish in a graduated cylinder with a known volume of water and measuring the displacement of that water. The weight of the specimen can be obtained by weighing the graduated cylinder with and without the fish or by calculating the weight from the fish volume assuming a density of 1g/ml. The respirometer volume, specimen volume and specimen weight are entered as user parameters on Page 3 (see below). The VO_2 in mgO_2/kg/hr is calculated automatically at the end of each circulation on Page 2 as:

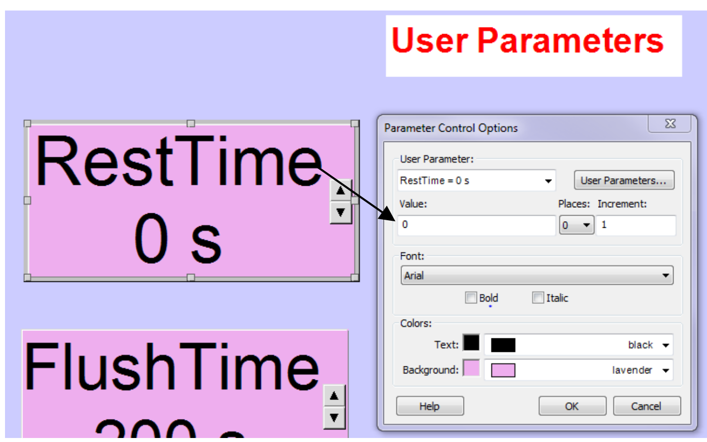
VO_2 = DOSlope*(Vr-Va)*60/m

VO_2=oxygen consumption (mg O_2/kg/hour)
DOSlope=rate of decrease of DO (mg O_2/L/min)
Vr=respirometer volume (L);
0.735 L – with large chamber, 0.215 L – with small chamber
m=animal mass (kg)

Page 3: The third page of the Logger Pro Experiment File shows “User Parameters”. This is where the user enters the length of time for the Rest – ***RestTime*** (initial adaptation period), Circulation – ***MeasureTime*** (measurement) and Flush – ***FlushTime*** (renewal) periods. Also the total respirometer volume – ***RespirVol***, as well as animal volume – ***SpecimenVol*** and weight – ***SpecimenWeight*** can be entered here. Finally, the fraction of the DO data obtained during circulation and used for slope calculation can be entered in the ***MeasureFraction*** parameter.



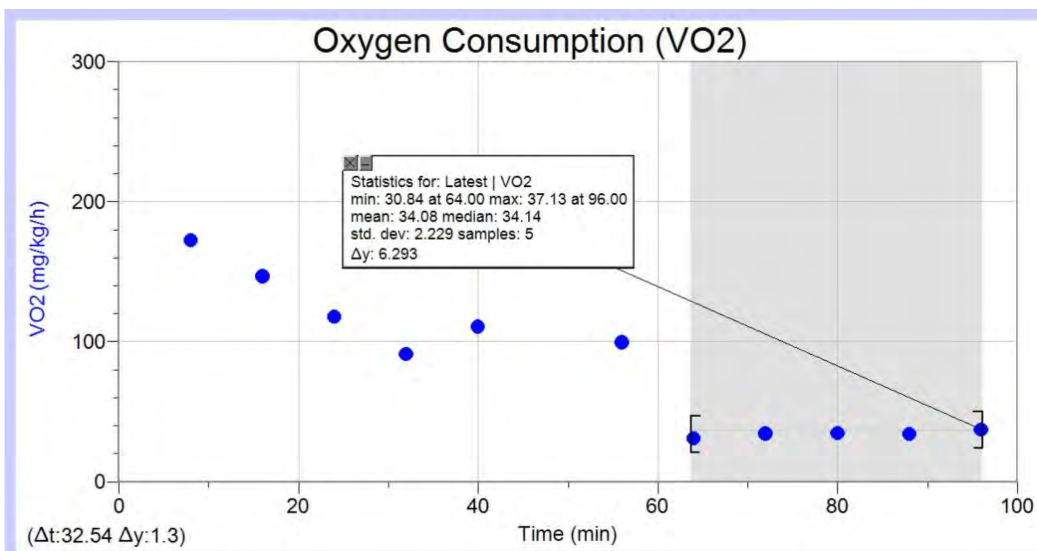
The value of a user parameter can be changed by toggling the up and down arrows in the meter, or by double clicking on the meter and inputting the required value as shown below.



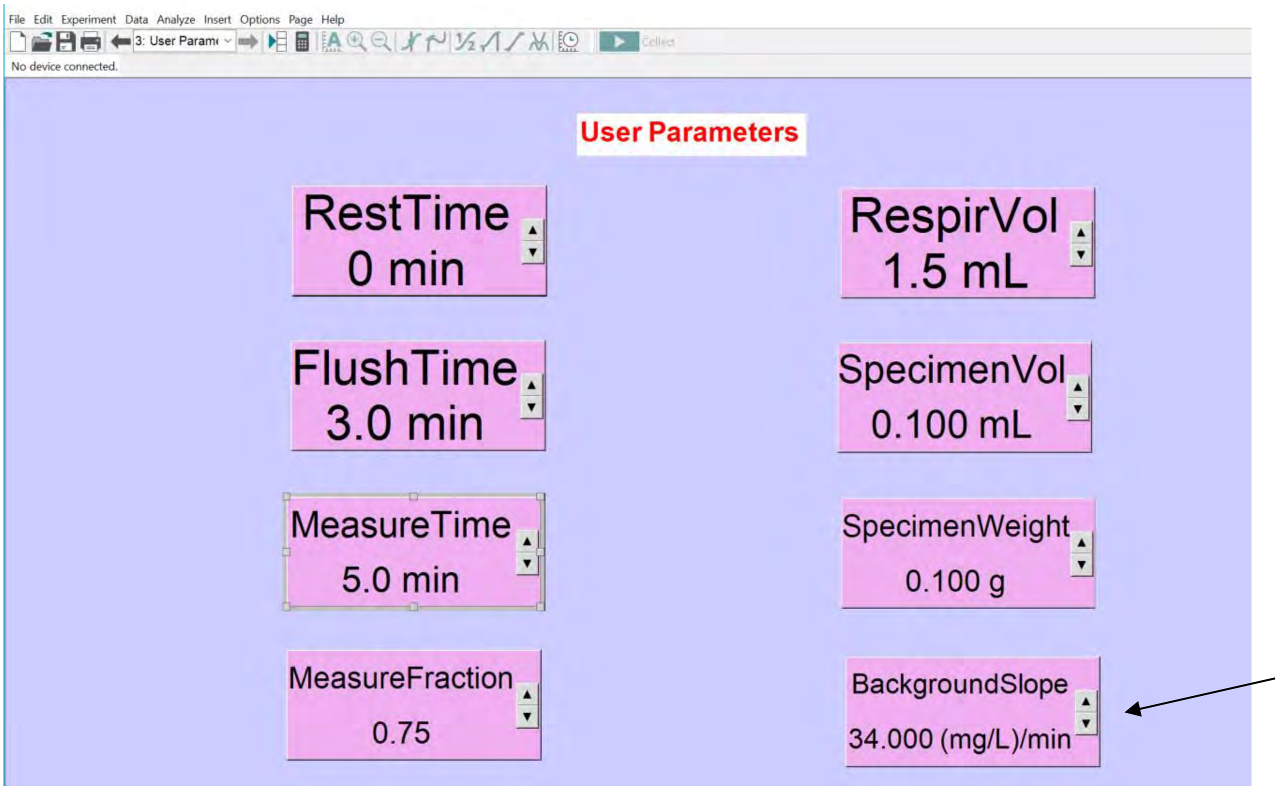
Default values are provided. Most of the User Parameter values should be entered before the start of the experiment and **not while the experiment is running**. **If user parameters are changed while an experiment is running this change will be applied to previously collected data**. The ***RespirVOl***, ***SpecimenVol***, ***SpecimenWeight*** can all be entered after the completion of the experiment and the VO2 data will be recalculated.

An additional User parameter ***BackgroundVO2*** is provided in the experimental file to correct the VO2 data for any background DO slopes that may be present when no specimen is inside the respirometer chamber. These background slopes are mainly due to contaminants either in the water or in the system often following the experiment and removal of the fish. It is therefore important to keep the system clean and the water clean. If the background slope is present it can be easily removed from the data obtained with the specimen present in the chamber by following these steps:

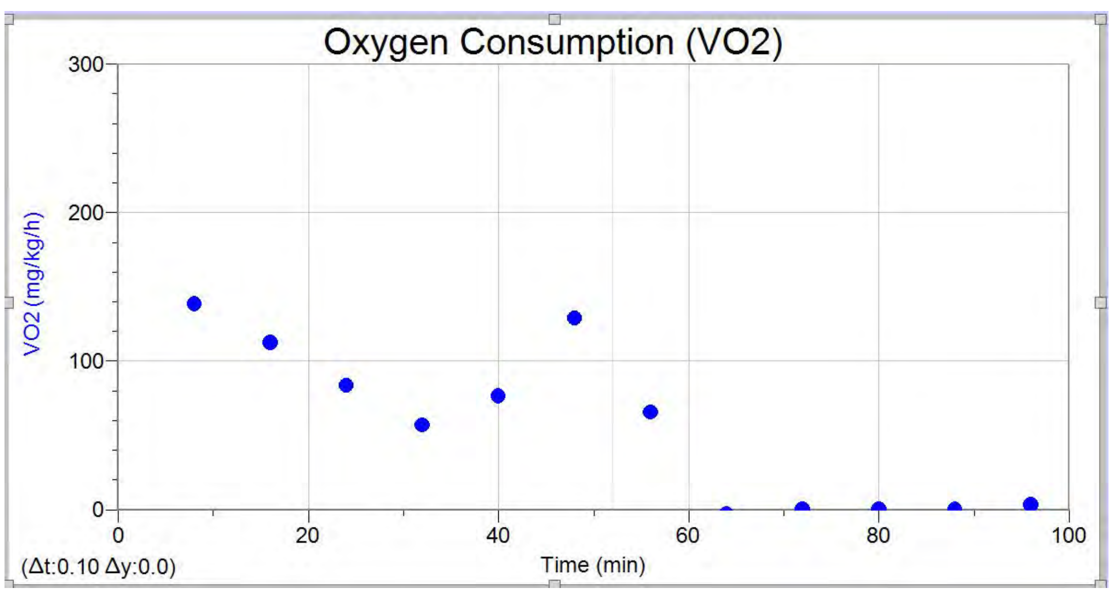
a. Set the BackgroundVO2 to zero before start of the experiment to determine the actual BackgroundVO2 from the collected data.
b. Proceed with the experiment and collect the data. If background slope in DO is observed, upon completion go to page 2 – VO2 calculations and determine the average background VO2 by highlighting with the mouse VO2 values when no specimen was present in the chamber (see image below) and click on the statistics icon in the top menu or select "Statistics" from "Analyze" drop down menu. The mean value for background DO is shown in the statistics window (34 in the example below).



c. Enter the value of background VO2 in the user parameter BacgroundVO2 of page 3 as shown below



d. Corrected VO2 data for background slope will be automatically displayed on page 2 in the VO2 graph:

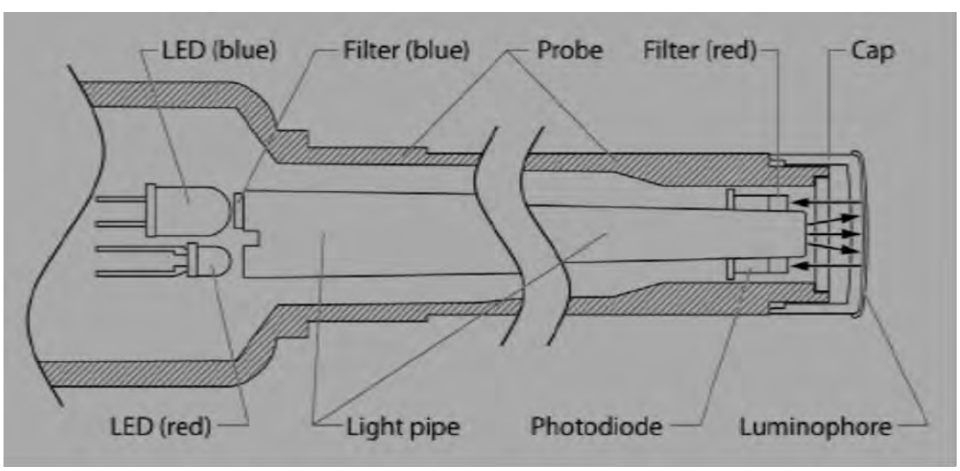


Optical DO Probe:

Principles of Use:

The Q-Box AQUA system employs an optical DO probe as shown in the following diagram. This type of DO probe is based on reversible quenching of both the intensity and lifetime of luminescence in a dye when oxygen is present. The probe cap encases the dye in a protective matrix which is permeable to oxygen diffusing from the water in which the probe is submersed. The intensity and lifetime are inversely related to the oxygen partial pressure in the water. This is a nonlinear relationship. Unlike Clark electrochemical sensors (both polarographic and galvanic) optical DO sensors do not consume oxygen and hence do not require liquid stirring. Also optical DO probes require much less frequent calibration than electrochemical sensors due to long term stability of the dye. They do not require filling with electrolyte solution and do not require warm-up like polarographic probes.

A blue LED excites the dye to a higher energy level. Collisions of oxygen molecules with the excited dye molecules transfer energy to the oxygen and cause the dye to emit longer wavelength red light. Although the luminescence intensity (i.e. red) is increased, a more stable signal is achieved by measuring the decrease in lifetime of the excited state in the dye caused by oxygen. Lifetime is not affected as much as intensity by bleaching or photo degradation of the dye over time. This lifetime is measured as the time between an excitation pulse from the blue LED and detection of the emitted red light by a photodiode. With more oxygen present, less time is required for the red light emission. Hence the lifetime decrease provides a measure of the **partial pressure of oxygen** in the water. Red LED flashes between the blue LED flashes, provide a reference reading.



A thermistor located in the DO probe automatically compensates for temperature changes, which affect both diffusion of oxygen through the cap as well as solubility of oxygen in water. The thermistor is the round silver metal spot near the probe tip, and it should be immersed to ensure correct sensing of the water temperature. The probe also contains a pressure sensor which compensates for pressure changes due to altitude, weather or water depth, since oxygen diffusion and solubility is also dependent on the partial pressure of oxygen. Consequently, the probe can be used at temperatures and pressures other that those during the original factory calibration or recalibration by the user.

The probe may be completely submerged to ~ 1.5 m (up to the box in the cable). An optional metal guard is available to protect the cap from damage and also to weigh down the buoyant probe for depth measurements.

Units of % (DO level relative to fully oxygenated water) or mg/L (absolute units of oxygen per liter of water) may be selected on the box in the cable. Please note that the experimental file is set up for use of the DO probe in mg/L units and a change to % saturation will require a new experimental setup.

The solubility of oxygen is dependent on temperature and pressure as shown in Table 1. Salinity also affects solubility as shown in Table 2. Solubility will be discussed further in the following Calibration section.

DO Probe Storage and Maintenance:

After use, dry the probe top with a tissue. Store the probe in the storage tube with a damp sponge in the bottom.

The probe cap containing the matrix and dye is replaceable. Avoid direct sunlight to prevent bleaching and reduced lifetime. Increased noise or a decrease in sensitivity indicates replacement is necessary. The caps are individually calibrated at the factory, therefore replacement caps are supplied with a microSD card containing the unique calibration code. To replace the cap and card:

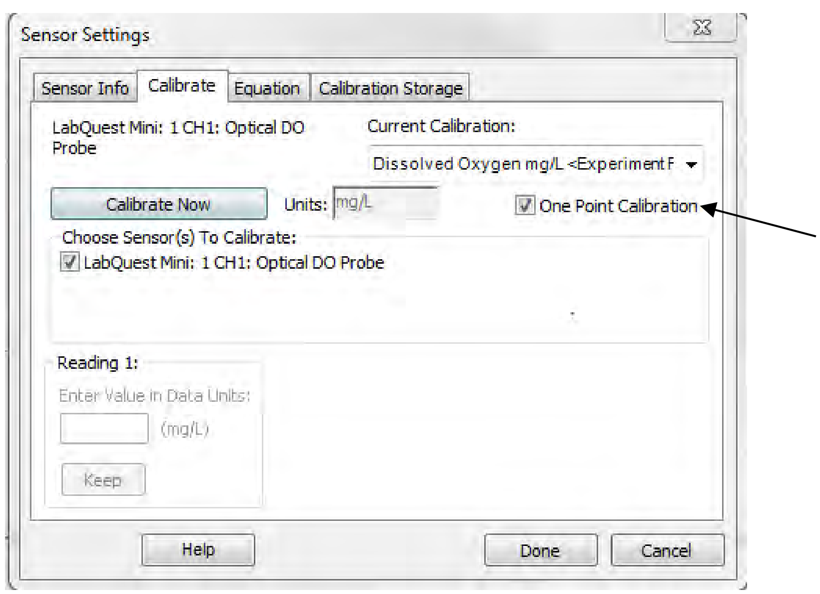
- disconnect the probe from the data interface
- remove the screw from the microSD cover on the box in the probe cable and replace the card
- unscrew the cap from the probe tip and replace with the new one
- *To reset the calibration:* set the units switch to %
- connect the probe to the LabQuest Mini and start Logger Pro
- add water to wet the sponge in the storage tube
- place the probe in the storage tube for at least 1 minute so the air becomes 100% saturated with water vapour
- press the reset button on the bottom of the microSD box for 3 seconds using a paper clip
- release the button - the reading should drop to 0%
- wait for the reading to change to 100% - this may take ~ 1 minute

DO Probe Calibration:

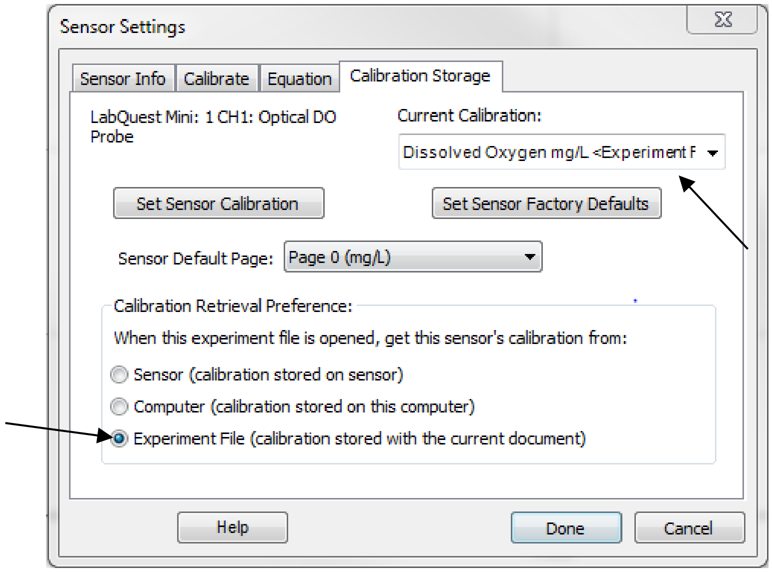
The optical DO probe does not require frequent calibration, however if necessary, a calibration procedure is provided as follows. Since the probe is nonlinear, it is calibrated at one known DO level (i.e. single point calibration) by using air-saturated water or, alternatively, water saturated air (100%). Water saturated with oxygen can be obtained by bubbling air through water, or by leaving water exposed to air for several hours. A more rapid method is to use air saturated with water vapour. This is obtained by wetting the sponge in the protective probe storage holder, inserting the probe in the holder and waiting ~ 1 minute. The calibration units can be mgO_2/L, or % air saturation, depending on the switch position on the box in the probe cable. Since the DO electrode is compensated for temperature, and pressure, the calibration performed in the lab (or at the factory) will be valid at other temperatures and pressures. The probe is not internally compensated for salinity. Therefore, salinity measurements provided by the salinity probe (S130) are used to correct the DO values in Logger Pro - see Salinity Correction for Measured DO on page 30.

DO probe calibration steps:

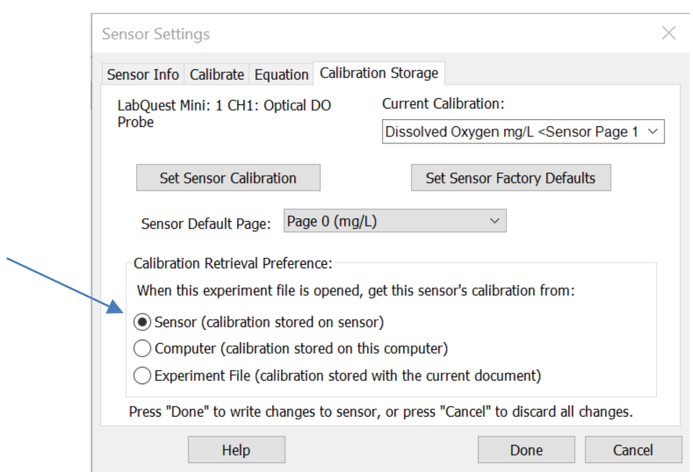
1. Load the “Q-Box AQUA Setup” file and ensure that the Dissolved Oxygen Probe is plugged into channel 1 of the LabQuest Mini 1 Interface.
2. A one-point calibration is initiated by the LoggerPro commands: ***Experiment>Calibrate>LabQuest Mini: 1 CH1: Optical DO Probe***. The following dialog box will open.
3. Check the box for "one point" calibration.



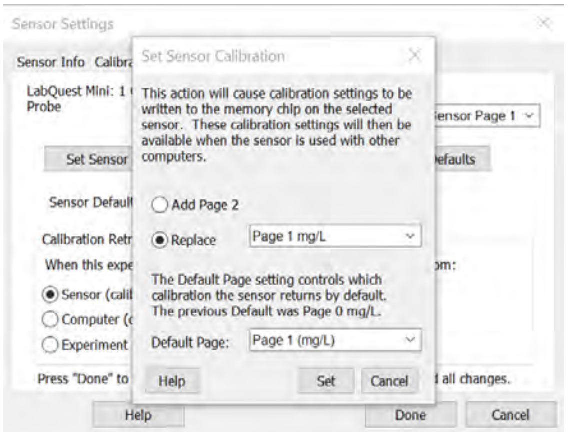
4. Ensure that the calibration storage is selected with the “Experiment file” if calibration is to be stored with the experiment

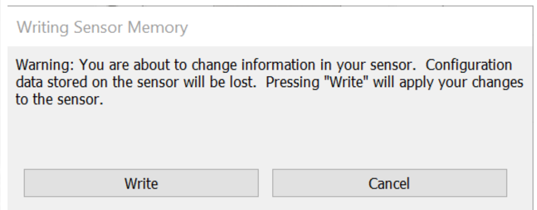


5. Alternatively, the new calibration may be stored on the sensor once it has been completed (see below).
6. Select ***Calibrate>Calibrate Now*** as shown in the above Sensor Settings window. The voltage output from the DO probe will be shown.
7. Place the probe in fully oxygenated water or in the storage tube with a wet sponge.
8. The voltage output from the DO electrode should stabilize at ~ 2 V. Enter as 'Reading 1', the appropriate value for DO of air-saturated water, at the temperature of the water bath measured by the S132 temperature probe, and at the atmospheric pressure measured by the S181 absolute pressure sensor. This value for DO in absolute units of mg/L is obtained from Table 1 below. Then click on 'Keep'. For example, at 20 °C and 760mmHg (101.325 kPa) this concentration is 9.17 mgO_2/L, as shown in Table 1. Data from Table 1 is plotted in Figure 1 indicating the temperature dependence of DO at a constant pressure (more oxygen dissolves in water open to the air as the temperature decreases).
9. Select ***Equation>Options*** from the Sensor Settings window menu and record the Slope and Intercept values for future reference.
10. If storing the calibration on the sensor, select in the calibration storage as "sensor" and press "Done"



11. A window will appear where the user selects replacement of the previous calibration and confirms to apply the changes.





12. When calibration is complete, click ‘Done’. The DO electrode is now calibrated. Save the file under a new name if you wish to save the calibration with the experimental file.

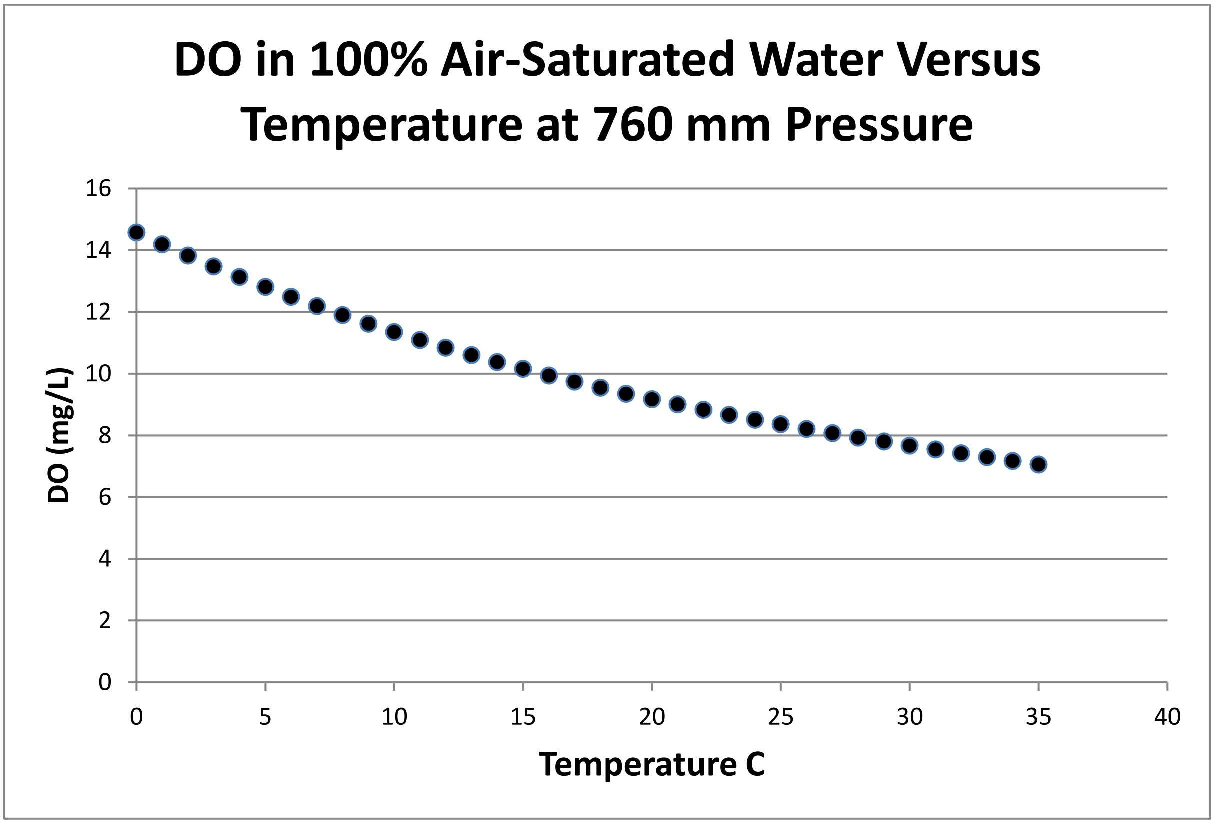
Note: Since the probe is temperature and atmospheric pressure compensated internally, it can be used at temperature and pressures different from those present during calibration.

Please note that temperature has a large effect on the solubility of O2 and therefore Do concentration in the water. As a result, it is important to maintain the temperature constant during experiments.

Table 1: Dissolved O_2 (mg/L) in air-saturated distilled water (at various temperatures (^oC) and atmospheric pressures (mmHg/kPa))

	770/ 102.658	760/ 101.325	750/ 99.992	740/ 98.659	730/ 97.325	720/ 95.992	710/ 94.659	700/ 93.326	690/ 91.992	680/ 90.659	670/ 89.326	660/ 87.993	650/ 86.659
0ºC	14.76	14.57	14.38	14.19	13.99	13.80	13.61	13.42	13.23	13.04	12.84	12.65	12.46
1ºC	14.38	14.19	14.00	13.82	13.63	13.44	13.26	13.07	12.88	12.70	12.51	12.32	12.14
2ºC	14.01	13.82	13.64	13.46	13.28	13.10	12.92	12.73	12.55	12.37	12.19	12.01	11.82
3ºC	13.65	13.47	13.29	13.12	12.94	12.76	12.59	12.41	12.23	12.05	11.88	11.70	11.52
4ºC	13.31	13.13	12.96	12.79	12.61	12.44	12.27	12.10	11.92	11.75	11.58	11.40	11.23
5ºC	12.97	12.81	12.64	12.47	12.30	12.13	11.96	11.80	11.63	11.46	11.29	11.12	10.95
6ºC	12.66	12.49	12.33	12.16	12.00	11.83	11.67	11.51	11.34	11.18	11.01	10.85	10.68
7ºC	12.35	12.19	12.03	11.87	11.71	11.55	11.39	11.23	11.07	10.91	10.75	10.59	10.42
8ºC	12.05	11.90	11.74	11.58	11.43	11.27	11.11	10.96	10.80	10.65	10.49	10.33	10.18
9ºC	11.77	11.62	11.46	11.31	11.16	11.01	10.85	10.70	10.55	10.39	10.24	10.09	9.94
10ºC	11.50	11.35	11.20	11.05	10.90	10.75	10.60	10.45	10.30	10.15	10.00	9.86	9.71
11ºC	11.24	11.09	10.94	10.80	10.65	10.51	10.36	10.21	10.07	9.92	9.78	9.63	9.48
12ºC	10.98	10.84	10.70	10.56	10.41	10.27	10.13	9.99	9.84	9.70	9.56	9.41	9.27
13ºC	10.74	10.60	10.46	10.32	10.18	10.04	9.90	9.77	9.63	9.49	9.35	9.21	9.07
14ºC	10.51	10.37	10.24	10.10	9.96	9.83	9.69	9.55	9.42	9.28	9.14	9.01	8.87
15ºC	10.29	10.15	10.02	9.88	9.75	9.62	9.48	9.35	9.22	9.08	8.95	8.82	8.68
16ºC	10.07	9.94	9.81	9.68	9.55	9.42	9.29	9.15	9.02	8.89	8.76	8.63	8.50
17ºC	9.86	9.74	9.61	9.48	9.35	9.22	9.10	8.97	8.84	8.71	8.58	8.45	8.33
18ºC	9.67	9.54	9.41	9.29	9.16	9.04	8.91	8.79	8.66	8.54	8.41	8.28	8.16
19ºC	9.47	9.35	9.23	9.11	8.98	8.86	8.74	8.61	8.49	8.37	8.24	8.12	8.00
20ºC	9.29	9.17	9.05	8.93	8.81	8.69	8.57	8.45	8.33	8.20	8.08	7.96	7.84
21ºC	9.11	9.00	8.88	8.76	8.64	8.52	8.40	8.28	8.17	8.05	7.93	7.81	7.69
22ºC	8.94	8.83	8.71	8.59	8.48	8.36	8.25	8.13	8.01	7.90	7.78	7.67	7.55
23ºC	8.78	8.66	8.55	8.44	8.32	8.21	8.09	7.98	7.87	7.75	7.64	7.52	7.41
24ºC	8.62	8.51	8.40	8.28	8.17	8.06	7.95	7.84	7.72	7.61	7.50	7.39	7.28
25ºC	8.47	8.36	8.25	8.14	8.03	7.92	7.81	7.70	7.59	7.48	7.37	7.26	7.15
26ºC	8.32	8.21	8.10	7.99	7.89	7.78	7.67	7.56	7.45	7.35	7.24	7.13	7.02
27ºC	8.17	8.07	7.96	7.86	7.75	7.64	7.54	7.43	7.33	7.22	7.11	7.01	6.90
28ºC	8.04	7.93	7.83	7.72	7.62	7.51	7.41	7.30	7.20	7.10	6.99	6.89	6.78
29ºC	7.90	7.80	7.69	7.59	7.49	7.39	7.28	7.18	7.08	6.98	6.87	6.77	6.67
30ºC	7.77	7.67	7.57	7.47	7.36	7.26	7.16	7.06	6.96	6.86	6.76	6.66	6.56
31ºC	7.64	7.54	7.44	7.34	7.24	7.14	7.04	6.94	6.85	6.75	6.65	6.55	6.45
32ºC	7.51	7.42	7.32	7.22	7.12	7.03	6.93	6.83	6.73	6.63	6.54	6.44	6.34
33ºC	7.39	7.29	7.20	7.10	7.01	6.91	6.81	6.72	6.62	6.53	6.43	6.33	6.24
34ºC	7.27	7.17	7.08	6.98	6.89	6.80	6.70	6.61	6.51	6.42	6.32	6.23	6.13
35ºC	7.15	7.05	6.96	6.87	6.78	6.68	6.59	6.50	6.40	6.31	6.22	6.13	6.03

Figure 1: Temperature Dependence of DO in Water at 760 mm Pressure



Salinity Correction for Measured DO

The calculations on Page 2 of the "Q-Box AQUA Setup" file are for DO in mg(O_2)/L(H_2O). Calibration in these absolute units (mg/L) requires consideration of temperature, pressure and salinity. As discussed above, when calibrating the dissolved oxygen probe in units of mg/L use the dissolved oxygen concentration in Table 1 that corresponds to the air-saturated value at the temperature and pressure during calibration. The temperature readings are obtained from the temperature probe (S132) and air pressure readings are obtained from the absolute pressure sensor (S181). Since the DO probe includes internal temperature and pressure compensation, once it is calibrated, a change in temperature or pressure is automatically accounted for when the units setting on the probe is absolute (i.e. mg/L).

Salinity significantly affects the solubility of oxygen in water. Seawater has a salinity of ~ 35 ppt, and at 20°C and 760 mm pressure, has ~ 19% lower DO compared to freshwater at the same temperature and pressure. The probe does not internally compensate for salinity, hence, the salinity correction to the DO measured by the optical DO probe is corrected in Logger Pro software using the salinity measured by the S130 salinity probe.

Dissolved O_2 concentration for air-saturated water at various salt concentrations, DOcor, can be calculated using the formula:

$$DOcor = DO-k*S \qquad (1)$$

Where, DOcor is the corrected concentration of dissolved oxygen (mg/L) in salt water solutions, DO is the dissolved oxygen concentration measured by the optical probe (T and P compensated), *S* is the salt concentration (ppt), measured by the S180 salinity probe and *k* (1/ppt) is a constant. The value of *k* varies according to the water temperature as shown in Table 2:

Note: At salinity levels less than 1 ppt, neglecting this salinity correction results in an error of less than 1 %.

Table 2: Salinity Correction Constant at Different Temperatures

Temp (°C)	K (1/ppt)	Temp (°C)	K (1/ppt)	Temp (°C)	K (1/ppt)
1	0.08796	11	0.06286	21	0.04854
2	0.08485	12	0.06104	22	0.04754
3	0.08184	13	0.05931	23	0.04662
4	0.07911	14	0.05757	24	0.0458
5	0.07646	15	0.05602	25	0.04498
6	0.07391	16	0.05456	26	0.04425
7	0.07135	17	0.05328	27	0.04361
8	0.06916	18	0.05201	28	0.04296
9	0.06697	19	0.05073		
10	0.06478	20	0.04964		

.

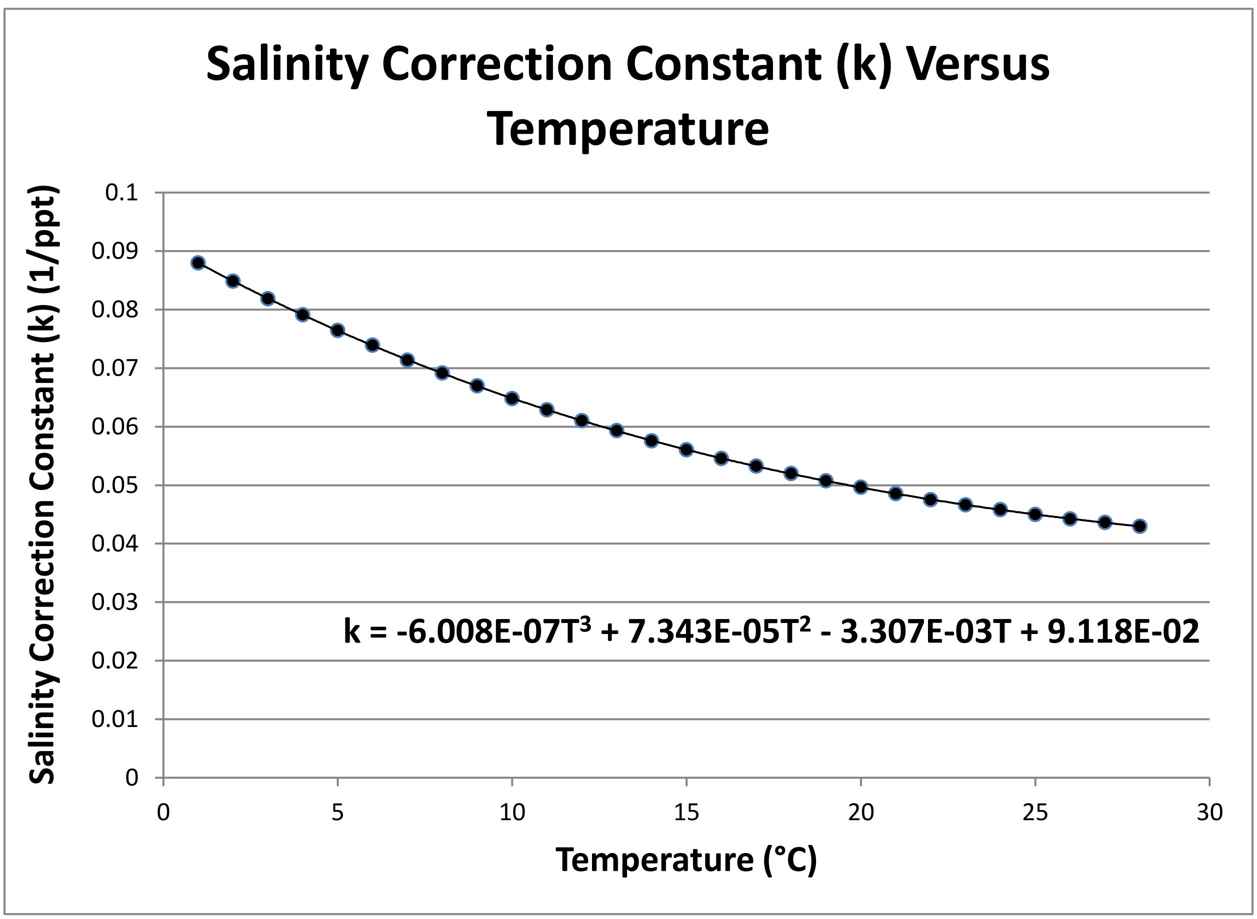
The data in Table 2 have been plotted and fitted to a third order polynomial as shown in Figure 2:

$$k = -6.008E\text{-}07T^3 + 7.343E\text{-}05T^2 - 3.307E\text{-}03T + 9.118E\text{-}02 \qquad (2)$$

Using the temperature measured by the S132 temperature probe, k can be calculated using equation (2). Using this calculated k and the salinity, S, measured by the S130 salinity probe, Equation (1) provides the salinity correction for dissolved oxygen measured by the DO probe. These calculations are performed by Logger Pro to obtain the salinity corrected DO, DOcor, (which is temperature and pressure compensated internally in the optical probe).

NOTE: the Q-Box AQUA package is designed for use with the salinity probe as the DOcor values use salinity readings. If the salinity probe is not used, the readings will not be correct and DO uncorrected should be used)

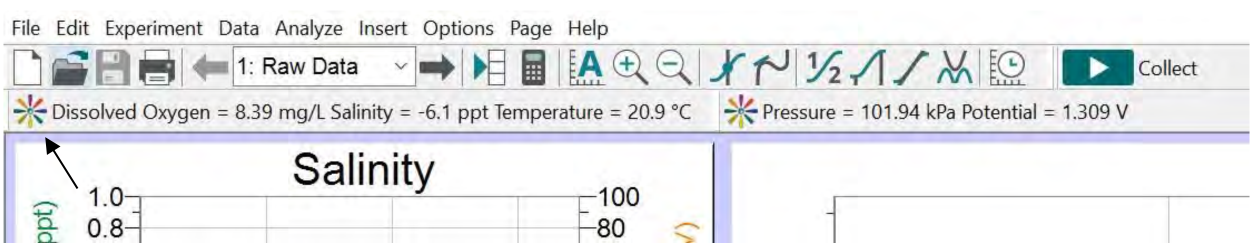
Figure 2: Salinity Correction Constant



Installing and Running Logger Pro 3

PC Users:

(1) To start, you will need to have a complete copy of Logger Pro 3 installed on the computer. Before starting the installation, make sure that all USB cables are disconnected from your computer. Failure to do so may cause an error in the installation of the USB drivers.
(2) Run the installation and do not change the default destination directory. Logger Pro 3 will be installed in C:/Program Files/Vernier Software/Logger Pro 3.
(3) The setup process will automatically load the USB drivers for connecting the LabQuest Minis to your computer.
(4) If you do not have QuickTime 6 (or greater) installed on your computer, it is advised that you install it when prompted. QuickTime will allow you to use the picture and movie features of Logger Pro 3.
(5) You will be prompted to connect the LabQuest Minis to the computer via the USB connection. Click 'Finish' to complete the installation process.
(6) Proceed to C404 to obtain the "Q-Box AQUA Setup" file.
(7) Double click the "**Q-Box AQUA Setup**" file (copied from Qubit Experiments folder of C404 disk to your computer) to start Logger Pro and data collection. If Logger Pro detects the LabQuest Mini interfaces, the Logger Pro screen will appear with two LabQuest Mini icons in the top left side



(9) If Logger Pro cannot detect the LabQuest Mini Interfaces, a message will appear "no device connected". Check that the LabQuest Minis are connected to the computer via the USB cables, and that the USB cables are connected to the computer. Exit the Logger Pro "**Q-Box AQUA Setup**" file, disconnect and reconnect these cables and restart the Experiment File. Reconnect the USB cables with USB of Mini1 first then USB of Mini 2.

Macintosh Users:

(1) To start, you will need to have a complete copy of Logger Pro 3 installed on the computer (You must be using at least OS 9.2). Run the "Complete Installation" and make sure that you have disconnected all TI GRAPH_LINK and USB cables. The most recent version of Logger Pro (3) is included with this package. Following instructions are the same as for PC users.

C404 Custom Experiment Files Installation:

Qubit Systems' C404 Custom Setup Files CD contains experimental files associated with this package, in this case "**Q-Box AQUA Setup**". The C404 disk also contains a manual for this package and the data interface (LabQuest Mini). These files can be copied to user specified location on the computer and the experimental file "**Q-Box-AQUA Setup**" should be placed in an accessible location or have a shortcut created on the desktop to the file. We highly recommend for the user to make a copy of the original file and keep it in a safe place on the computer in case the original is accidentally altered.

Using the S130 Salinity Probe

The salinity probe measures the electrical conductivity, C of a liquid in order to calculate its salinity, S. It does this by measuring the current carried by ions between its two electrodes, when a voltage is applied between the electrodes. It provides the total concentration of ions in solution but does not identify the ions. It actually measures conductance (i.e. 1/resistance), G in units of microsiemens, µS. The conductivity is calculated as:

$$C = G*k_c \qquad (3)$$

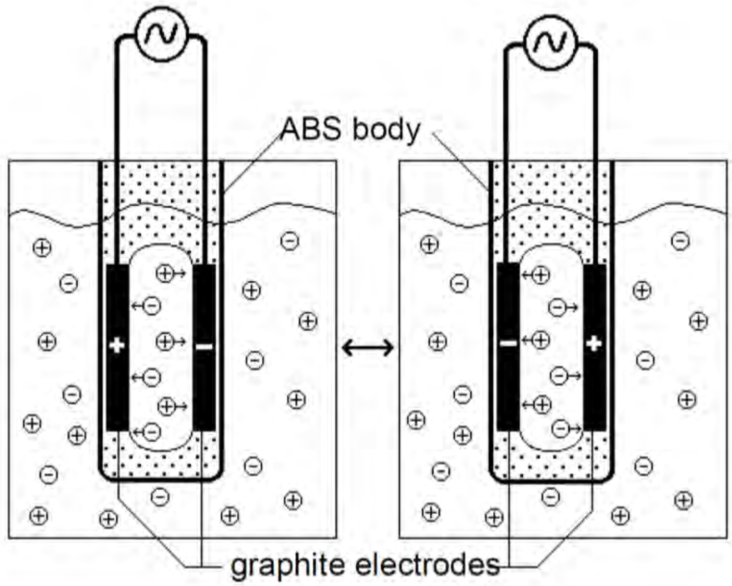
where k_c is the cell constant for the probe. The cell constant for the probe is:

$$k_c = d/A \qquad (4)$$

where d is the electrode separation and A is the electrode area. For the S122 salinity probe, the cell constant is:

$$k_c = 1\ cm/0.1\ cm^2 = 10\ cm^{-1}$$

To prevent electrolysis and polarization of the electrodes, an AC voltage rather than DC is applied between the two electrodes. As shown in the figure below, the direction of ion flow is reversed on each cycle of the applied AC voltage.



There is a relationship between conductivity, C, (µS/cm) and concentration of a specific ion or salt defined as salinity, S, measured in mg/L (ppm) or in parts per thousand (ppt = ppm/1000). This relationship between conductivity and salinity depends on the ion as shown in Table 3. For sodium chloride, the major salt in seawater, it is *approximately* a 2:1 ratio and is very nearly a direct relationship:

$$C = k_s * S \qquad (5)$$

Table3: Relationship Between Conductivity (µS/cm) and Salinity (mg/L) for Various Ions

Ion	Conversion Constant, K_s (µS*L/(cm*mg))
Bicarbonate	0.715
Calcium	2.60
Carbonate	2.82
Chloride	**2.14**
Magnesium	3.82
Nitrate	1.15
Potassium	1.84
Sodium	**2.13**
Sulfate	1.54

In summary, the S130 salinity probe obtains the solution conductance, G, from the ionic current between its two electrodes, then combining equations 3 and 5, calculates salinity, S as:

$$S=G*k_c/k_s \qquad (6)$$

When using the S130 salinity probe, ensure the electrodes are submerged. The handle is not waterproof. Rinse and dry the probe tip with a tissue between measurements. The probe can be stored dry after rinsing and drying.

The sensor may be recalibrated using a 2-pont calibration by clicking on ***Experiment>Calibrate>LabQuest Mini: 1 CH2: Salinity*** and following the instructions. The zero point can be obtained with the probe in air. The second point is obtained by placing the probe in a standard saltwater calibration solution. A 35 ppt standard solution (equivalent to seawater) can be prepared by adding 33.03 g of NaCl and adding distilled water to make 1 liter of solution. Use reagent grade rather than iodized salt.

The salinity sensor has an internal thermistor to automatically compensate for temperature changes between 5 and 35 °C. Readings are automatically referenced to a conductivity at 25 °C, thus the sensor at other temperatures will give the same conductivity as if it were at 25 °C. Therefore, the probe can be calibrated in the lab and used at other temperatures in the field.

Note that the Q-Box -AQUA package requires the use of the salinity probe to correct the DO readings for changes in salinity. If a salinity probe is not used, the DOcor readings that are displayed and used in calculations of VO2 will not be correct.

When using the salinity probe ensure that the probe is not fully submerged in the water. The top of the probe with the cable should be kept above the water level to prevent damage to the probe.

Using the S132 Temperature Probe

The temperature probe sensor is a semi-conductor device (20 kΩ NTC thermistor) mounted on the end of a stainless-steel probe. The handle is not waterproof so only the steel part should be submerged. It has an operational range from $-40^{o}C$ to $+135^{o}C$. The sensor can be placed in the water bath for monitoring the temperature of the water in the system. It is suitable for continuous use in saltwater. **Do not fully submerge the temperature sensor in the water. The top of the probe where the cable is attached should not be submerged to prevent water damage.**

The sensor is recognized automatically, and calibration is loaded when the “Q-Box AQUA Setup” file is opened. The temperature sensor should not require calibration, however if necessary, it can be recalibrated (three point) in Logger Pro by clicking on ***Experiment>Calibrate>LabQuest Mini: 1 CH3: Stainless Steel Temperature*** and following the instructions. The thermistor's resistance, R decreases nonlinearly with increasing temperature as described by the Steinhart-Hart equation:

$$T = [K_0 + K_1(\ln(1000R)) + K_2(\ln(1000R^3)]^{-1} - 273.15 \qquad (6)$$

where T is the temperature (°C), R is the thermistor resistance (kΩ), K_0=1.02119E^{-3}, K_1=2.22468E^{-4} and K_3=1.33342E^{-7}. When connected to the LabQuest Mini interface, R is measured, and T is calculated in Logger Pro using the above equation.

Using the S181 Absolute Pressure Sensor

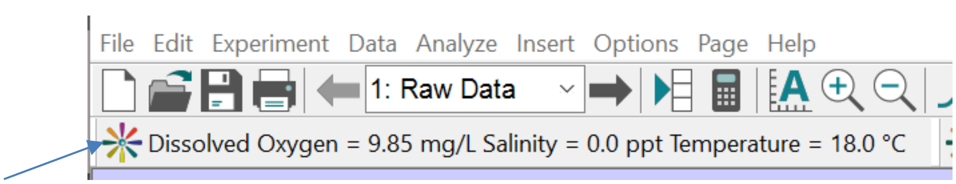
The S181 is an absolute pressure sensor with a silicon-based electronic sensor. It measures changes in pressure and produces a voltage output in the range of 0-2V that corresponds to pressures of 15-115 kPa. This sensor is factory calibrated and only needs to be plugged into the LabQuest Mini interface. The sensor is temperature-compensated and has a fast response time (1ms). **Do not submerge the sensor under water**. It is designed for measurements of atmospheric pressure only. The atmospheric pressure readings can be used when calibration the DO probe in mg/L.

Using the PL175 Liquid Pump

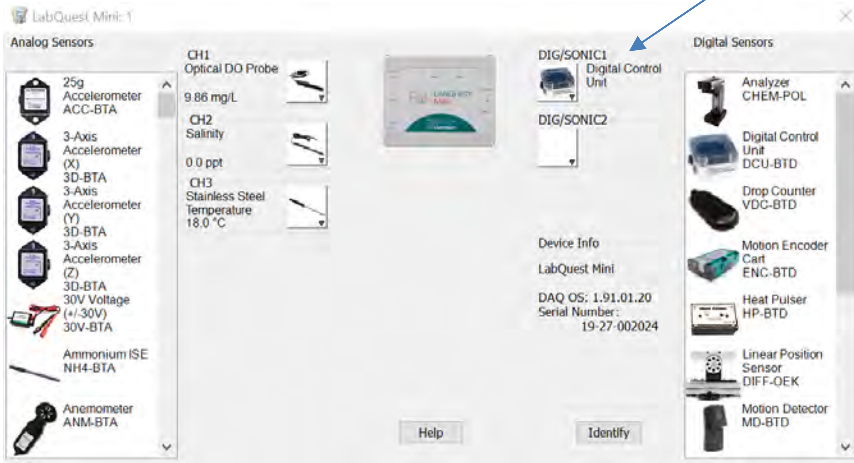
The PL175 is a liquid pump that provides flows of water in the range of 0-1.4 L/min (no load). When used with the Q-Box AQUA system, the resistance of all the components result in a maximum flow of approximately 1 L/min. The speed of the pump can be regulated with the speed control knob on the front of the instrument. The speed of the pump may also be monitored in the software as Flow voltage output. This output is specific to each setup and should be corelated with specific flow rates through the system. Connect the tubing from the outlet of the 3-way valve to the “IN” port of the pump. When finished using the pump, disconnect all the tubing and blow out any water that may be in the lines, before storing the pump in the Q-Box case. Please note that when setting up the system the liquid pump may be turned upside down briefly to release any bubbles that may get trapped inside.

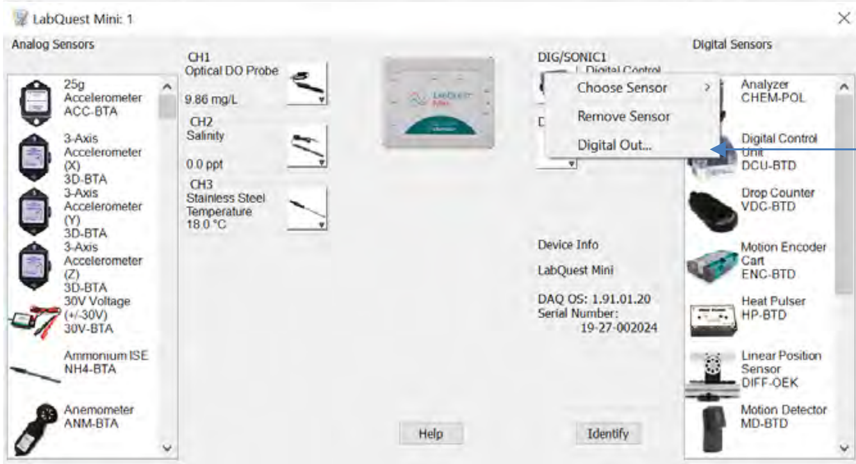
Using C200 Digital Control Unit DCU

The C200 Digital Control Unit works together with the A236 3-way liquid valve to change the flow of water from Flush (when respirometer chamber is flushed with freshly oxygenated water) to circulation, when decline in DO due to respiration of the animal is measured. The A236 valve is plugged into D1 input on the DCU. When activated by the software the red LED light above D1 will light up. When trouble shooting the A236 valve and the DCU, one may open the software and check the settings by clicking on the first star (representing Mini 1) in the top menu.

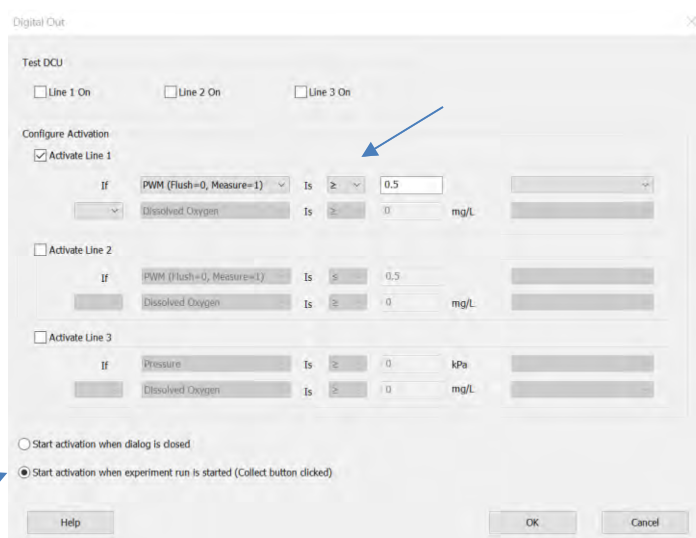


The following window will appear. Click on the Digital Control unit and select “Digital Out” from the drop-down menu.





The window below shows the DCU programing for the control of the 3-way solenoid valves. If this programing has been changed the function of the system may be affected. Please ensure that the correct programing is restored.



Specifications

S122 Optical DO Probe

Range: mg/ml		0 to 20 mg/L
%		0 to 100%
Accuracy: mg/L		+/- 0.2 mg/L below 10 mg/L
		+/- 0.4 mg/L above 10 mg/L
%		+/- 2%
Resolution:		0.006 mg/L
Response Time:		90% of final reading in 40 sec
Temperature compensation:		automatic from 0 to 50 °C
Pressure compensation:		automatic from 228 mmHg to 1519 mmHg
Salinity compensation:		externally corrected for in LoggerPro software
Stirring Required?		no
Stored calibration constants (mg/L):	slope	4.444 (mg/L)/V
	intercept	-0.4444
Stored calibration constants (%):	slope	22.222 %/V
	intercept	-2.2222
Dimensions:	probe tip diameter	16.6mm OD
	probe tip length	100 mm
	probe handle length	120 mm
	length of cable	1.36 m

S130 Salinity Sensor

Range:		0 to 50 ppt (0 to 50,000 ppm)
Resolution:		0.02 ppt (20 ppm)
Accuracy:		+/- 1% of full scale reading
Response Time:		98% of full scale reading in 5 sec
Temperature compensation:		automatic from 5 to 35 °C
Cell constant:		10 cm^{-1}
Electrodes:		parallel platinum
Dimensions:		12 mm OD; 150 mm length
Calibration constants:	slope	16.3 ppt/V
	intercept	0

S132 Temperature Sensor

Range:	-40 to 135 °C (-40 to 275 °F)
Resolution:	0.17 °C (-40 to 0 °C) 0.03 °C (0 to 40 °C) 0.1 °C (40 to 100 °C) 0.25 °C (100 to 135 °C)
Accuracy:	+/- 0.2 °C at 0 °C; +/- 0.5 °C at 10 °C
Sensor:	kΩ NTC thermistor
Response time (for 90% change in reading)	10 sec (in water with stirring) 400 sec (in still air) 90 sec in moving air
Dimensions	Stainless steel body: length 10.5 cm; 4.0 mm diameter

S181 Pressure Sensor

Sensor:	monolithic silicon pressure sensor
Response time:	1 ms
Range:	15 to 115 kPa
Voltage Output:	0 to 2 V
Temperature compensated:	yes

<u>ACKNOWLEDGEMENT:</u>

QUBIT SYSTEMS gratefully acknowledges Vernier Software Inc. for the information contained in the above tables and sensor specifications.

Qubit Systems Warranty Information

QUBIT warrants all its instruments to be free from defects in materials or workmanship for a period of **one year** from the date of invoice/shipment from QUBIT.

If at any time within this warranty period the instrument does not function as warranted, return it and QUBIT will repair or replace it at no charge. The customer is responsible for shipping and insurance charges (for the full product value) to QUBIT. QUBIT is responsible for shipping and insurance on return of the instrument to the customer.

No warranty will apply to any instrument that has been (i) modified, altered, or repaired by persons unauthorized by QUBIT; (ii) subjected to misuse, negligence, or accident; (iii) connected, installed, adjusted, or used otherwise than in accordance with the instructions supplied by QUBIT.

The warranty is return-to-base only, and does not include on-site repair charges such as labour, travel, or other expenses associated with the repair or installation of replacement parts at the customer's site.

QUBIT repairs or replaces the faulty instruments as quickly as possible; maximum time is one month.

QUBIT will keep spare parts or their adequate substitutes for a period of at least five years.

Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.

QUBIT also offers out-of-warranty repairs. These are usually returned to the customer on a cash-on-delivery basis.

Wear & Tear Items are excluded from this warranty. The term Wear & Tear denotes the damage that naturally and inevitably occurs as a result of normal use or aging even when an item is used competently and with care and proper maintenance.

Return Procedure

Before returning any instrument to QUBIT:

Please consult the operating manual or contact Qubit to ensure that the instrument(s) is in fact faulty and has not just been set up improperly.

Contact QUBIT before sending anything back. We will issue an RMA number and provide shipping instructions. QUBIT will refuse any goods that are returned without an RMA number or which are sent in a manner outside of QUBIT'S stipulations.

If you have encountered a program failure, we would need a printed copy of any faults you have seen, including how to reproduce them. Include these in the return package along with your mailing address.

Include a copy of the Invoice on which the product was shipped to you.

All returns must be shipped prepaid. Unpaid packages will not be accepted.

In case of questions contact QUBIT by

E-mail: info@qubitsystems.com, by phone: (01)-613 384-1977, or by fax: (01)-613 384- 9118.