Q-Box RP1LP Low Range Respiration Package (Enhanced)

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Overview of the Enhanced Q-Box RP1LP Low Range Respiration Package

The enhanced Q-Box RP1LP package can be used to measure the metabolic rate of small animals or less active animals such as reptiles, amphibians, insects and other invertebrates in both open, and stop flow modes. A system diagram is shown below. **Open flow** measurements are used for more metabolically active samples**.** Although the open mode gives continuous, fast response data, it has lower sensitivity than the stop flow configuration. **Open** flow uses the **difference** between the sample and reference gases to calculate CO_2 production ($\dot{V}CO_2$) and oxygen consumption ($\dot{V}O_2$). For very small or metabolically inactive specimens, the system can be used in a **stop flow** configuration. Since the gas analyzers measure partial pressure, their readings are pressure corrected for changes to pressure due to weather, or any back pressure created in the system. Pressure in the gas system is measured by the Q-S102 O_2 Analyzer pressure sensor. The **stop flow** and **open flow** methods have the advantage over **closed flow** systems that the gas analyzers remain at atmospheric pressure. By using a pair of 3-way solenoid valves controlled by a pulse width modulated signal (PWM; generated by Logger Pro software) via a digital control unit (DCU), flow through the chamber is periodically **stopped** when the solenoid valves are **energized.** During this time the chamber is bypassed to provide background measurements of CO_2 and O_2. Meanwhile, respiration gases from the specimen either accumulate or are depleted in the chamber and when solenoid valves become **de-energized** the chamber is flushed with background gas and respiration gases are swept to the gas analyzers for measurement. The **stop** flow method uses the **integral** of the measured bolus of gases that build up or are depleted during the stop time to calculate CO_2 production ($\dot{V}CO_2$) and oxygen consumption ($\dot{V}O_2$). The enhanced RP1LP system includes automation of the open and stop flow methods including all the calculations in the software.

The gas is pumped through the system at a known flow by the Q-P103 Gas Pump. The flow is adjusted and measured by the Q-G266 Flow Monitor. The concentration of CO_2 and O_2 in the reference (i.e. carrier) gas supplied to the animal chamber, is determined during chamber bypass by the Q-S151 infrared CO_2 gas analyzer (0-2000ppm range), and the fuel cell Q-S102 O_2 Analyzer (0-25% range). The concentration of CO_2 and O_2 in the gas flowing through the chamber (modified by specimen respiration), is determined when the 3-way solenoid valves (A380-E) are deenergized and the chamber is flushed. Analog voltages from all of the sensors are converted to digital signals via two LabQuest Mini interfaces (6 analog + 4 digital channels). Control A380-E solenoid valves is programmed in Logger Pro software experimental file (Enhanced Q-Box RP1LP Setup) in conjunction with a DCU (C200), connected to a digital channel on a data interface. Data are displayed, recorded and manipulated on a PC or Macintosh computer using Logger Pro. Metabolic rates are calculated by the software from the changes in O_2 and CO_2 for both open and stop flow.

The enhanced Q-Box RP1LP also includes a temperature sensor (S174) which can be placed inside the animal chamber, to investigate the effect of temperature on metabolic rate. Metabolic rates measured at different temperatures can be corrected by the user to a standard temperature, assuming a given Q_{10}. In addition, the enhanced Q-Box RP1LP can be used to investigate animal metabolic responses to exercise, to different diets, to pharmaceuticals and to various concentrations of O_2 and CO_2 in the carrier gas supplied to the chamber. Gas mixtures can be supplied via 2 large gas bags (G122 included). For longer experiments such as hibernation studies, gas can be obtained from a commercial tank with a regulator. Qubit also manufactures gas mixing equipment that can be used for this application.

This enhanced Package can be used for measurements of CO_2 and O_2 in fungi and micro-organisms. Other potential applications include measurements of metabolism from aquatic suspensions. For example, yeast or bacterial growth can be studied, by bubbling air through a culture suspension to analyze the headspace gas. Gas can also be analyzed from a soil chamber.

Components of the Enhanced Q-Box RP1LP

Q-S151 CO_2 Analyzer (Range: 0-2000 ppm)

Q-S102 O_2 Analyzer with pressure sensor for O_2 pressure correction (Range: 0-100%, 0-25%)

Drying columns for Q-S151 and Q-S102 with DRIERITE (blue) (3x) (Q11784)

Small drying column

CO_2 scrubbing column with soda lime (white) (Q13025)

Q-G266 Flow Monitor (Range: 0 -1 LPM)

Q-P103 Gas Pump (1 LPM no load)

G115-E Flow-through Chamber for enhanced package (3.8 cm ID x 20 cm L)

G117 variable volume Glass and Aluminum flow through chamber (1.9 cm ID, adjustable length) for small samples

C200 Digital Control Unit (DCU)

A380-E Three-way solenoid valve assembly for Stop Flow

S174 Thermistor Temperature Probe for enhanced Q-Box RP1LP

G122 Large Gas Bags (30 Litres x 2)

Integrated C610 LabQuest Mini Data Acquisition Interfaces (2x), 6 analog + 4 digital channels

C901 Logger Pro Data Acquisition Software

C404 Customized Setup files

Q-Box Accessory Kit (includes tubing, filters, connectors, wool, wrench for needle valve adjustment, screwdriver, qubitac sealant)

Manual

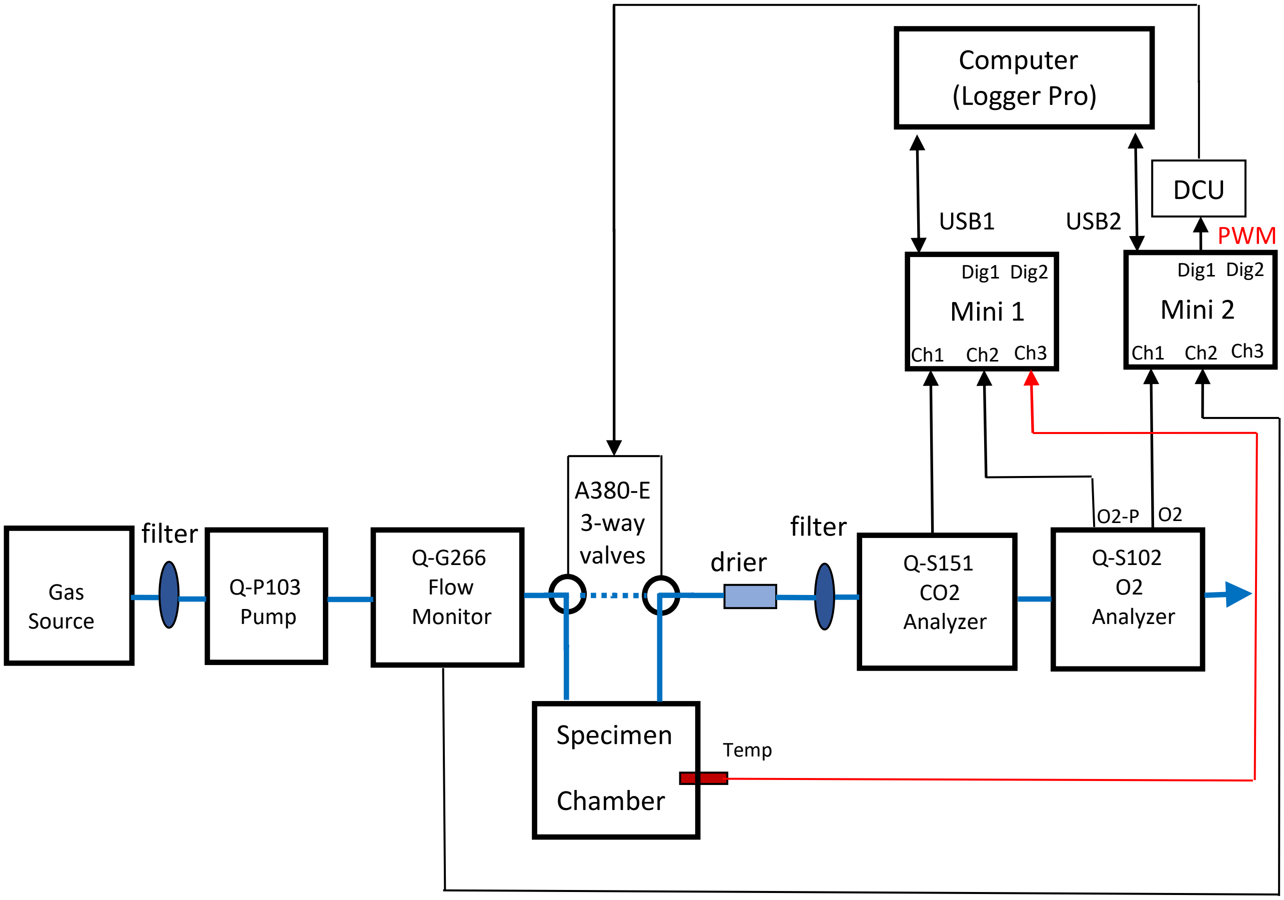
Individual power supplies for all the sensors (for use in stand-alone mode)

Rugged weather-proof case

A249 Battery Pack for field work (**OPTIONAL**)

Schematic of Enhanced Q-Box RP1LP Setup

Note: heavy blue lines indicate gas flow; thin black lines indicate electrical connections. The 3-way solenoids are shown in the failsafe default state (not energized; PWM=0) with flow through the chamber. PWM is the pulse width modulated signal programmed in software that controls the 3-way solenoids via the DCU (digital control unit).



The method of stop and open flow used in this package is explained below using the following CO_2 and O_2 time plots obtained with 8 mealworms inside a G117 chamber adjusted to 6.3 ml. In the Stop Flow setup with **PWM=0 during collection** (and when the **software is not collecting data, i.e.** during setup of an experiment or during a power failure), the 3-way solenoid valves are not energized. The carrier gas (CO_2-scrubbed and dried outside air in this example) flows through the chamber. Note this state is **default** so that when the solenoids are not powered (e.g. during a power outage), this is a safety feature to ensure the specimen has carrier gas flowing past it to avoid excessive CO_2 buildup and O_2 depletion. This is very important during setup of the experiment. When solenoid valves become energized and the chamber gas is swept through the analyzers, a CO_2 peak and an O_2 trough appear on the graphs as a result of CO_2 build up and O_2 depletion during the previous **stop phase** (i.e. **PWM=1**; bypass of the chamber with specimen). Once the CO_2 peak and O_2 trough have passed through the analyzers, the measured gas concentrations will return to the steady state open flow values. If the specimen is active, these values may be different enough from the reference levels that **open flow** calculations of O_2 consumption (**$\dot{V}O_2$**) and CO_2 production (**$\dot{V}CO_2$**) may be obtained from this **difference**. In the **stop flow** setup, **$\dot{V}O_2$** and **$\dot{V}CO_2$** are calculated from the **area** under the peak (CO_2) or above the trough (O_2).

In the enhanced Q-Box RP1LP package, **$\dot{V}O_2$ and $\dot{V}CO_2$ are computed continuously** (i.e. for every sample time) during the **open flow** configuration and only **once per stop/flush cycle** - at the end of the stop peak/trough during the **stop flow** configuration.

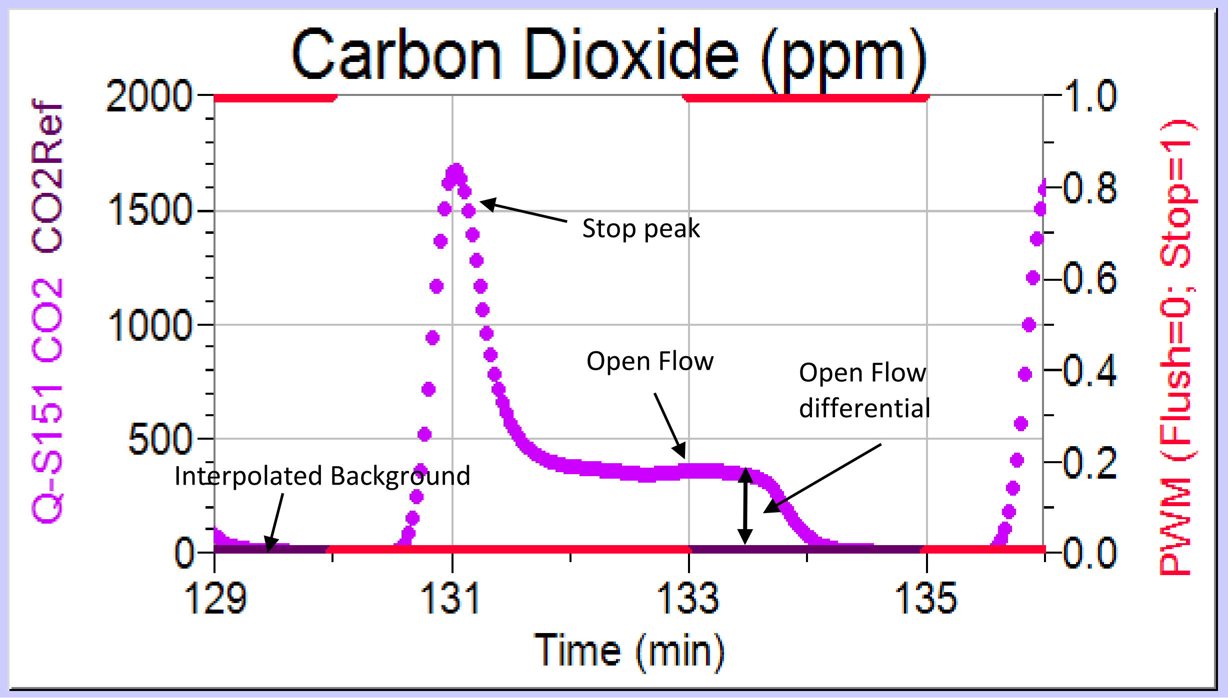
When **PWM=1** the 3-way solenoid valves are **energized** by the DCU to **bypass** carrier gas around the chamber to the analyzers to give a background measurement of CO_2 (0 ppm) and O_2 (20.95 %). **Interpolation between the background samples** is performed in the software to **remove** any **drift** in the background. This is especially useful for long experiments where temperature, pressure or humidity may change. Consequently, although stop flow has a better sensitivity for small samples such as insects, it has a slower time response compared to open flow. Open flow is more suited to continuously following larger specimen activity.

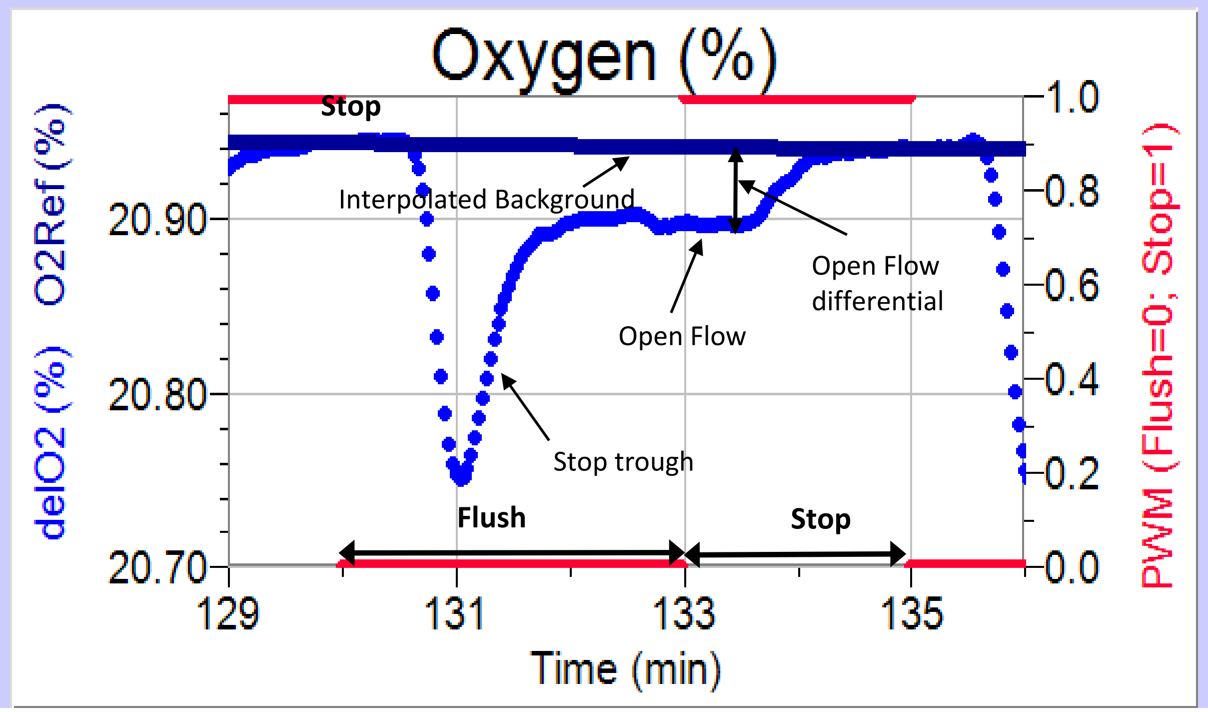
Important Notes:

1) **Automatic calculations** of **$\dot{V}O_2$** and **$\dot{V}CO_2$** for open flow and stop flow are presented on Pages 2 and 3, respectively, of the experimental setup file in Logger Pro. These automatic calculations permit long term monitoring of respiration. 2) If **$\dot{V}CO_2$** measurements are not possible to obtain during **stop flow** configuration**,** i.e. CO2 peak may exceed the 2000 ppm limit of the CO_2 analyzer (when the specimen is very active or stop flow period is too long) the open flow configuration may be more appropriate to use. 3) This package may also be used as a standard Q-Box RP1LP package when active specimens are studied (i.e. without the solenoid valves and digital control unit; see standard Q-Box RP1LP Low range respiration manual for instructions). 4) The O_2 and CO_2 changes during **stop flow** configuration may be increased by a **longer stop time and smaller chamber volume**. 5) During **open flow** configuration differences between the reference and sample gases may be enhanced by **reducing the gas flow** through the system. 6) For accurate recording of the data during **stop flow** configuration, gas flow should be low enough (~ 25 ml/min) so that the gas sensors (response time ~ 20 sec) to register the peaks being swept through them. 7) In addition to the automatic calculation of **$\dot{V}O_2$** and **$\dot{V}CO_2$** on Pages 2, 3 of the Logger Pro experimental file, templates for **manual calculation** are provided on Pages 4, 5. These are most useful for educational purposes and manual check on the automatic calculations.

It is extremely important to properly set the user parameters on Logger Pro Page 1 so that the automatic calculations are performed correctly for a specific experiment flow, flush and stop time etc.

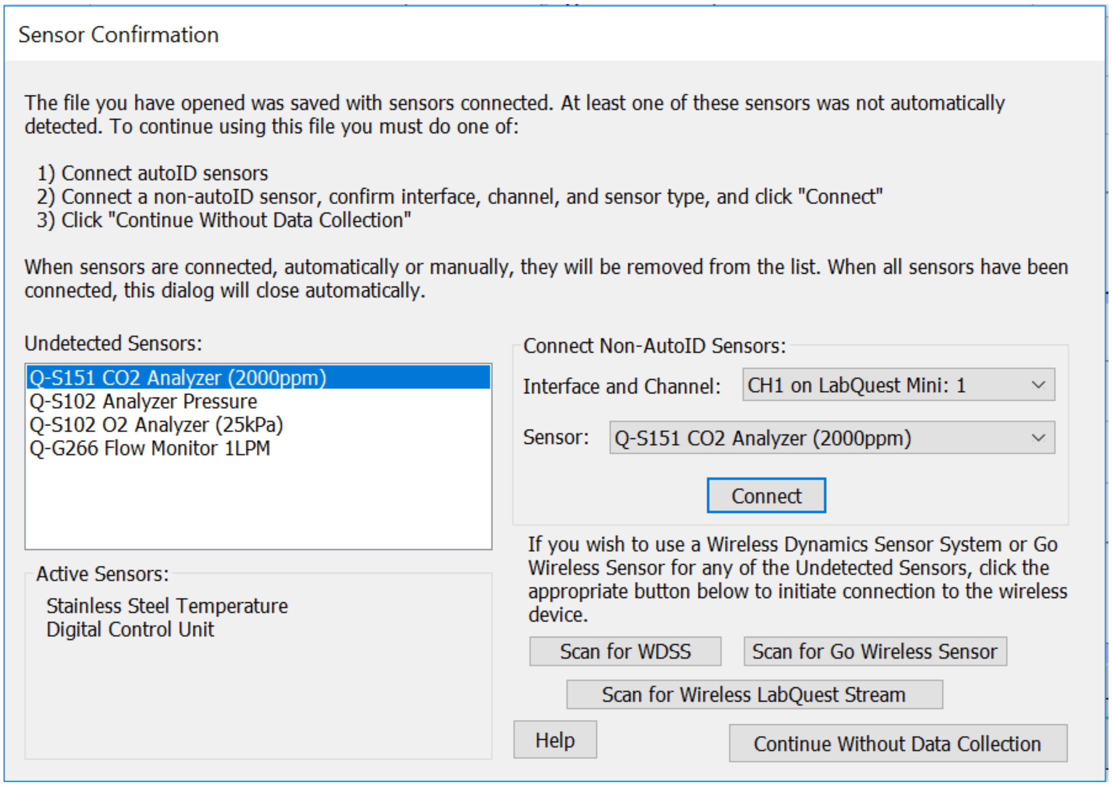
Stop Flow and Open flow Signals in Enhanced Q-Box RP1LP





Quick Start Up Steps:

1. Load Logger Pro 3 software onto the computer (see page 35).
2. Load C404 customized experimental files (see page 37).
3. Connect the Q-Box to a power supply (or A249 Battery pack for field use).
4. Turn on the pump (Q-P103), flow monitor (Q-G266), CO2 analyzer (Q-S151) and O2 analyzer (Q-S102).
5. Allow the Q-S151 CO_2 and Q-S102 analyzers to warm up for at least 30 min.
6. Connect the DCU to the 3-way solenoid valve assembly (A380-E).
7. Connect two USB cables from the Q-Box frame to the computer. Two audible sounds may be heard as the two interfaces are recognized. **Ensure that USB1 is plugged in first and USB2 is plugged in second, with time in between so the two interfaces are recognized in the correct order.**
8. Open the "**Enhanced Q-Box RP1LP Setup**" file copied from the C404 disk, to start Logger Pro software. the following sensor connection window will be shown:



Sensor Confirmation

The file you have opened was saved with sensors connected. At least one of these sensors was not automatically detected. To continue using this file you must do one of:

1) Connect autoID sensors
2) Connect a non-autoID sensor, confirm interface, channel, and sensor type, and click "Connect"
3) Click "Continue Without Data Collection"

When sensors are connected, automatically or manually, they will be removed from the list. When all sensors have been connected, this dialog will close automatically.

Undetected Sensors:
Q-S151 CO2 Analyzer (2000ppm)
Q-S102 Analyzer Pressure
Q-S102 O2 Analyzer (25kPa)
Q-G266 Flow Monitor 1LPM

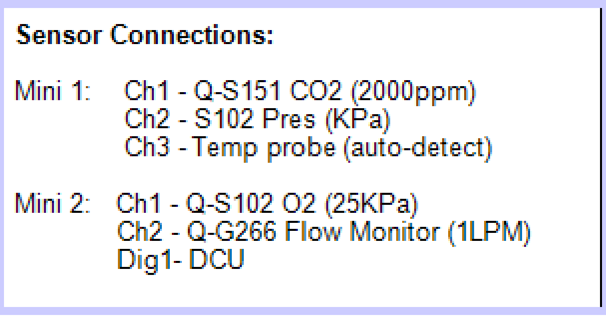
Active Sensors:
Stainless Steel Temperature
Digital Control Unit

Connect Non-AutoID Sensors:
Interface and Channel: CH1 on LabQuest Mini: 1
Sensor: Q-S151 CO2 Analyzer (2000ppm)

If you wish to use a Wireless Dynamics Sensor System or Go Wireless Sensor for any of the Undetected Sensors, click the appropriate button below to initiate connection to the wireless device.

9. Assign the sensors to appropriate channels on the 2 data interfaces as follows:
    a. Ch. 1 LabQuest Mini 1 - Q-S151 CO_2 Analyzer
    b. Ch. 2 LabQuest Mini 1 - Q-S102 P (gas pressure kPa)
    c. Ch. 1 LabQuest Mini 2 - Q-S102 O2 Analyzer (O2 kPa)
    d. Ch. 2 LabQuest Mini 2 - Q-G266 Flow Monitor (V)
    e. Dig.1 LabQuest Mini 2 - DCU

Note: Ch. 3 LabQuest Mini 1 automatically recognizes the temperature sensor and the digital control unit. The configuration of the sensor connections is shown in a text box at the bottom of Page 1 in Logger Pro.



Sensor Connections:

Mini 1: Ch1 - Q-S151 CO2 (2000ppm)
Ch2 - S102 Pres (KPa)
Ch3 - Temp probe (auto-detect)

Mini 2: Ch1 - Q-S102 O2 (25KPa)
Ch2 - Q-G266 Flow Monitor (1LPM)
Dig1- DCU

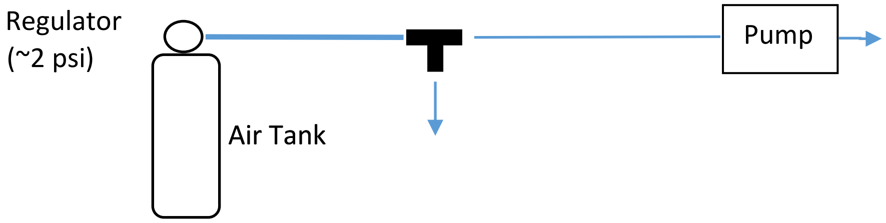
Note: If Logger Pro does not recognize the two data interfaces (LabQuest Minis) in correct order and switches them, this will likely result in unrealistic readings for the sensors, since they will be assigned to the wrong channels and hence have incorrect calibrations. If this occurs, the user should first open the experimental Setup file without USB1 and USB2 connected. Once the sensor confirmation screen opens, USB1 cable may be connected first (allow few seconds for the software to recognize it - a star appears in the top left corner of the menu) then USB2 cable connection may follow. This method should force the minis to be recognized in the correct order.

Check the system plumbing by removing the tray with all the sensors from the Q-Box, and place it outside of the box as shown in the photo below. The system should be configured as shown by the photo and diagram below.

10. Connect the carrier (i.e. reference) gas source (gas bag or other constant source of CO_2) to the “in” port on the Gas Pump via a blue particulate filter.

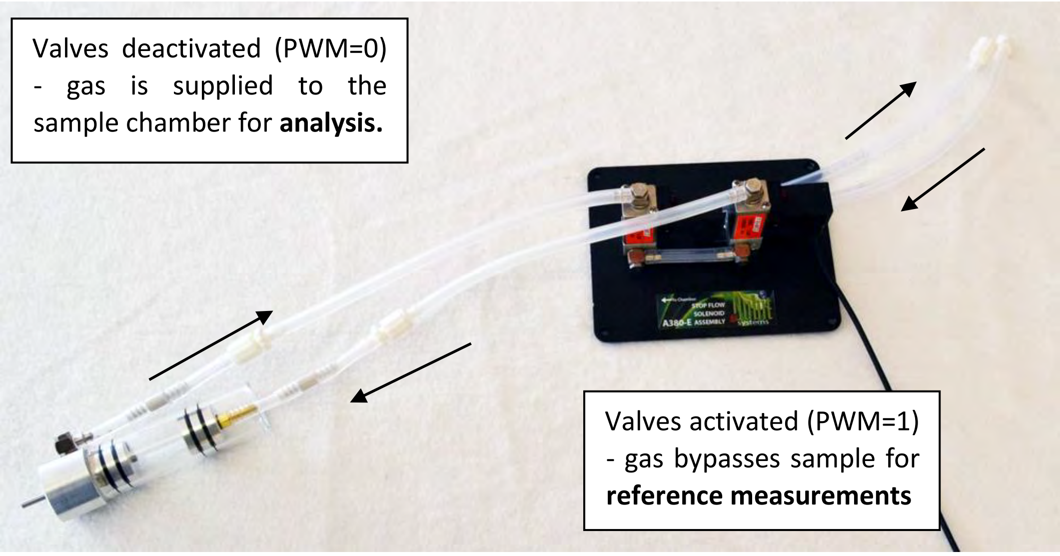
The gas source can be a gas bag of CO_2-free air filled from a commercial tank of air. An alternative is to use dried **outside air** (dried with the Drierite column) which **has a constant CO_2 level of ~ 400 ppm and O_2** concentration **of 20.95%. Do not use inside room air which has significant fluctuations of CO_2 due to human breath.** Dried outside or inside air can be scrubbed of CO_2 using the soda lime column, if CO_2-free air is needed.

Note: If the gas source is pressurized (e.g. tank) do not connect pressurized gas to the pump. Instead, as shown below, reduce the regulator pressure to a couple of psi and connect the gas line to a luer "tee" piece. Connect the pump to one of the other "tee" ports and let excess gas vent (feel on skin) from the third port of the tee piece. To conserve gas, if a rotameter is available, place it before the "tee" and adjust the flow with its valve to ~ twice that required by the pump. The pump then supplies gas at whatever flow is determined by the pump and flow monitor valves.



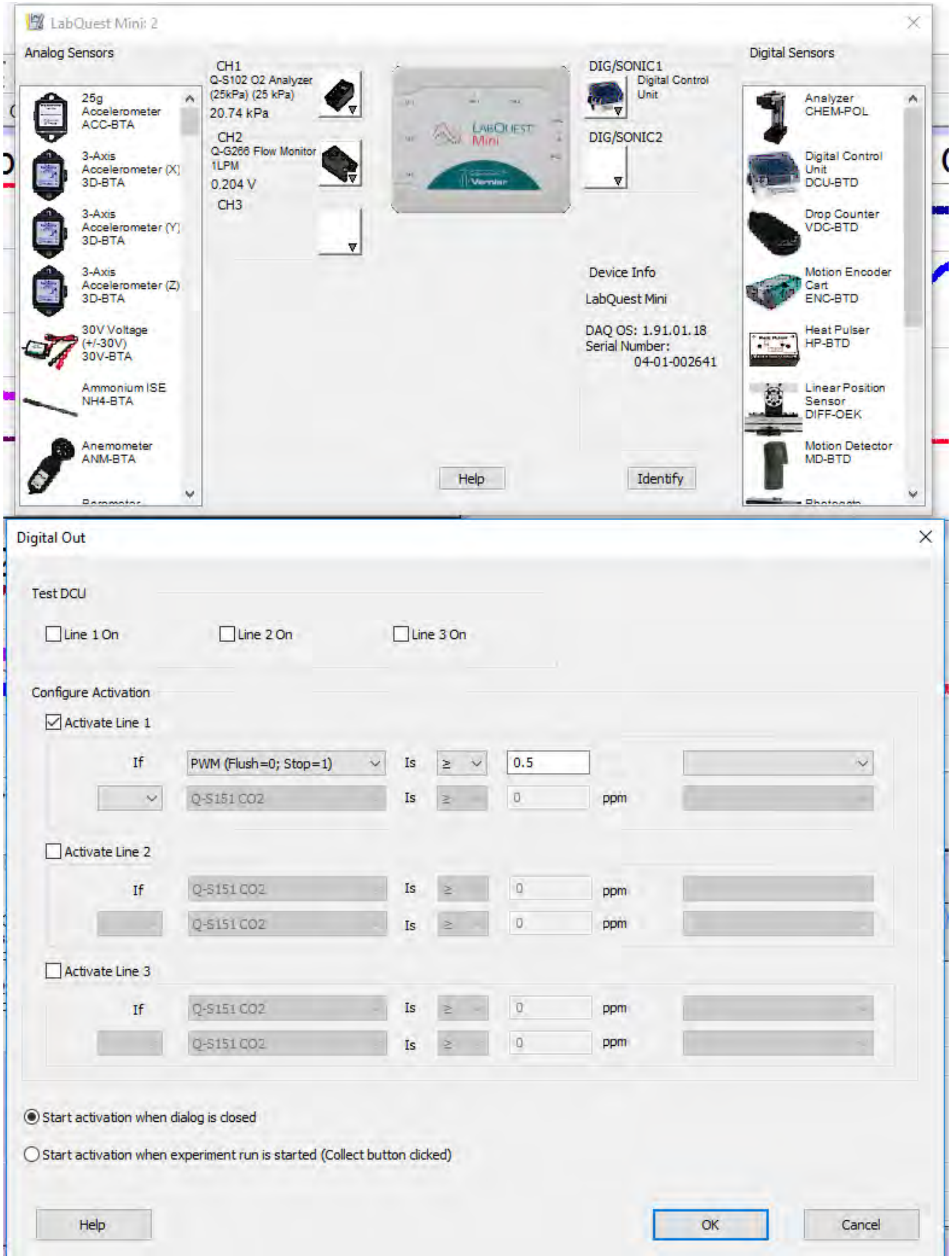
11. Connect the "out" port of the Gas Pump to the "in" port of the Q-G266 Flow Monitor.

Note: The 3-way solenoid valve assembly (A380-E) are installed as in the above schematic diagram of the enhanced RP1LP (Page 5) to direct the flow of gas either through the animal chamber (de-energized; PWM=0) to obtain a measurement, or to bypass the chamber (energized; PWM=1) to obtain reference levels.


Valves deactivated (PWM=0) - gas is supplied to the sample chamber for **analysis.**

Valves activated (PWM=1) - gas bypasses sample for **reference measurements**

The screen shot below shows how the DCU is programmed to control the 3-way solenoid valves. This may be viewed by clicking on the star icon for Mini 2 under the top menu in Logger Pro. Next click on the DCU symbol and open the Digital Out. The DCU/3-way solenoid combination can be manually tested by checking "Line 1 On".



CH1 Q-S102 O2 Analyzer (25kPa) (25 kPa) 20.74 kPa

CH2 Q-G266 Flow Monitor 1LPM 0.204 V

CH3

DIG/SONIC1 Digital Control Unit

DIG/SONIC2

Device Info
LabQuest Mini
DAQ OS: 1.91.01.18
Serial Number: 04-01-002641

Test DCU

☐ Line 1 On ☐ Line 2 On ☐ Line 3 On

Configure Activation

☑ Activate Line 1

If PWM (Flush=0; Stop=1) Is ≥ 0.5

Q-S151 CO2 Is ≥ 0 ppm

☐ Activate Line 2

If Q-S151 CO2 Is ≥ 0 ppm

Q-S151 CO2 Is ≥ 0 ppm

☐ Activate Line 3

If Q-S151 CO2 Is ≥ 0 ppm

Q-S151 CO2 Is ≥ 0 ppm

● Start activation when dialog is closed

○ Start activation when experiment run is started (Collect button clicked)

12. Connect the "out" port of A380-E to the "in" port of the Q-S151 CO_2 Analyzer via the Drying Column (blue DRIERITE) and a blue filter.

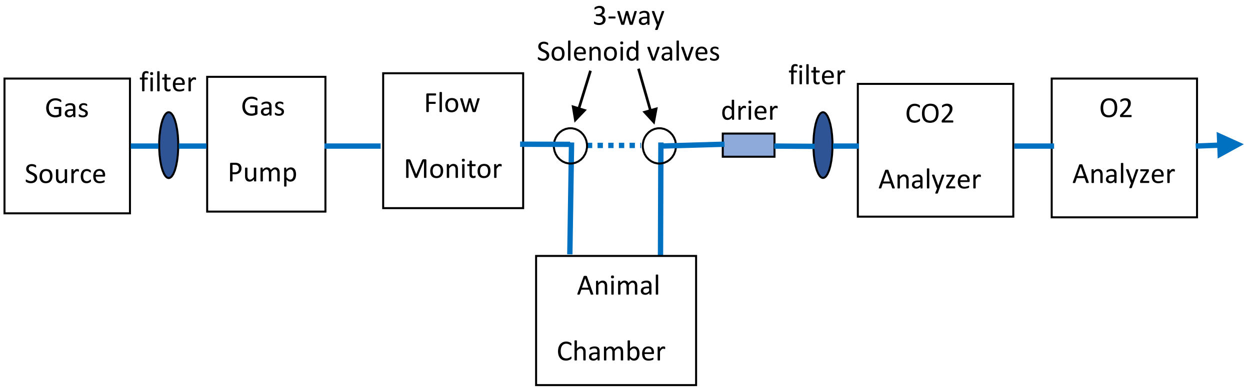
Note: Brand new Drierite may absorb and then slowly release some CO_2, thereby widening CO_2 stop flow peaks. It is strongly recommended to use Magnesium

perchlorate rather than Drierite to remove water vapour released by the specimen, in order to avoid O_2 dilution by this water vapour.

Only one drying column needs to be used when the Q-S151 and Q-S102 are used in series. A second drying column is provided for stand-alone use of the Q-S102. Two columns in series are recommended with very wet samples.

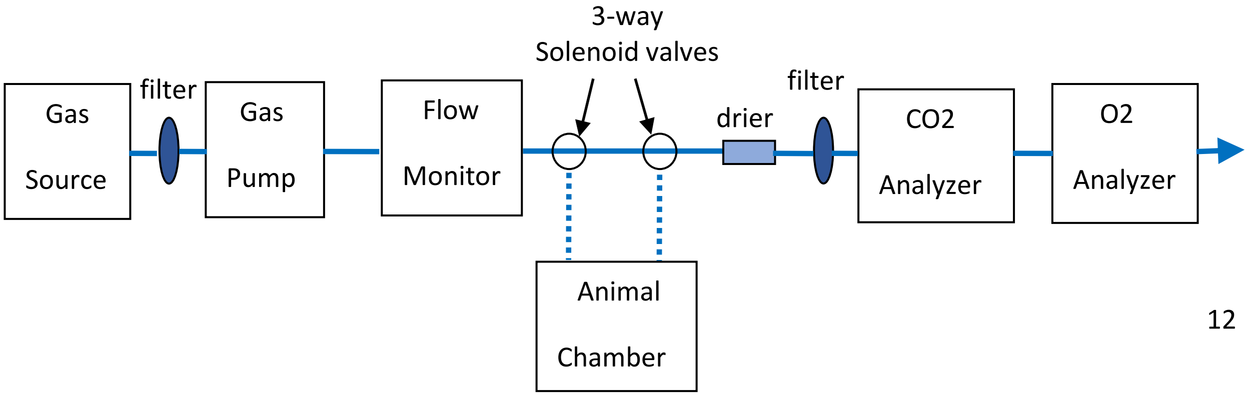
a. Connect the "out" port of the Q-S151 CO_2 Analyzer to the "in" port of the Q-S102 O_2 Analyzer.
b. Gas vents from the O_2 analyzer via the "out" port.
c. Two blue filters should be placed in the gas line, one before the pump and one before the CO_2 analyzer, to prevent particulate dust from the columns entering the analyzers.

Open Flow (PWM=0): The chamber is **flushed** with reference gas to sweep out the CO_2 and O_2 from the chamber. The accumulation of CO_2 will be graphed as a positive bolus whereas the reduction in O_2 is seen as a negative bolus or trough.



O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) are computed from the **difference** between sample and reference gases for O_2 and CO_2 respectively in the **open flow** calculation. For the **stop flow** computation, they are calculated from the **area** (i.e. integral) of the stop flow peaks for O_2 and CO_2.

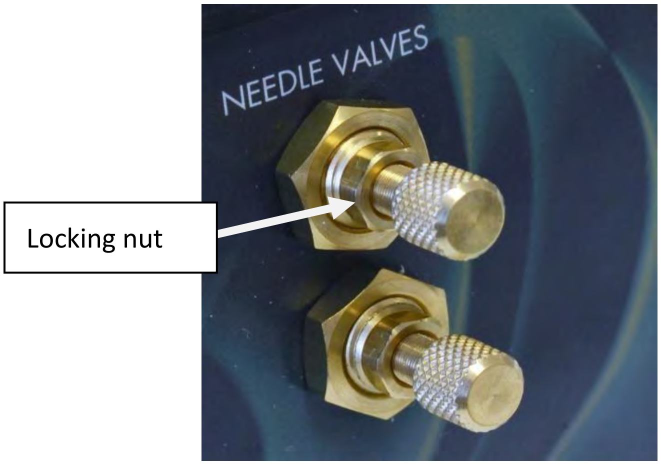
Stop Flow (PWM=1): The chamber is **bypassed** (i.e. gas flow through the chamber is **stopped**). CO_2 will increase and O_2 will decrease in the chamber due to the animal's respiration.



13. **Set the gas flow through the system.**

It is recommended that the **gas sensors be calibrated at the same flow and temperature as that used in experimental measurements**. To allow time for the gas sensors (response time ~ 20 sec) to respond to passing stop flow peaks, the flow should be low (~ 25 ml/min). **In the open flow or steady state signal, after the stop flow peak has passed, the CO_2 and O_2 differentials (with respect to the reference gas) depend inversely on flow, hence <u>larger differences</u> can be obtained by <u>lowering the flow</u>.** A higher flow should be used when higher rates of respiration are measured. The flow rates through the system are set with the Flow monitor (Q-G266) valve. The gas pump has been set at manufacture and when used with the Q-Box RP1LP does not need any adjustments.

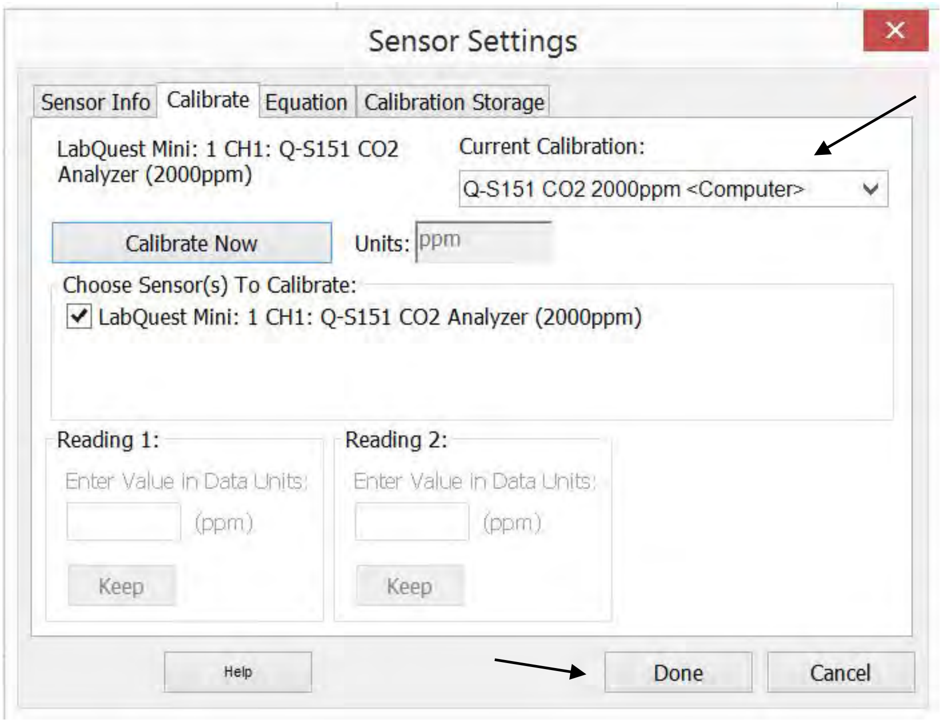
To set the flow through the system, use the valve on Q-G266 Flow Monitor. First, unlock the locking nut of the valve with the 8 mm wrench provided in the accessory pack. Next, fully close the valve (clock-wise) and observe a 0 L/min flow in the Q-G266 Flow meter on bottom left hand side of Logger Pro Page 1. Then, slowly open the valve (counter clock-wise) until desired flow rate is reached. Use the 8mm wrench, to lock the by turning the locking nut on the Flow Monitor. This will avoid accidental change of flows. Flow is recorded in software. The tray holding all the sensors can then be placed back in the Q-Box.



The Q-G266 Flow Monitor and S174 Temperature Sensor are factory calibrated and no additional calibrations are required. **The calibration of the Q-S151 CO_2 Analyzer and Q-S102 O_2 Analyzer should be checked at the start of each day of experiments.** Both Analyzers have linear responses, hence they only require 2 point calibrations. The first point should be zero concentration, and the second point (span) should be a standard CO_2 or O_2 concentration near the upper part of the range at which the analyzer will be used.

14. Calibration of the Q-S151 CO_2 analyzer (two point calibration):

a. Set the CO_2 analyzer in the 0 – 2000 ppm range which is the default range used with the Enhanced Q-Box RP1LP package.
b. Supply CO_2-free air to the analyzer by attaching the soda lime column to the outlet of the flow monitor. Attach the outlet of the soda lime column to the inlet of the drying column. Both the soda lime and drying columns should be vertical to ensure maximal contact of the gas with the particles. The outlet of the drying column should be attached to the inlet of the CO_2 analyzer. Soda lime will scrub CO_2 from air and provide the zero reading (the first point of the calibration).
c. From the menu in Logger Pro Software, select ***Experiment>Calibrate>LabQuest Mini 1>Q-S151 CO2 Analyzer (2000ppm)***. A dialog box will appear as shown below. Select the calibration storage as "Computer" then click "Done".



LabQuest Mini: 1 CH1: Q-S151 CO2 Analyzer (2000ppm)

Current Calibration: Q-S151 CO2 2000ppm <Computer>

Units: ppm

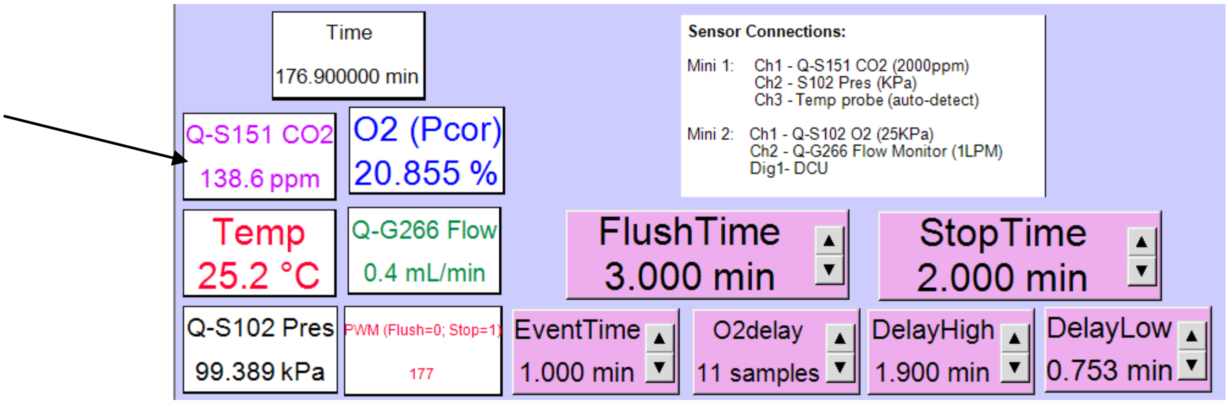
Choose Sensor(s) To Calibrate:

☑ LabQuest Mini: 1 CH1: Q-S151 CO2 Analyzer (2000ppm)

Reading 1: Enter Value in Data Units: (ppm)

Reading 2: Enter Value in Data Units: (ppm)

d. When the reading on the CO_2 analyzer is stable, use the small green screwdriver provided to adjust the "CO_2 Zero" control on the analyzer to set its digital display to 000 CO_2. The Q-S151 CO2 meter in the software Page 1 should also read zero.



Time: 176.900000 min

Sensor Connections:

Mini 1: Ch1 - Q-S151 CO2 (2000ppm)
Ch2 - S102 Pres (KPa)
Ch3 - Temp probe (auto-detect)

Mini 2: Ch1 - Q-S102 O2 (25KPa)
Ch2 - Q-G266 Flow Monitor (1LPM)
Dig1- DCU

Q-S151 CO2: 138.6 ppm

O2 (Pcor): 20.855 %

Temp: 25.2 °C

Q-G266 Flow: 0.4 mL/min

FlushTime: 3.000 min

StopTime: 2.000 min

Q-S102 Pres: 99.389 kPa

PWM (Flush=0; Stop=1): 177

EventTime: 1.000 min

O2delay: 11 samples

DelayHigh: 1.900 min

DelayLow: 0.753 min

e. If the zero reading is highly out of range (by more than 40 ppm), or if the maximum or minimum position of the “CO_2 Zero” has been reached (i.e. turning the control has no effect), use the “Coarse Zero” adjustment on the back of the instrument to bring zero within range. **Use the “Coarse Zero” with caution, since very small adjustments result in large changes. Also, there is a delay in response to changes in “Coarse Zero”**. If use of the Coarse Zero is necessary, first adjust the fine zero to the middle of its range. Do this by turning the “CO_2 Zero” potentiometer on the front of the analyzer clockwise or counter-clockwise to the end of its range (it will click when this is reached). Then turn the potentiometer twelve complete turns in the opposite direction. The CO_2 Zero control is now centered. Use the Coarse Zero to bring the reading close to Zero. Then use the “CO_2 Zero” on the front of the analyzer to make the final zero adjustment.

f. Remove the soda lime column from the gas line. Attach the “in” port of the Q-P103 Gas Pump to the gas bag or tank with known CO_2 concentration. Allow the reading to stabilize.

g. If the Q-S151 display does not show the concentration of CO_2 that is in the calibration gas, adjust the "CO_2 Span" control until the display shows the CO2 concentration supplied to the analyzer. The CO_2 readings in the Logger Pro experimental file should match that on the digital display of the CO_2 analyzer.

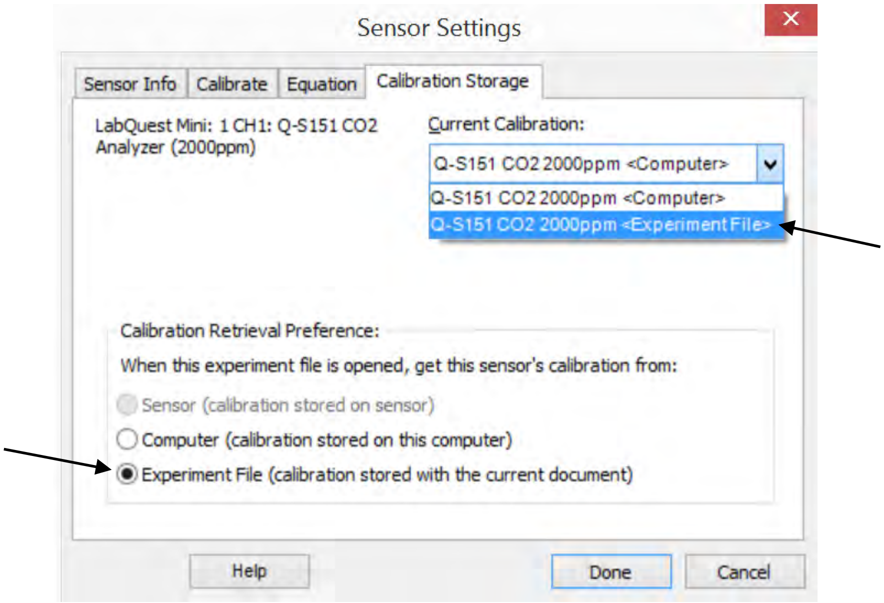
h. If a significant adjustment was made to the "CO_2 Span", return to the zero check and ensure the zero reading has not shifted.

i. If the readings on the Q-S151 digital display are significantly different from those on the Q-S151 CO_2 meter in the software, first check that the correct range (2000ppm) is selected on the CO_2 analyzer and the correct CO_2 range (Q-S151 CO2 Analyzer 2000ppm) has been selected in the experimental file. If these are correct and the readings are mismatched by more than ~10ppm, then proceed to calibration of the CO_2 analyzer in the software as described below.

Calibration of the CO_2 analyzer in software (to be used only if the display reading and the software readings are significantly mismatched)

a. Supply CO_2-free air to the analyzer by attaching the soda lime column to the outlet of the flow monitor. Attach the outlet of the soda lime column to the inlet of the drying column. Both the soda lime and drying columns should be vertical to ensure maximal contact of the gas with the particles. The outlet of the drying column should be attached to the inlet of the CO_2 analyzer. Soda lime will scrub CO_2 from air and provide the zero reading (the first point of the calibration).

b. From the menu in Logger Pro Software, select ***Experiment>Calibrate>LabQuest Mini 1>Q-S151 CO2 Analyzer (2000ppm)***. A dialog box will appear as shown below. Select the calibration storage as "Experiment File" so the calibration is saved with the current file.



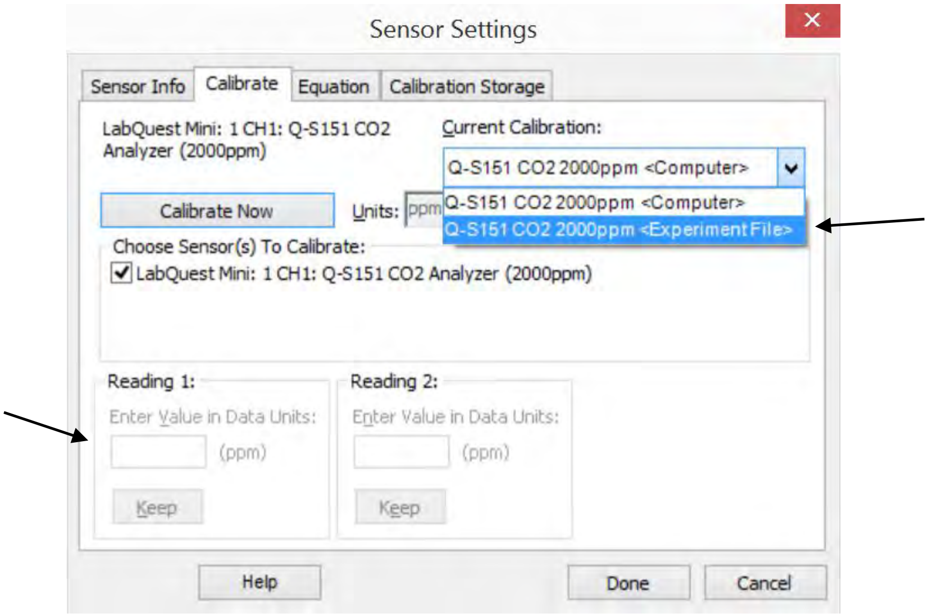
LabQuest Mini: 1 CH1: Q-S151 CO2 Analyzer (2000ppm)

Current Calibration: Q-S151 CO2 2000ppm <Computer>

Q-S151 CO2 2000ppm <Computer>
Q-S151 CO2 2000ppm <Experiment File>

Calibration Retrieval Preference:
When this experiment file is opened, get this sensor's calibration from:
○ Sensor (calibration stored on sensor)
○ Computer (calibration stored on this computer)
● Experiment File (calibration stored with the current document)

c. Proceed to "Calibrate" in the same window (see below). Ensure the Current Calibration is selected as ***Q-S151 CO_2 Analyzer 2000ppm <Experiment>***. This selection will ensure that the calibration is saved with the Experiment file.



LabQuest Mini: 1 CH1: Q-S151 CO2 Analyzer (2000ppm)

Current Calibration: Q-S151 CO2 2000ppm <Computer>

Q-S151 CO2 2000ppm <Computer>
Q-S151 CO2 2000ppm <Experiment File>

Units: ppm

Choose Sensor(s) To Calibrate:
☑ LabQuest Mini: 1 CH1: Q-S151 CO2 Analyzer (2000ppm)

d. When the reading on the CO_2 analyzer is stable, use the small green screwdriver provided to adjust the "CO_2 Zero" control on the analyzer (if needed) to set the digital display to read 000 CO_2. In software, enter "Reading 1" as 0 and click "Keep"

e. Remove the soda lime column from the gas line. Attach the "in" port of the Q-P103 Gas Pump to a gas bag or tank with known CO_2 concentration.

f. If the Q-S151 display does not show the correct concentration of CO_2 in the calibration gas, adjust the display using the "CO_2 Span" control. When the Q-S151 CO_2 display shows the correct CO_2 concentration, enter that concentration as "Reading 2" in software and click "Keep" then "Done". **Save the experimental file under a new name so the new calibration is saved with the current file.** The CO_2 readings in Logger Pro software should now match those on the digital display of the CO_2 analyzer.

15. Calibration of the Q-S102 O_2 analyzer:

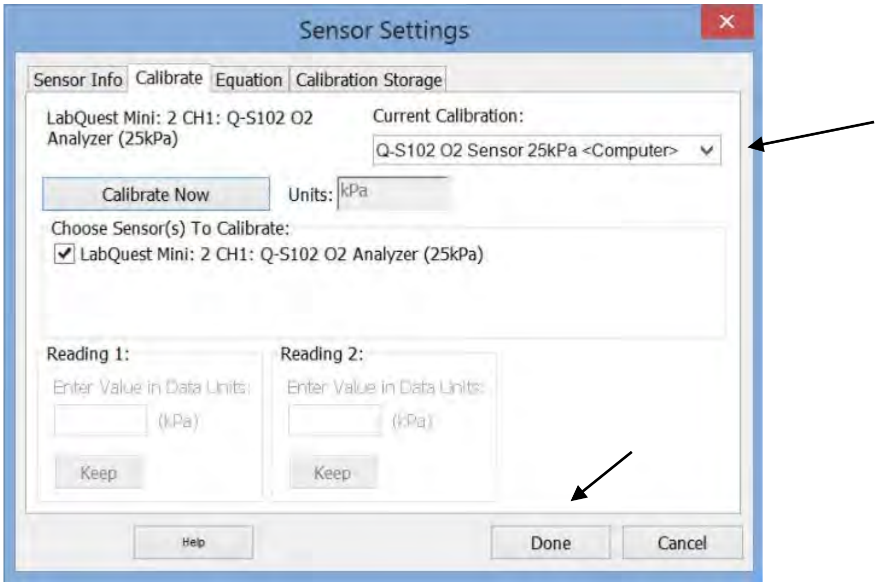
Note the Q-S102 analyzer measures partial pressure of O_2 (Q-S102 O2; kPa) as well as total gas pressure in the analyzer (Q-S102 Pres; kPa). Normally Q-S102 Pres is close to atmospheric pressure if the analyzer vents to atmosphere. The partial pressure of O_2 will follow atmospheric pressure changes (i.e. weather) and altitude, whereas the concentration is constant and independent of pressure variation. The analyzer O_2 partial pressure reading is corrected in software for changes in total gas pressure to produce O_2 (**Pcor**) reading in % units. These corrected readings (see calculation below) are displayed in the software in a meter.

O2 (Pcor) = ((Q-S102 O2)/(Q-S102 Pres))*100

Where Q-S102 O2 is the O_2 partial pressure, and *Q-S102 Pres* is the (total) gas pressure measured inside the QS102 O_2 analyzer.

To check and adjust the calibration of the Q-S102, follow these steps:

a. Set the O_2 analyzer in the 0 – 25 KPa range. This is the default range for measurements of respiratory O_2 consumption with the Q-Box RP1LP. Ensure the current calibration is selected as Q-S102 O2 Sensor 25KPa< Computer> then click Done.



LabQuest Mini: 2 CH1: Q-S102 O2 Analyzer (25kPa)

Current Calibration: Q-S102 O2 Sensor 25kPa <Computer>

Units: kPa

Choose Sensor(s) To Calibrate:

☑ LabQuest Mini: 2 CH1: Q-S102 O2 Analyzer (25kPa)

Reading 1: Enter Value in Data Units: (kPa)

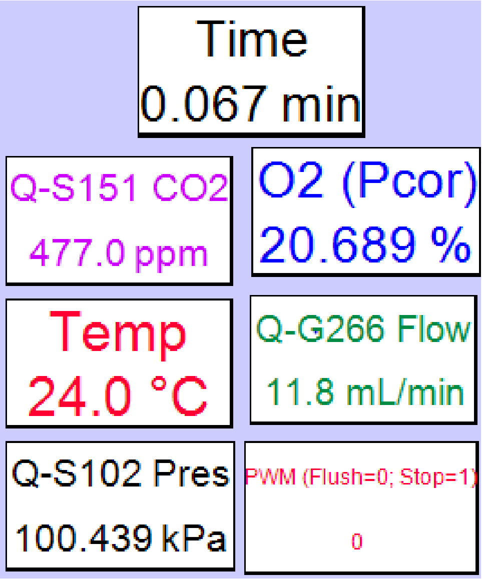
Reading 2: Enter Value in Data Units: (kPa)

b. Supply O_2-free gas (e.g. N_2) to the analyzer by attaching the O_2-free gas source (i.e. gas bag) to the “In” port of the Q-P103 Pump. As the O_2-free gas enters the Q-S102 O_2 Analyzer, O2 (Pcor) in the software on Page 1 will decrease toward zero (the first point of the calibration)

c. When the reading in the software is stable, use the small green screwdriver to adjust the “O_2 Zero” control on the analyzer until the O2 (Pcor) meter in the software reads 0.0 % O_2.

d. Attach the “In” port of the Q-P103 Gas Pump to a source of gas with known O_2 concentration (i.e. gas bag or air tank). Alternatively, dried room air contains 20.95% O_2.

e. When the O2 (Pcor) reading in software becomes stable, adjust the “O_2 Span” control on the analyzer until the reading of O2 (Pcor) corresponds to the O_2 concentration supplied to the analyzer.



Time
0.067 min

Q-S151 CO2
477.0 ppm

O2 (Pcor)
20.689 %

Temp
24.0 °C

Q-G266 Flow
11.8 mL/min

Q-S102 Pres
100.439 kPa

PWM (Flush=0; Stop=1)
0

O2 concentration Corrected for Changes in gas Pressure (%)

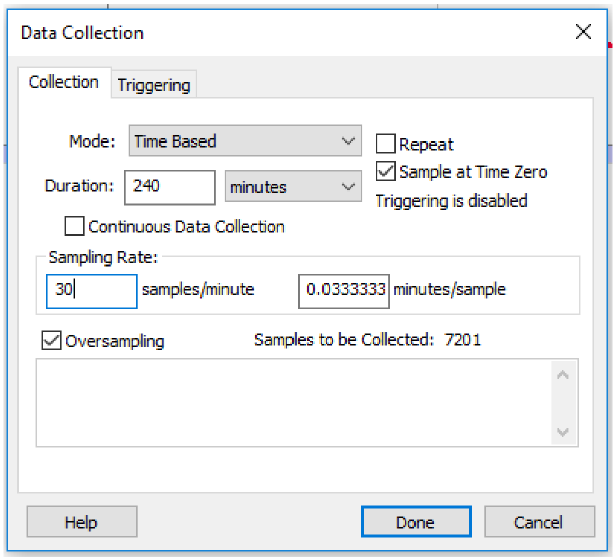
Total gas pressure at the O2 sensor (kPa)

f. If a significant adjustment had to be made to “O_2 Span”, return to the zero check and ensure that the zero reading on the analyzer has not shifted. It is not necessary to do the zero check while in the calibration mode of the software.

16. Assemble the flow through animal chamber by placing the S174 temperature probe (with O-rings) in the opening of the end cap as shown. If the temperature probe requires additional sealing around the opening, use the blue Qubitac sealant provided in the accessory kit. If the temperature probe is not used, ensure the hole is plugged by the included metal rod with o-rings. Insert the rod or temperature probe to the bottom of the channel so small specimens cannot enter this channel from the chamber.

17. Place the animal(s) in the chamber and attach the chamber and the solenoid assembly to the system as shown below.

18. Before collecting data with Logger Pro software, select ***Experiment > Data Collection*** in the main menu. The following dialog box will appear:



Mode: Time Based

Duration: 240 minutes

☐ Repeat

☑ Sample at Time Zero

Triggering is disabled

☐ Continuous Data Collection

Sampling Rate:

30 samples/minute

0.0333333 minutes/sample

☑ Oversampling

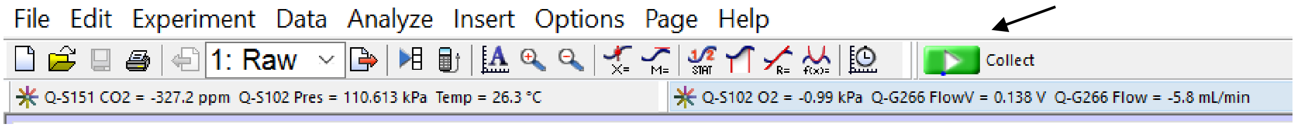
Samples to be Collected: 7201

Input experiment length and data sampling rate as required. For long experiments, the sample rate may need to be lowered to accommodate the large amount of data collected and processed online.

NOTE: Do not change the units of time i.e. keep minutes as the unit.

Select: ***File> Save As*** to save the experiment settings under the file name selected so that the original Experiment file (Enhanced Q-Box RP1LP Setup) is not over- written.

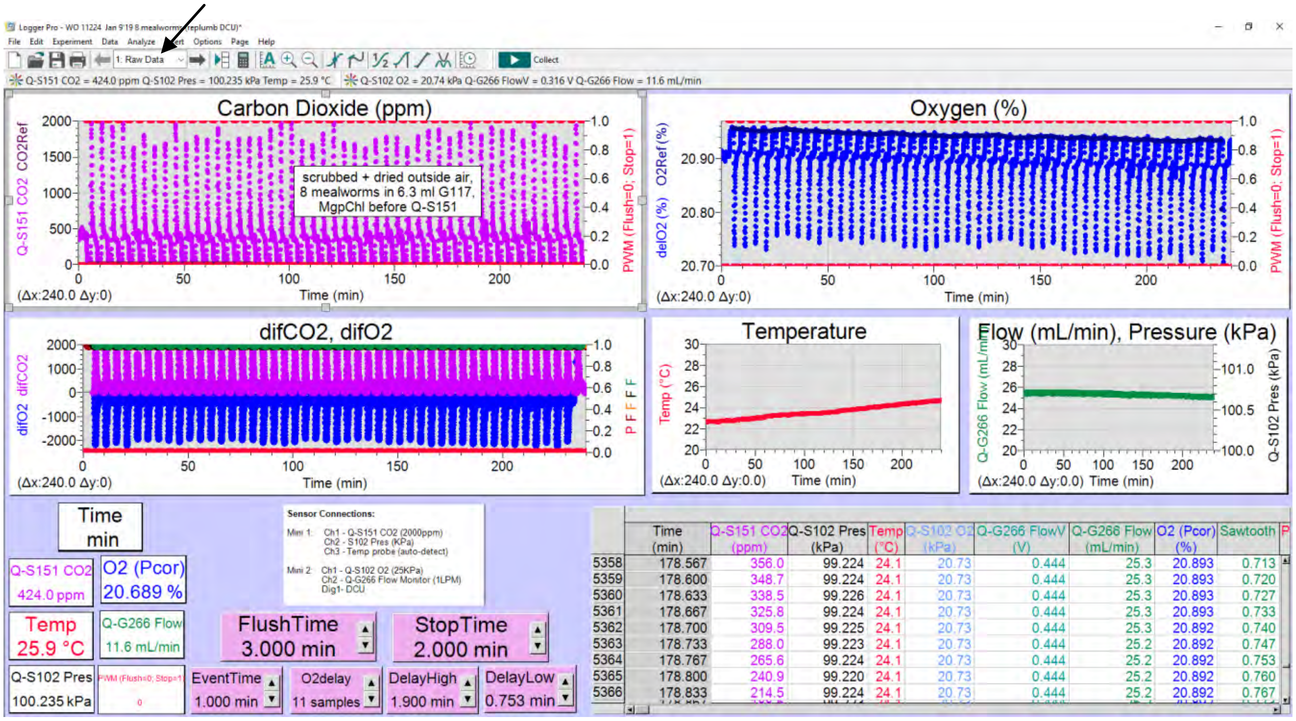
19. Start data collection by clicking the green "Collect" button, and stop data collection by clicking the red "Stop" button (green turns red during data collection)



Q-S151 CO2 = -327.2 ppm Q-S102 Pres = 110.613 kPa Temp = 26.3 °C

Q-S102 O2 = -0.99 kPa Q-G266 FlowV = 0.138 V Q-G266 Flow = -5.8 mL/min

20. Logger Pro displays data on Page 1 "Raw Data" as they are collected in graphs, meters and spreadsheet columns. Sample data from an experiment with 8 mealworms in a G117 chamber adjusted to 6.3 ml is shown below. CO_2-scrubbed and dried outside air was used as the carrier gas flowing at 25 ml/min. A 13 ml magnesium perchlorate column was used to dry the sample gas entering the gas analyzers.



Sensor Connections:

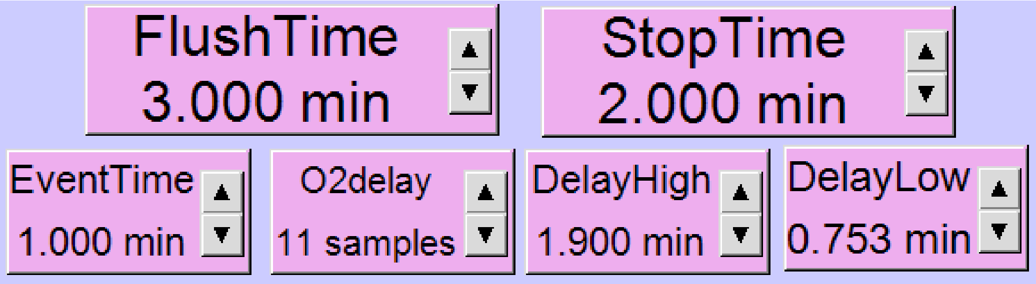
Mini 1: Ch1 - Q-S151 CO2 (2000ppm)
Ch2 - S102 Pres (KPa)
Ch3 - Temp probe (auto-detect)

Mini 2: Ch1 - Q-S102 O2 (25KPa)
Ch2 - Q-G266 Flow Monitor (1LPM)
Dig1- DCU

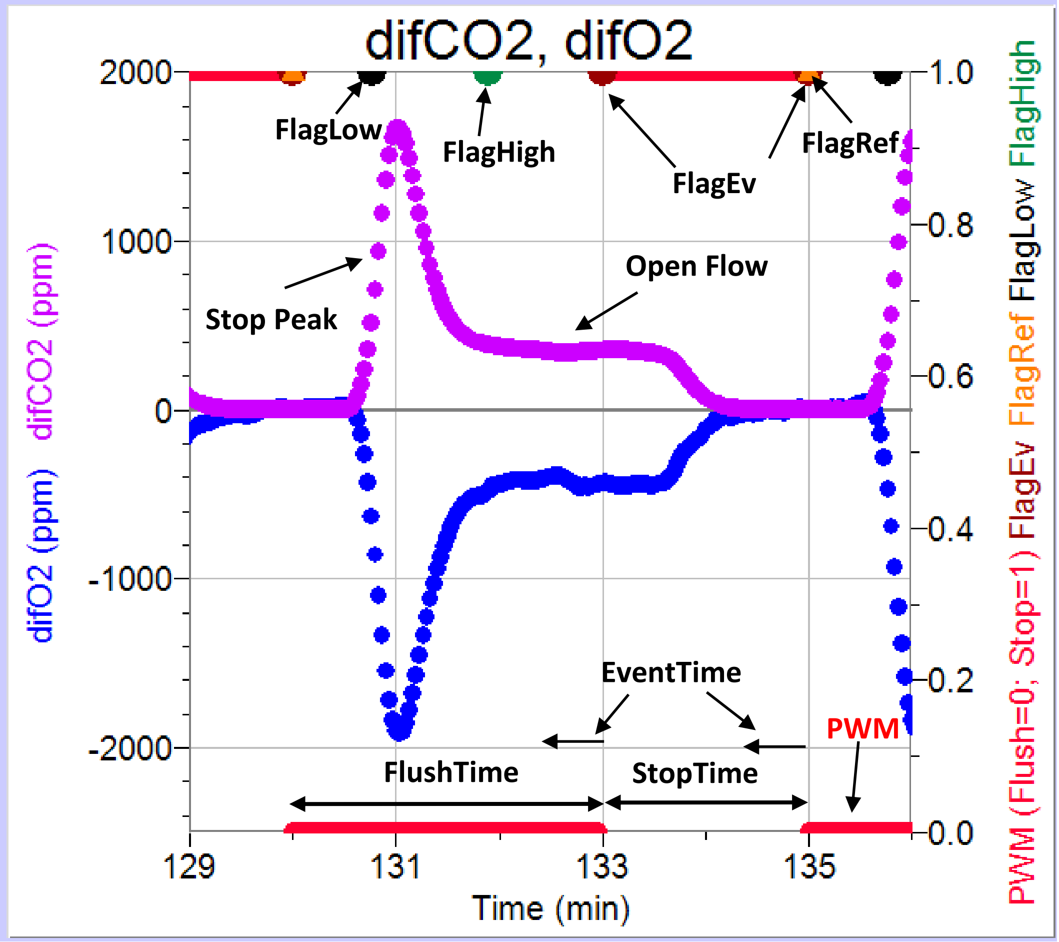
Q-S151 CO2 424.0 ppm
O2 (Pcor) 20.689 %
Temp 25.9 °C
Q-G266 Flow 11.6 mL/min
Q-S102 Pres 100.235 kPa
PWM (Flush=0; Stop=1) 0
FlushTime 3.000 min
StopTime 2.000 min
EventTime 1.000 min
O2delay 11 samples
DelayHigh 1.900 min
DelayLow 0.753 min

	Time (min)	Q-S151 CO2 (ppm)	Q-S102 Pres (kPa)	Temp (°C)	Q-S102 O2 (kPa)	Q-G266 FlowV (V)	Q-G266 Flow (mL/min)	O2 (Pcor) (%)	Sawtooth
5358	178.567	356.0	99.224	24.1	20.73	0.444	25.3	20.893	0.713
5359	178.600	348.7	99.224	24.1	20.73	0.444	25.3	20.893	0.720
5360	178.633	338.5	99.226	24.1	20.73	0.444	25.3	20.893	0.727
5361	178.667	325.8	99.224	24.1	20.73	0.444	25.3	20.893	0.733
5362	178.700	309.5	99.225	24.1	20.73	0.444	25.3	20.892	0.740
5363	178.733	288.0	99.223	24.1	20.73	0.444	25.2	20.892	0.747
5364	178.767	265.6	99.224	24.1	20.73	0.444	25.2	20.892	0.753
5365	178.800	240.9	99.220	24.1	20.73	0.444	25.2	20.892	0.760
5366	178.833	214.5	99.224	24.1	20.73	0.444	25.2	20.892	0.767

21. Before proceeding with an experiment the user must ***<u>very carefully</u>*** set the user parameters on Page 1 of the experimental Setup file. This ensures data can be automatically used in open and stop flow calculations and these are performed correctly. The user parameters may be changed by toggling the arrows of the right or by double clicking and typing the desired value.


FlushTime 3.000 min
StopTime 2.000 min
EventTime 1.000 min
O2delay 11 samples
DelayHigh 1.900 min
DelayLow 0.753 min

This is most easily done by collecting a short period of data and expanding the differential graph on Page 1 of the setup file to show a single flush-stop cycle as shown below.



FlushTime (PWM=0): Choose this time period to be long enough so that after the stop peaks have passed through the gas analyzers, steady state open flow is achieved.

StopTime (PWM=1): Select this time to be long enough to obtain suitable stop peaks without exceeding the 2000 ppm limit of the CO2 analyzer (unless only the open flow measurements are of interest for an active animal).

Note: There is a washout time after switching (~1 min in data shown above) before fresh gas reaches the gas analyzers. Both the flush and stop times will be influenced by carrier gas flow and specimen activity.

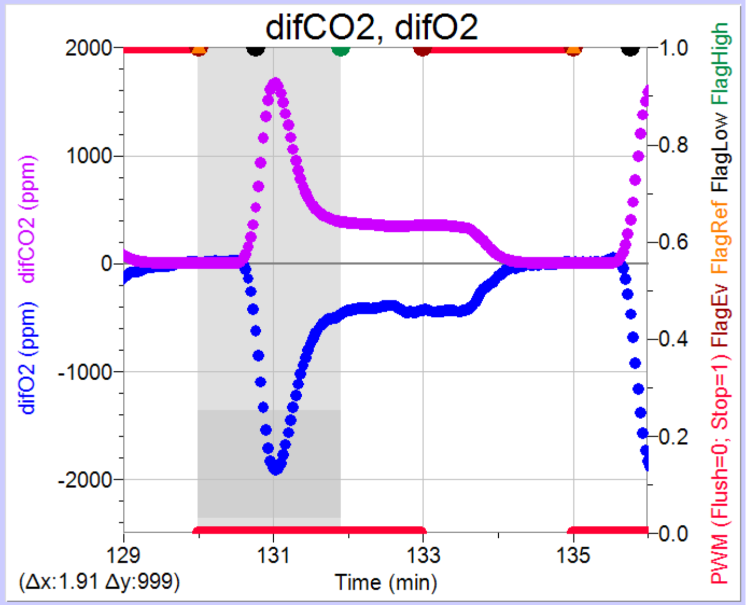
EventTime: At the end of the flush and stop phases, data is averaged over the EventTime prior to the switch. This event average is recorded at the time of switching shown by the large dark red dots at the top of the above graph (i.e. **FlagEv**). The event flag at the end of the stop phase (i.e. end of flush-stop cycle) is shown by an orange triangle (i.e. **FlagRef**).

O2delay: Since the O_2 analyzer follows the CO_2 analyzer in the setup, gas reaches the O_2 analyzer after passing through the CO_2 analyzer. Thus the O_2 signal is delayed with respect to the CO_2. Calculations of $\dot{V}CO_2$ and $\dot{V}O_2$ require both CO_2 and O_2 signals, hence these must be aligned (i.e. O2 shifted) by setting the **O2delay** user parameter. Alignment is done by placing a vertical line (menu: Analyze>Examine) on the CO2 peak and adjusting O2delay by how many sample points were measured. As this number is increased or decreased the O2 shifts into alignment with the CO2 signal.

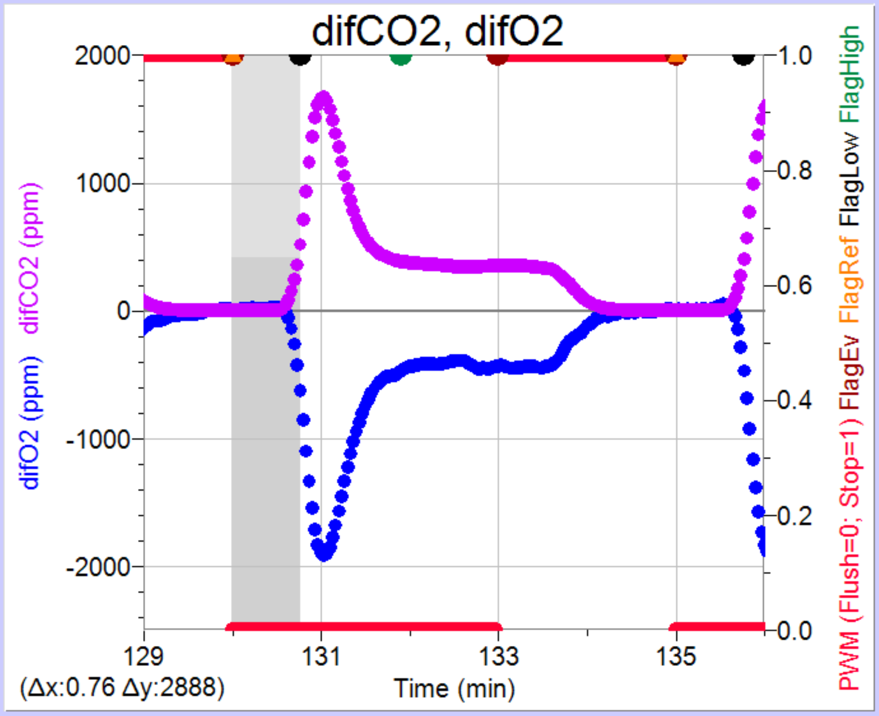
Note: Delay of the O_2 signal depends on gas flow and length of tubing between the gas analyzers so it will differ with experiments.

DelayHigh: The **DelayHigh** and **DelayLow** user parameters pertain to stop flow calculations and determine how accurately peak integrals are automatically computed for use in the stop flow calculations of $\dot{V}CO_2$ and $\dot{V}O_2$ on Page 3 of the setup file. **DelayHigh** can be adjusted by placing the cursor cross hairs on the time axis at the beginning of the flush phase (PWM=0) holding the left mouse button and sweeping to a time after the stop peaks where steady state has been achieved. The swept area will be greyed as shown in the image below. Determine the Δx from the lower left time axis (in the graph below it is 1.91) and adjust **DelayHigh** to this value. The end of DelayHigh is indicated by a green dot on the top of the graph (i.e FlagHigh).

Note: The placement of FlagHigh is not critical since the concentration levels of CO_2 and O_2 are fairly constant after the stop peak recording, unless the specimen has rapid changes in respiration.



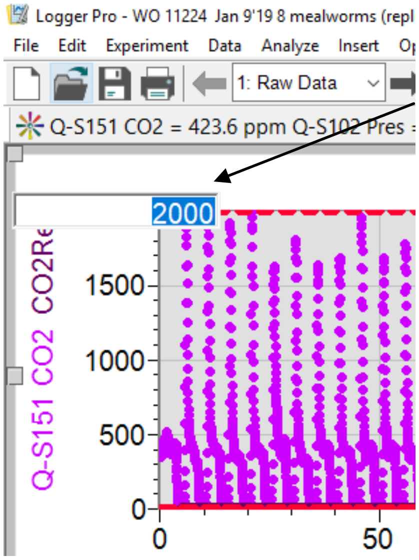
DelayLow: **This user parameter must be chosen extremely carefully so the area under CO_2 peak or above O_2 through is correctly calculated.** As explained above in determining **DelayHigh**, use the cursor to sweep data from the beginning of the flush phase (PWM=0) to a point on the left side of the CO2 peak where steady state open flow levels are being measured (see the data shown below). The value of Δx shown in the lower left side is the **DelayLow** (0.76 in example below). Enter this value or adjust **DelayLow** user parameter to this value. The end of DelayLow period is indicated by a black dot at the top of the graph (i.e. FlagLow).



Now the user can now proceed with collecting data for an experiment.

Note: FlushTime and StopTime parameters cannot be changed during or after completion of an experiment. These user parameters have to be set before start of the experiment. O2delay, DelayHigh and DelayLow parameters may be adjusted after completion of an experiment.

22. During the experiment, the range of the x and y axes can be adjusted by clicking on the lowest or highest number and typing in the desired new value as shown in the following diagram.



23. During collection of data, calculations of $\dot{V}CO_2$ and $\dot{V}O_2$ are done **automatically** in Logger Pro for both open flow differences and stop flow peaks/trough areas in the flush phase**. Open Flow calculations are graphed for each sample time** (i.e. instantaneously) during the flush phase (i.e. FlagHigh to FlagEv) and are displayed on Page 2 of the setup experimental file. These calculations use differences in concentration of CO2 and O2 measured during flush phase and interpolated reference gas concentrations from those measured during stop periods. A long flush time will provide a long open flow period after the stop flow peak/trough is recorded, where activity of a sample can be followed continuously.

The stop flow measurements can be optimized with long stop times. Calculations of $\dot{V}CO_2$ and $\dot{V}O_2$ are done using the area of the stop flow peak/trough and are displayed on page 3 of the setup experimental file. Stop flow measurements are more useful for smaller and less active specimens. **One stop flow calculation per cycle** is recorded and graphed at the end of each peak (i.e. **at FlagHigh**). As a result, the total cycle time (stop plus flush time) determines the time response for stop flow measurements. As a result, the time resolution is sacrificed for sensitivity in stop flow calculations. If frequent stop flow measurements are required for best time response then a short flush time should be selected which is just sufficient to record the full peak/trough. Only peak area and stop time are required for stop flow calculations of $\dot{V}CO_2$ and $\dot{V}O_2$. Chamber volume should be minimized to be just large enough to comfortably contain the specimen. Reducing chamber volume will not change the peak area but the peak height will increase and the peak width will decrease with chamber volume reduction allowing Santiago estop flow calculations. Gas flow should be low (~25 ml/min) so the gas analyzers (~20 sec response time) can respond to the peak washing through.

Upon completion of the experiment, the data can also be **analyzed manually** (i.e. offline), in Logger Pro using the various analysis tools in the ***Analyze*** menu, or by selecting the analysis icons in the top menu. Calculations for open flow can be done manually in the Logger Pro template **on Page 4 (open flow calculations)** or for stop flow **on Page 5 (stop flow calculations)**. These manual calculation templates are intended for educational applications where the students can see all the steps of the computations. These manual calculations also provide a secondary check on the **automatic calculations** which **are dependent on the user parameters** set on Page 1.

Note: The data can also be directly exported as a text file (***File > Export As > text***), which can then be opened in a worksheet such as Excel for further analysis and calculations.

Calculations of Metabolic Rates ($\dot{V}CO_2$ and $\dot{V}O_2$):

Overview

Measurements of metabolic rates are expressed as rates of CO_2 produced per unit time ($\dot{V}CO2$), or as O_2 consumed per unit time ($\dot{V}O_2$). The units are µl/min which can be converted by the user to µmol/min. Respiratory Quotient, RQ, is the ratio of CO_2 produced to O_2 consumed (i.e.

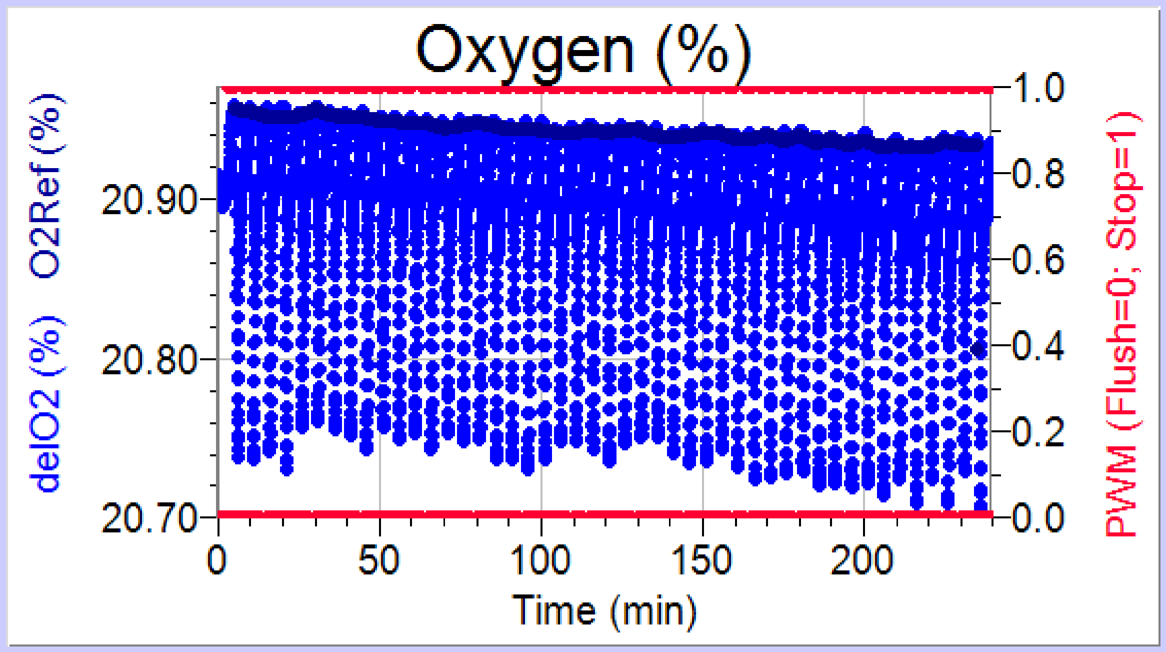
RQ=$\dot{V}CO_2/\dot{V}O_2$). RQ is directly related to the animal's diet and metabolic condition. RQ is usually less than 1.0, which means that more O_2 is consumed than CO_2 produced.

The incurrent CO_2 and O_2 concentrations (i.e. reference gas entering the chamber) are measured throughout the stop (i.e. bypass) phase, averaged over the EventTime and recorded at the end of the stop phase (i.e. at FlagRef). These event averages are interpolated to provide **CO2Ref** and **O2Ref** for every sample time. The excurrent concentrations (i.e. respiration-modified gas exiting the chamber) are measured during the flush phase. The interpolated reference values are subtracted to provide the differentials (i.e. **difCO2, difO2**). These differentials are used to calculate the open flow $\dot{V}$CO2 and $\dot{V}O_2$. Only peak volumes (calculated using differential flow) and stop time are required for stop flow computation of $\dot{V}$CO2 and $\dot{V}O_2$.

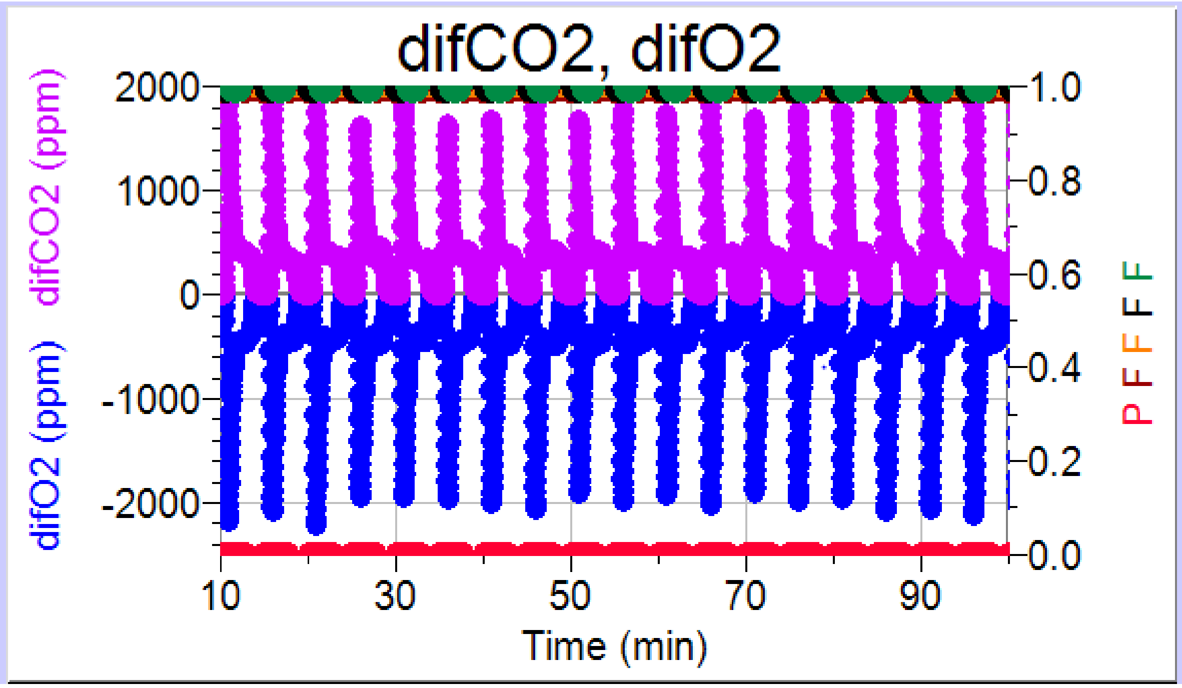
Open Flow Calculations of $\dot{V}CO_2$ and $\dot{V}O_2$

Open Flow (Automatic; Page 2 of Setup file):

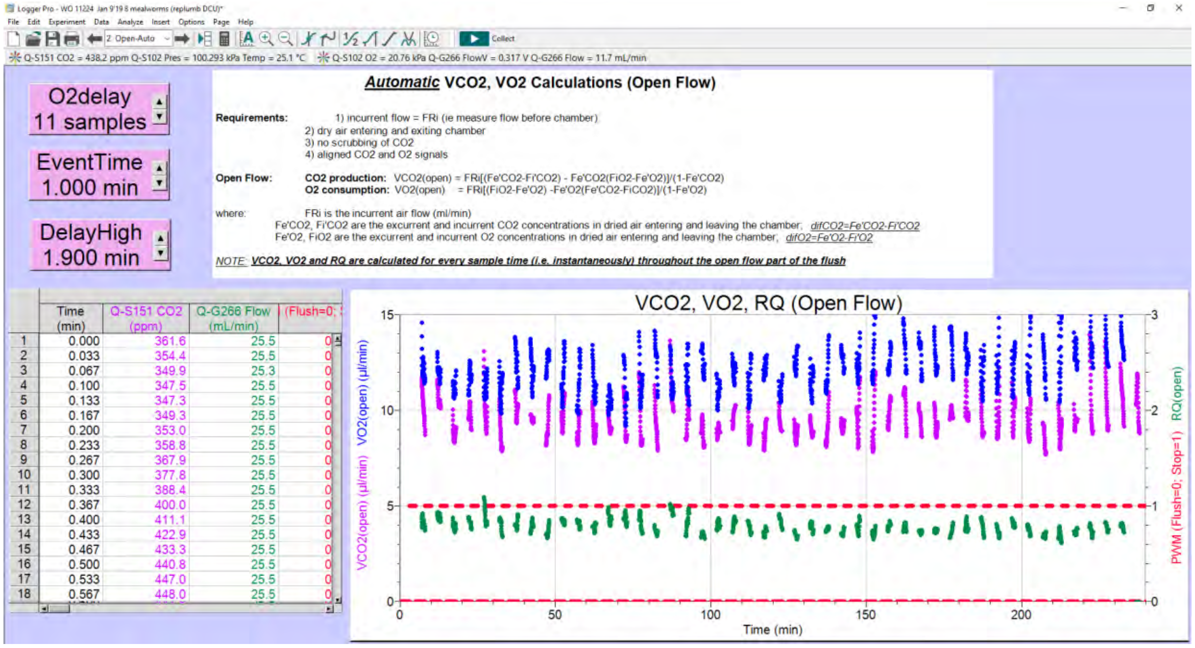
The automatic open flow measurements are useful for long term, high time resolution monitoring of respiration where the animal is active enough for the open flow signal (i.e. difference between reference and sample gases) to be accurately measured. In the **automatic, open flow calculations (Page 2 in Setup Experimental file)**, the instantaneous (i.e. at each sample time) incurrent concentrations of gases are obtained from the background measurements during the stop (i.e. bypass) periods. **These reference samples are linearly interpolated to provide a continuous (i.e. at each sample time) background levels to remove any drift in the signal.** This is done for both CO_2 and O_2 but is most useful for the O_2 data since O_2 differentials (ppm) are measured in the air background of ~21%=210,000 ppm. CO_2 background in outside air is only ~400 ppm. The CO2 analyzer has a resolution of ~ 1 ppm whereas the O2 resolution is ~ 40 ppm. The IR CO2 sensor is temperature controlled whereas the fuel cell O2 sensor has passive temperature control and is more prone to temperature-induced drift. The interpolated O_2 background is shown by the dark blue O2Ref(%) line in the following screen capture for an experiment with 8 mealworms in a 6.3 ml G117 chamber using CO_2-scrubbed and dried outside air flowing at 25 ml/min. The excurrent levels are obtained at each sample time during the open flow steady state part of the flush periods after the stop peak has washed through the gas analyzers. The equations used to calculate **$\dot{V}CO_2$ and $\dot{V}O_2$** are the same as those presented below for the manual open flow calculations on Page 4 in the Setup experimental file.



CO_2 and O_2 differentials (excurrent minus interpolated(incurrent)) are shown in the following differential graph from Page 1 (Raw Data) of Setup file. Any background drift has been removed by the interpolation between the reference samples obtained in the stop periods. These differences (difCO2, difO2) form the basis of both open and stop flow calculations of $\dot{V}CO_2$ and $\dot{V}O_2$.



The screen capture below shows the automatic open flow calculations found on Page 2 of the Setup file. Note the $\dot{V}CO_2$, $\dot{V}O_2$ and RQ values are retained only for the open flow portion of the flush phases after the stop peaks have washed trough the gas analyzers (i.e. between FlagHigh and FlagEv).



Q-S151 CO2 = 438.2 ppm Q-S102 Pres = 100.293 kPa Temp = 25.1 °C Q-S102 O2 = 20.76 kPa Q-G266 FlowV = 0.317 V Q-G266 Flow = 11.7 mL/min

Automatic **VCO2, VO2 Calculations (Open Flow)**

Requirements:
1) incurrent flow = FRi (ie measure flow before chamber)
2) dry air entering and exiting chamber
3) no scrubbing of CO2
4) aligned CO2 and O2 signals

Open Flow:
CO2 production: VCO2(open) = FRi[(Fe'CO2-Fi'CO2) - Fe'CO2(FiO2-Fe'O2)]/(1-Fe'CO2)
O2 consumption: VO2(open) = FRi[(FiO2-Fe'O2) -Fe'O2(Fe'CO2-FiCO2)]/(1-Fe'O2)

where:
FRi is the incurrent air flow (ml/min)
Fe'CO2, Fi'CO2 are the excurrent and incurrent CO2 concentrations in dried air entering and leaving the chamber; *difCO2=Fe'CO2-Fi'CO2*
Fe'O2, FiO2 are the excurrent and incurrent O2 concentrations in dried air entering and leaving the chamber; *difO2=Fe'O2-Fi'O2*

NOTE: ***VCO2, VO2 and RQ are calculated for every sample time (i.e. instantaneously) throughout the open flow part of the flush***

	Time (min)	Q-S151 CO2 (ppm)	Q-G266 Flow (mL/min)	(Flush=0;
1	0.000	361.6	25.5	0
2	0.033	354.4	25.5	0
3	0.067	349.9	25.3	0
4	0.100	347.5	25.5	0
5	0.133	347.3	25.5	0
6	0.167	349.3	25.5	0
7	0.200	353.0	25.5	0
8	0.233	358.8	25.5	0
9	0.267	367.9	25.5	0
10	0.300	377.8	25.5	0
11	0.333	388.4	25.5	0
12	0.367	400.0	25.5	0
13	0.400	411.1	25.5	0
14	0.433	422.9	25.5	0
15	0.467	433.3	25.5	0
16	0.500	440.8	25.5	0
17	0.533	447.0	25.5	0
18	0.567	448.0	25.5	0

Open Flow (Manual; Page 4 of Setup file):

Manual open flow **calculations** are intended for **short term respiration experiments** or for **teaching** purposes where the open flow method is applicable i.e. the difference in signal between reference and sample is substantial. Since the **flow monitor** in the Q-Box RP1LP package is **upstream** of the CO_2 and O_2 analyzers, and CO_2 or O_2 are not removed from the excurrent gas stream, the following equations are used to calculate $\dot{V}CO_2$ and $\dot{V}O_2$:

Lighton, J.R.B. (2008): "Measuring Metabolic Rates. A Manual for Scientists. Oxford University Press
(http://warthog.ucr.edu/WartHogPage/LAX%20website/respirometry.html)

Dilution of CO_2 due to the concentration of O_2, is accounted for in the $\dot{V}CO_2$ calculation and the concentration of CO_2 is accounted for in the $\dot{V}O_2$ calculation as shown in the following equations:

$$\dot{V}\mathbf{CO_2 = FR * ((FeCO_2 – FiCO_2) – FeCO_2 * (FiO_2 – FeO_2))/(1 - FeCO_2)}$$

$$\dot{V}\mathbf{O_2 = FR * ((FiO_2 – FeO_2) – FeO_2 * (FeCO_2\text{-}FiCO_2))/(1 – FeO_2)}$$

Where $FeCO_2$ is the fractional excurrent CO_2 concentration, and $FiCO_2$ is the fractional incurrent CO_2.

$$\mathbf{FeCO_2 = eCO_2 \text{ in ppm}/1000,000}$$

$$\mathbf{FiCO_2 = iCO_2 \text{ in ppm}/1000,000}$$

FeO_2 is the fractional excurrent O_2 concentration, and FiO_2 is the fractional incurrent O_2

$$\mathbf{FeO_2 = eO_2 \text{ in } \%/100}$$

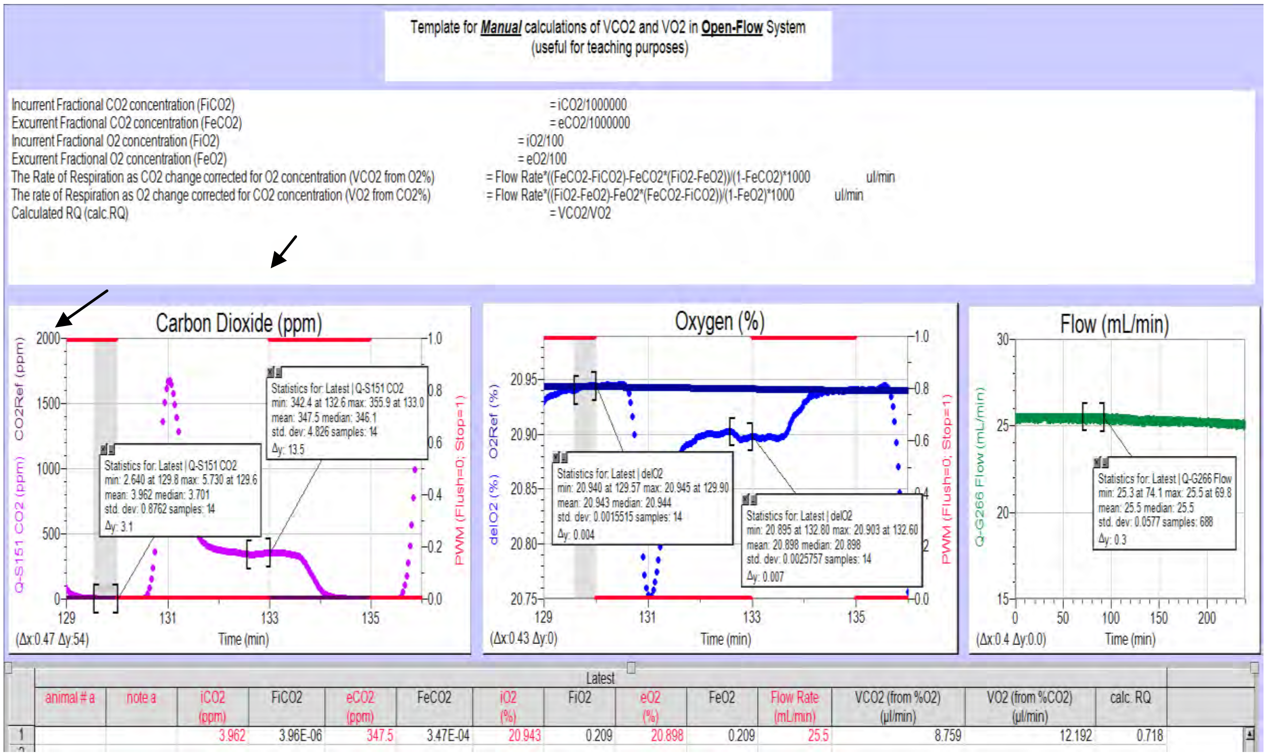
$$\mathbf{FiO_2 = iO_2 \text{ in } \%/100}$$

Respiratory Quotient, RQ , is the ratio of CO_2 production to the O_2 consumption which can be related to the food metabolized.

$$\mathbf{RQ = \dot{V}CO_2/\dot{V}\ O_2}$$

The following screen shows Page 4 of the setup file where manual open flow calculations of $\dot{V}CO_2$, $\dot{V}O_2$ and RQ are done. The example shown is for 8 mealworms in a 6.3 ml chamber. The reference gas was outside air scrubbed of CO_2 with soda lime and dried with Drierite. The gas exiting the chamber was dried with a 13 ml Magnesium perchlorate column before entering the gas analyzers. The carrier gas flow was 25 ml/min.

To complete the manual calculations on Page 4, the values in **red columns of the table** (iCO2, eCO2, iO2, eO2 and Flow Rate) must be **entered manually**. These values are obtained directly from the raw data of Carbon Dioxide (ppm) and Oxygen (%) graphs shown on Page 4 (copied from Page 1). The user has to manually highlight the data collected in the bypass (i.e. stop) and flush phases respectively. Next, select the statistics icon from the menu (Analyze>Statistics) and obtain the averages from the statistics window as shown in the image below. Enter these average values into the appropriate red columns in the table. When the required input values are entered, the calculations (black columns) will populate automatically.



Template for ***Manual*** calculations of VCO2 and VO2 in **Open-Flow** System
(useful for teaching purposes)

Incurrent Fractional CO2 concentration (FiCO2) =iCO2/1000000
Excurrent Fractional CO2 concentration (FeCO2) =eCO2/1000000
Incurrent Fractional O2 concentration (FiO2) =iO2/100
Excurrent Fractional O2 concentration (FeO2) =eO2/100
The Rate of Respiration as CO2 change corrected for O2 concentration (VCO2 from O2%) = Flow Rate*((FeCO2-FiCO2)-FeCO2*(FiO2-FeO2))/(1-FeCO2)*1000 ul/min
The rate of Respiration as O2 change corrected for CO2 concentration (VO2 from CO2%) = Flow Rate*((FiO2-FeO2)-FeO2*(FeCO2-FiCO2))/(1-FeO2)*1000 ul/min
Calculated RQ (calc.RQ) =VCO2/VO2

Statistics for: Latest | Q-S151 CO2
min: 2.640 at 129.8 max: 5.730 at 129.6
mean: 3.962 median: 3.701
std. dev: 0.8762 samples: 14
Δy: 3.1

Statistics for: Latest | Q-S151 CO2
min: 342.4 at 132.6 max: 355.9 at 133.0
mean: 347.5 median: 346.1
std. dev: 4.826 samples: 14
Δy: 13.5

Statistics for: Latest | delO2
min: 20.940 at 129.57 max: 20.945 at 129.90
mean: 20.943 median: 20.944
std. dev: 0.0015515 samples: 14
Δy: 0.004

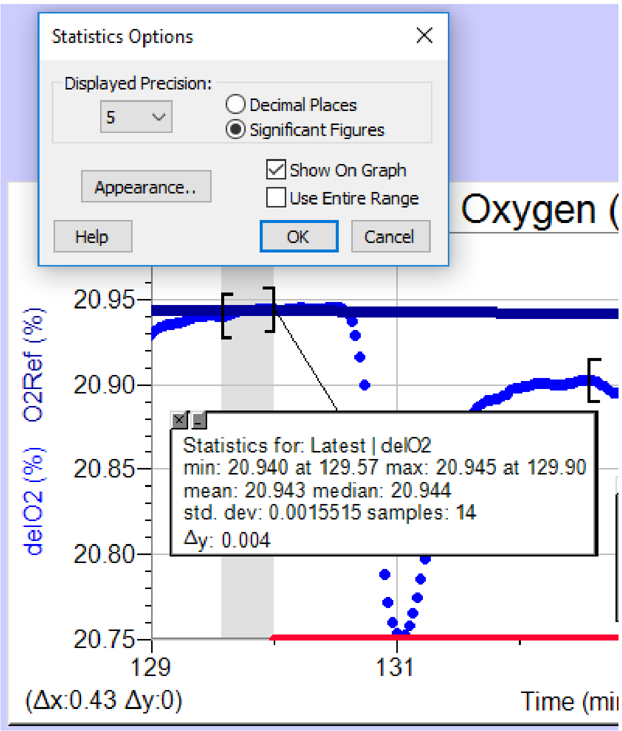
Statistics for: Latest | delO2
min: 20.895 at 132.80 max: 20.903 at 132.60
mean: 20.898 median: 20.898
std. dev: 0.0025757 samples: 14
Δy: 0.007

Statistics for: Latest | Q-G266 Flow
min: 25.3 at 74.1 max: 25.5 at 69.8
mean: 25.5 median: 25.5
std. dev: 0.0577 samples: 688
Δy: 0.3

Latest

	animal # a	note a	iCO2 (ppm)	FiCO2	eCO2 (ppm)	FeCO2	iO2 (%)	FiO2	eO2 (%)	FeO2	Flow Rate (mL/min)	VCO2 (from %O2) (µl/min)	VO2 (from %CO2) (µl/min)	calc. RQ
1			3.962	3.96E-06	347.5	3.47E-04	20.943	0.209	20.898	0.209	25.5	8.759	12.192	0.718

Note: All graphs can be selected by clicking on each graph while holding down the shift key. Then select the data of interest on one of the graphs (e.g. Q-S151 CO_2 values during the steady state response of the animal) by holding down the shift key and dragging the mouse over the region of interest on that graph. Note that data on the other selected graphs for that time period will also be highlighted. When the data have been selected on all the graphs, the appropriate analysis tool can be chosen (i.e. "Statistics" analysis) while still holding the shift key. The statistics will then be shown in a separate window for the selected data on each of the graphs. The number of decimal places or the size of the font to be displayed can be changed by right clicking on the analysis window as shown below.



Statistics Options

Displayed Precision: 5

○ Decimal Places

● Significant Figures

☑ Show On Graph

☐ Use Entire Range

Statistics for: Latest | delO2
min: 20.940 at 129.57 max: 20.945 at 129.90
mean: 20.943 median: 20.944
std. dev: 0.0015515 samples: 14
Δy: 0.004

Stop-Flow Calculations of ($\dot{V}CO_2$ and $\dot{V}O_2$)

Stop Flow (Automatic; Page 3 of Setup file):

When measuring very low metabolic rates (e.g. in single small insects), the system can be used in Stop flow mode and the **stop-flow calculations** will provide the data. Although stop flow provides higher sensitivity, it has a much lower time resolution than the open flow method since the stop flow peak/trough occurs only once per stop/flush cycle. The automatic calculations provide a means of monitoring low respiration rates over long periods. Depletion of O_2 as well as the accumulation of CO_2, are then measured as boluses when the chamber is flushed and these gases are swept out to the gas analyzers. The stop flow peak/trough can be enhanced by increasing the stop time. Note the flow must be low enough (~25 ml) so that the gas analyzers have sufficient time to respond to the peak/trough washing through.

For stop flow calculations, difCO2, difO2 are multiplied by the gas flow at each sample time to produce **difFlow** graphs. difO2Flow is converted into positive values to facilitate integration of the area, i.e. Neg(difFlowO2) = -difFlowO2. This results in the difFlowO2 trough being converted to a peak. The use of difFlow accounts for any change in flow, and more importantly, difFlowCO2 and Neg(difFlowO2) can be directly integrated to give respired CO_2 and O_2 **volumes**, respectively. Page 3 of the Setup file is shown below for automatic stop flow calculations. The same mealworm raw data discussed above for open flow calculations was used. $\dot{V}CO_2$, $\dot{V}O_2$ and RQ for stop flow peaks are automatically computed on Page 3 of the Setup file using the same calculations as explained below in the discussion of manual stop flow calculations (Page 5 of the Setup file). The computations are performed once per stop/flush cycle at the end of each stop peak (i.e. at **FlagHigh**) and plotted in the VCO_2, VO_2 and RQ graphs on Page 3 of the Setup file. These automatic graphs are useful for long term monitoring of respiration in small specimens.

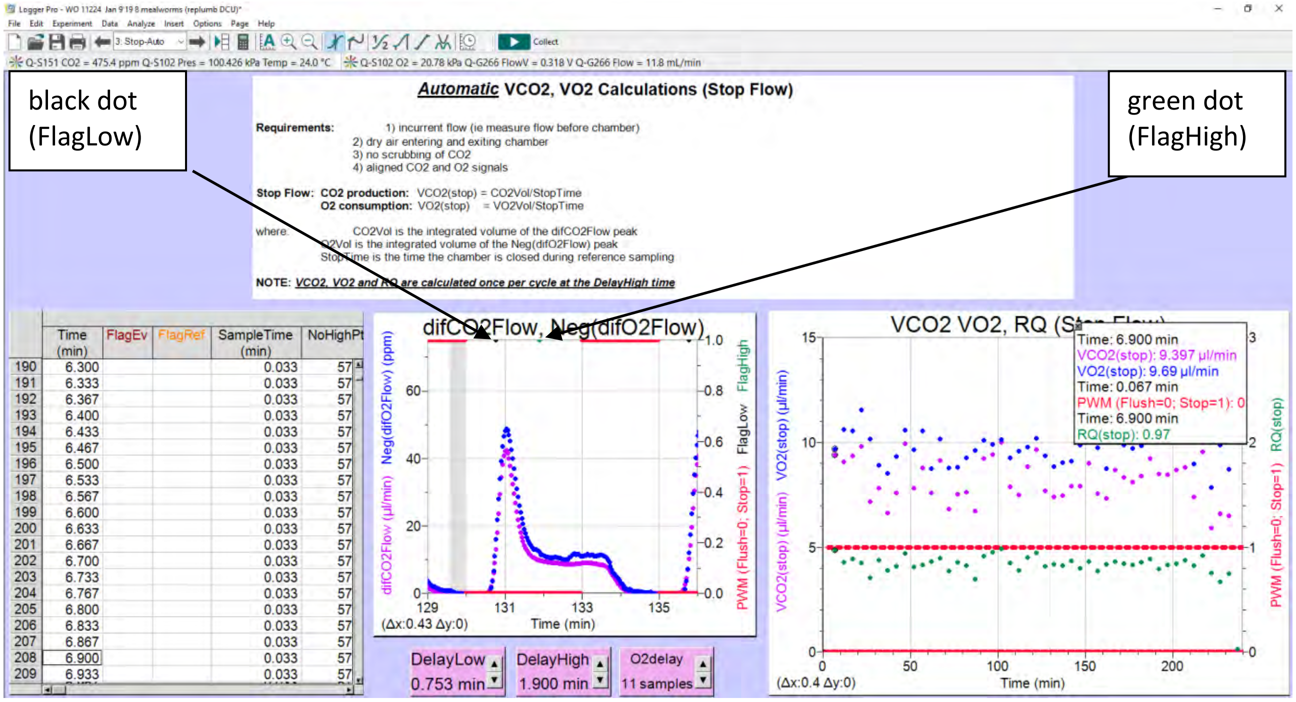
NOTE:

To obtain accurate peak integration, the user parameters on Page 3 of the Setup file (the same ones shown on Page 1) (**DelayLow, DelayHigh an O2delay**) must be *carefully* adjusted by the user, either by toggling the arrows or selecting and typing in the desired value. If as discussed earlier, these parameters were adjusted by collecting a short trial run before the actual experiment and using a differential peak from the graph on Page 1, further adjustment will unlikely be necessary on Page 3. If calculated data is not reasonable these parameters (DelayHigh, DelayLow and O2delay) should be checked and they may be adjusted after data collection has been completed.

The black dot (**FlagLow**) on the difFlow graph on Page 3 (see graph below) corresponds to the **DelayLow** user parameter position and may be adjusted by reducing the value of **DelayLow** parameter (so the the left side of the peaks correspond to the steady state open flow level similar to the right side of the peak). The green dot (**FlagHigh**) on this graph corresponds to the **DelayHigh** user parameter position and should be adjusted on the right side of the peaks to correspond with the beginning of the steady state level after the peak has passed the gas analyzers. This procedure is required to ensure that only the area of the peaks without the open flow steady state gas levels will be used in calculations.

The **O2delay** user parameter must also be *carefully adjusted* to align the CO_2 and O_2 data, so that in calculations requiring both CO_2 and O_2, these are selected from the correct period in time. This misalignment is due to the two gas analyzers being in series with CO_2 being measured first and O_2 second. The gas flow through the system, and tubing length between the CO_2 and O_2 analyzers will also contribute to this misalignment. The user can toggle this parameter until the correct number of samples is selected and the **difFlowCO2** and **Neg(difFlowO2)** peaks are aligned.

The stop peak areas will be calculated and graphed once per flush-stop cycle at the FlagHigh position as shown in the $\dot{V}CO_2$, $\dot{V}O_2$ and RQ graph on Page 3 of the Setup file.



black dot (FlagLow)

green dot (FlagHigh)

Q-S151 CO2 = 475.4 ppm Q-S102 Pres = 100.426 kPa Temp = 24.0 °C Q-S102 O2 = 20.78 kPa Q-G266 FlowV = 0.318 V Q-G266 Flow = 11.8 mL/min

Automatic **VCO2, VO2 Calculations (Stop Flow)**

Requirements: 1) incurrent flow (ie measure flow before chamber)
2) dry air entering and exiting chamber
3) no scrubbing of CO2
4) aligned CO2 and O2 signals

Stop Flow: **CO2 production:** VCO2(stop) = CO2Vol/StopTime
O2 consumption: VO2(stop) = VO2Vol/StopTime

where: CO2Vol is the integrated volume of the difCO2Flow peak
O2Vol is the integrated volume of the Neg(difO2Flow) peak
StopTime is the time the chamber is closed during reference sampling

NOTE: ***VCO2, VO2 and RQ are calculated once per cycle at the DelayHigh time***

	Time (min)	FlagEv	FlagRef	SampleTime (min)	NoHighPt
190	6.300			0.033	57
191	6.333			0.033	57
192	6.367			0.033	57
193	6.400			0.033	57
194	6.433			0.033	57
195	6.467			0.033	57
196	6.500			0.033	57
197	6.533			0.033	57
198	6.567			0.033	57
199	6.600			0.033	57
200	6.633			0.033	57
201	6.667			0.033	57
202	6.700			0.033	57
203	6.733			0.033	57
204	6.767			0.033	57
205	6.800			0.033	57
206	6.833			0.033	57
207	6.867			0.033	57
208	6.900			0.033	57
209	6.933			0.033	57

DelayLow 0.753 min
DelayHigh 1.900 min
O2delay 11 samples

Time: 6.900 min
VCO2(stop): 9.397 µl/min
VO2(stop): 9.69 µl/min
Time: 0.067 min
PWM (Flush=0; Stop=1): 0
Time: 6.900 min
RQ(stop): 0.97

Stop Flow (Manual; Page 5 of Setup file):

Manual stop flow calculations are intended for short term experiments measuring low respiration, and also for teaching purposes. For calculations of $\dot{V}CO_2$ and $\dot{V}O_2$ in stop-flow, integrals of the CO_2 and O_2 peaks in the difFlowCO2 and Neg(difO2Flow) graphs, respectively, as well as the stop time must be known.

$$\dot{V}\mathbf{CO_2} = \mathbf{CO2Vol/StopTime}$$

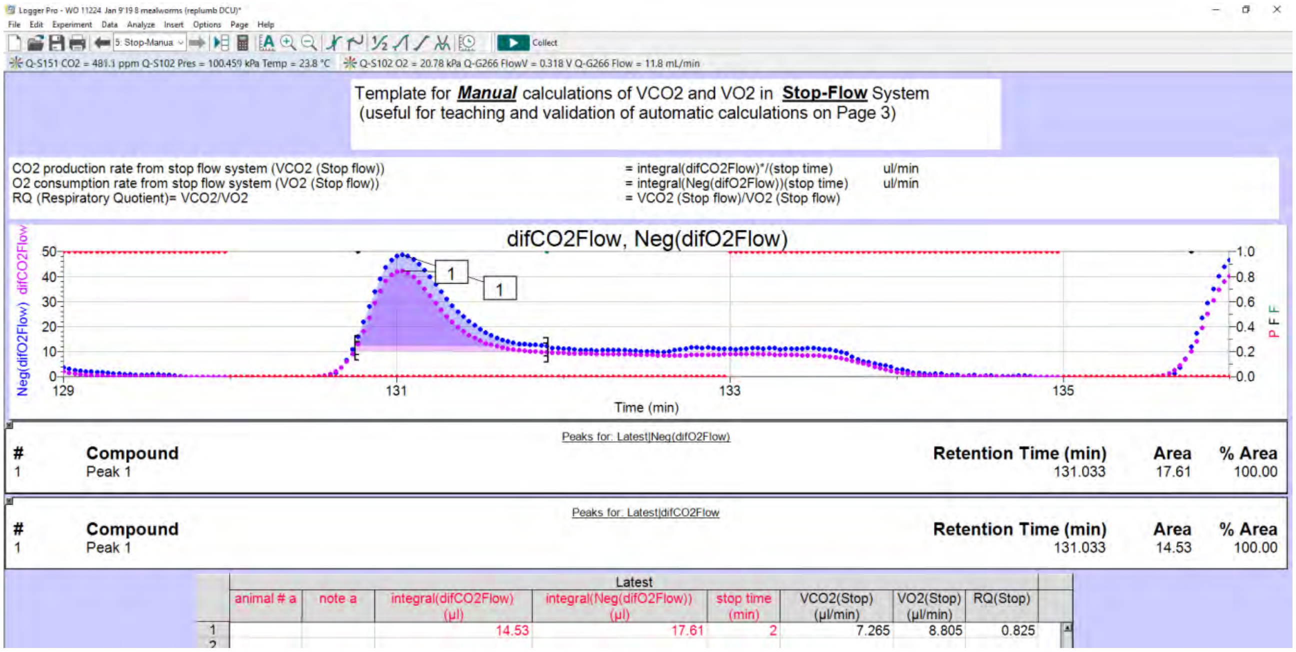
$$\dot{V}\mathbf{O_2} = \mathbf{O2Vol/StopTime}$$

$$\mathbf{RQ} = \dot{V}\mathbf{CO_2}/\dot{V}\mathbf{O_2}$$

CO2Vol and the **O2Vol** are the respired volumes of CO_2 and O_2, respectively. These volumes are provided by integrating the stop peaks in the difCO2Flow and Neg(difO2Flow) graphs. StopTime (min) is the user parameter from Page 1 indicating the time that flow through the chamber is stopped (bypassed) to produce the stop peak. This value must be entered in the table on Page 5 of the Setup file. The following expanded graphs (Page 5 of setup file) for the same mealworm data discussed above, shows the integrals for the $difCO_2Flow$ and Neg(difO2Flow) peaks. The integrals (i.e. areas under the peaks) were calculated using the Logger Pro analysis tool: menu: *Analyze>Peak Integration*

Note: 1) The *"Integral" analysis tool does not subtract the background levels of CO2 and O2 that are measured during open flow portion of the cycle* and therefore cannot be used to integrate the peaks generated in stop flow, unless the background level (i.e. open flow level after the stop peak) is essentially zero (e.g. very small specimens).

2) Ensure the user parameters DelayLow, DelayHigh and O2Delay are *carefully adjusted* as discussed above for the automatic stop flow calculations on Page 3 of the setup file. Enter the required data in the red columns of the table. The Black columns will be automatically calculated.



Q-S151 CO2 = 481.1 ppm Q-S102 Pres = 100.459 kPa Temp = 23.8 °C Q-S102 O2 = 20.78 kPa Q-G266 FlowV = 0.318 V Q-G266 Flow = 11.8 mL/min

Template for ***Manual*** calculations of VCO2 and VO2 in **Stop-Flow** System
(useful for teaching and validation of automatic calculations on Page 3)

CO2 production rate from stop flow system (VCO2 (Stop flow)) = integral(difCO2Flow)*/(stop time) ul/min
O2 consumption rate from stop flow system (VO2 (Stop flow)) = integral(Neg(difO2Flow))(stop time) ul/min
RQ (Respiratory Quotient)= VCO2/VO2 = VCO2 (Stop flow)/VO2 (Stop flow)

Peaks for: Latest|Neg(difO2Flow)

#	Compound	Retention Time (min)	Area	% Area
1	Peak 1	131.033	17.61	100.00

Peaks for: Latest|difCO2Flow

#	Compound	Retention Time (min)	Area	% Area
1	Peak 1	131.033	14.53	100.00

	Latest							
	animal # a	note a	integral(difCO2Flow) (µl)	integral(Neg(difO2Flow)) (µl)	stop time (min)	VCO2(Stop) (µl/min)	VO2(Stop) (µl/min)	RQ(Stop)
1			14.53	17.61	2	7.265	8.805	0.825
2								

Installing and Running Logger Pro 3

PC Users:

(1) To start, a complete copy of Logger Pro 3 must be installed on the computer. Before starting the installation, make sure all USB cables are disconnected from the 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 Mini or other interfaces to the computer.

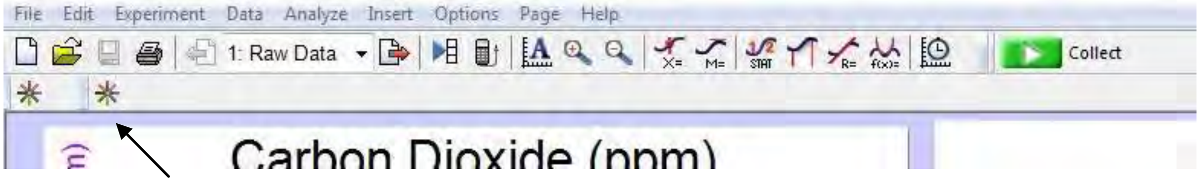
(4) If QuickTime 6 (or greater) is not installed on the computer, install it when prompted. QuickTime will allow use of the picture and movie features of Logger Pro 3.

(5) You will be prompted to connect the LabQuest Mini or other interfaces to the computer via the USB connection. The LabQuest Minis can be connected to the computer by connecting the USB1 and USB2 cables **(in that order)** from the Q-Box frame into the computer

(6) Click 'Finish' to complete the installation process.

(7) Proceed to C404 installation (below) before opening the Logger Pro with the “Enhanced Q-Box RP1LP Setup” file.

(8) Double click the “**Enhanced Q-Box RP1LP Setup**” file (create a shortcut on the desktop once moved from the C404 disk for easy access) to start Logger Pro and data collection. If Logger Pro detects the two LabQuest Mini interfaces, the Logger Pro screen will appear with two stars (icons for LabQuest Minis) in the top left corner.



(9) If Logger Pro cannot detect the LabQuest Mini Interfaces, a message will appear “no device connected”. Check that the LabQuest Minis are attached to the computer directly via USB connectors 1 and 2 on the frame of the Q-Box. The LED power lights on the Minis should be green. No LED light indicates that power is not supplied to the Minis - check power connections. A red LED on the minis indicates that power is on but there is no communication between the interfaces and the computer. In this case, exit the “Enhanced Q-Box RP1LP Setup” file and disconnect the USB cables from the computer. Reconnect the USB cables with **USB1 connected first and USB2 second**. This order is important as **the computer will assign the LabQuest Minis as 1 or 2 according to the order in which they were connected**. Allow the computer to recognize the Minis and then reopen the “Enhanced Q-Box RP1LP Setup” file.

Macintosh Users:

(1) To start, a complete copy of Logger Pro 3 must be installed on the computer (You must be using at least OS 9.2). Run the “Complete Installation” and ensure all TI GRAPH_LINK and USB cables are disconnected. The most recent version of Logger Pro (3) is included with this package. The following instructions are the same as those for PC users.

Note: Logger Pro Software may be upgraded to the latest version, free of charge, as long as the original purchased version (3.0 or higher) is on the computer already. Most recent version of logger pro may be obtained at the following link: http://www.vernier.com/support/updates/logger-pro/

C404 Custom Setup Files Installation:

Qubit Systems' C404 Custom Setup Files disk contains Experimental files (designed by Qubit Systems) for this package. For Enhanced RP1LP packages, the **Enhanced Q-Box RP1LP Setup** and the standard **Q-Box RP1LP Setup** (for use in open flow measurements of respiration) files are included. When using the standard Q-Box RP1LP Setup the DCU and the A380-E solenoid assembly are not used. The C404 disk also contains the manual for this package, the standard package and the manual for the LabQuest mini data interface. These files can be copied to a user specified location on the computer and the experimental file of interest "**Enhanced Q-Box RP1LP Setup**" should be placed in an accessible location or have a shortcut created on the desktop to the file. We highly recommend the user makes a copy of the original file and keeps it in a safe place on the computer in case the original is accidentally altered.

Using the Q-S151 CO_2 Analyzer

The Q-S151 CO_2 Analyzer is a non-dispersive infrared gas analyzer (NDIR IRGA). It has a gas "in" port with a female luer-lock connector and a gas "out" port with a male luer-lock connector. Tygon tubing with a luer-lock connector is provided to join the Q-S151 to the rest of the gas exchange system. The gas supplied to the IRGA passes through a sealed wave guide and vents from the "out" port. The Q-S151 can be used as a stand-alone analyzer in an open or stop-flow gas exchange system. It can be used with other gas analyzers downstream providing they do not cause a significant backpressure. Increasing pressure significantly beyond that at which the IRGA was calibrated, produces erroneously high readings.

The maximum flow of gas into the Q-S151 should not exceed 650 mL/min. The flow, is provided by the Q-P103 Gas pump and monitored by the Q-G266 Flow Monitor. It is recommended the user calibrates the Q-S151 at the flow used during the experiments. For **open flow** measurements, start with a **flow of 100-200ml/min** and reduce it if the differential signal is low. In **stop-flow measurements**, the recommended flow is **25-50ml/min.** This flow may have to be reduced for very small volume chamber.

Gases entering the Q-S151 must be clean and dry, since particulate matter may absorb infrared light and cause erroneous readings. Water vapour will not interfere with the IR absorption measurement of CO_2, but water vapour will dilute the CO_2 concentration slightly. The Q-S151 CO_2 analyzer is supplied with a drying column containing DRIERITE. This drying agent removes moisture from the analysis gas before it enters the CO_2 analyzer. Wool plugs at the base and top of the column prevent particulate matter from leaving the column.

The Q-S151 CO_2 Analyzer requires 12 Volts DC power to operate. A 120/220 AC power adaptor (included) provides 12 Volts DC. For continuous use, keeping the Q-S151 powered up will maintain the calibration longer.

Caution: Use only a 12 VDC adapter with the correct AC line voltages. A 120/220 VAC 50/60 Hz to 12 VDC adapter is supplied by QUBIT SYSTEMS.

The Q-S151 requires a 2 to 3 minute warm-up period before the CO_2 level will be displayed. During warm-up, the LCD will flash numbers briefly and display the number 1. After warm up, the LCD will display a very high reading, which will slowly decline to the CO_2 level in the supplied gas stream.

For most accurate and stable readings, warm up the Q-S151 for 15 - 30 min before use. If the Q-S151 is to be used on a regular basis, it should be left powered on via the 12 VDC adapter.

Altitude and Barometric Pressure Correction

The Q-S151 is factory calibrated at sea level. When using the IRGA at elevations other than sea level, calibrate at the elevation at which the analyzer will be used. If this is not possible, correct the CO_2 reading of the analyzer according to the table below. For example, when using a factory-calibrated unit at an altitude of 2,500 feet, multiply the displayed CO_2 reading by 89% (1,000 ppm x 0.89 = 890 ppm actual).

Altitude (feet)	**Pressure (inches Hg)**	**Pressure (mm Hg)**	**Pressure (psia)**	**% Display Reading**
0 sea level	29.92	759.78	14.70	100
500	29.38	746.04	14.43	97.97
1000	28.86	732.84	14.18	95.94
1500	28.34	719.64	13.92	93.91
2000	27.82	706.43	13.66	91.88
2500	27.32	693.73	13.42	89.84
3000	26.82	681.04	13.17	87.81
3500	26.32	668.34	12.93	85.78
4000	25.82	655.65	12.68	83.75
4500	25.36	643.96	12.46	81.72
5000	24.90	632.28	12.23	79.69

For other altitudes, use the following equation:

Percentage of measured CO_2 = $[1 - (4.06234 \times 10^{-5} \times \text{Altitude in feet})] \times 100$

Using the Drying Column and Soda Lime Column

The Q-S151 is designed to analyze dry gas samples. A desiccant column is provided which is filled with DRIERITE to dry the gas before analysis. The column has glass wool at both ends to prevent particulates from being carried through the system. It is supplied ready for use. The column should be mounted **vertically** on the Q-S151.

DRIERITE contains an indicator that is blue when the column is functional and pink when the DRIERITE is saturated with absorbed water vapour. When spent, the DRIERITE should be replaced or reconditioned. To **recondition**, remove the **DRIERITE** from the column and place it in a drying oven at **210 ˚C for 1 hour**, or until the blue coloration reappears. The replacement DRIERITE is #8 mesh, order #23005 from DRIERITE.com.

Note: Brand new Drierite may bind and release small amounts of CO_2 slowly, thereby washing out stop flow peaks. The user may therefore wish to use magnesium perchlorate to remove water vapour when measuring very low rates of respiration. Drying gas before measurements is necessary to avoid O_2 dilution. However, magnesium perchlorate will turn into liquid if it absorbs too much water vapour, so it must be changed frequently. It is recommended that a trap is installed downstream if using magnesium perchlorate. **Qubit Systems is not responsible for any damage caused to any components of the Q-Box RP1LP due to magnesium perchlorate entering any part of the system.**

CO_2-free gas can be obtained by pumping air through a column containing soda lime. A small amount of glass wool should be placed at the ends of the column to contain small particles, and the column should then be filled with soda lime using a spatula. When the column is full, it should be tapped to settle the soda lime, and then topped off. The soda lime column should be used in a **vertical** position to ensure the gas has maximal contact with the crystals when flowing through the column**.**

The soda lime provided with the Q-S151 has a coloured indicator to show when it is saturated with absorbed CO_2. The soda lime should be replaced when most of it has changed from its original white colour to a pale purple. This colour change is subtle, and the purple coloration often does not persist, but appears as a band in the column at the junction between active and inactive soda lime. Replacement supplies may be obtained from Fisher Scientific (product #S200I-3).

Note: remove the soda lime column from the system once calibration has been completed.

Warning: Soda lime can cause severe burns. Users should read and comply with the Material Safety Data Sheet on soda lime.

Troubleshooting the Q-S151:

Span or zero out of range may cause “1” to appear on the display:

Turn the “CO_2 Span” all the way down (counter clockwise) and use the “Coarse Zero” on the back of the CO_2 analyzer to display 0 with zero CO_2 gas flowing through the system. Once the zero display is read, attach a known CO_2 concentration to the system and use “CO_2 Span” to adjust the display on the CO_2

analyzer to the correct CO_2 concentration. Return to the zero CO_2 check with zero CO_2 gas running through the system, and if necessary, make small adjustments with the "CO_2 Zero".

Ensure no material (liquid or dust) has entered the analyzer:

If material has entered the analyzer, it will block the internal light path and "1" will be shown on the display. The unit will need to be returned to QUBIT Systems for repair.

Ensure the Gas Flow Path in the Analyzer is Open:

Check that there are no internal leaks in the analyzer. Connect a 10mL syringe to a tube on the 'OUT Port' of the CO_2 analyzer and plug the 'IN Port'. Pull a slight vacuum with the syringe. If there is no leak, the plunger should not move. If there is an internal leak, contact QUBIT Systems for further instructions. If the analyzer is exposed to extreme pressures, a leak can result.

Ensure the Pump is not Leaking.

A faulty pump can leak room air (possible high ppm CO_2) and increase the CO_2 reading. To test the pump, turn it on and plug the inlet. Connect a tube to its outlet and place the tube in a glass of water. There should be no bubbles. If there are bubbles, the pump is leaking internally and should be repaired or replaced.

Ensure the Soda Lime CO_2 Scrubbing Column is used to provide Zero CO_2 gas to the analyzer:

Do not confuse the soda lime column with the drying column filled with DRIERITE. Soda Lime is white, whereas DRIERITE is blue. If the drying column is mistaken as the CO_2 scrubbing column, CO_2 may enter the analyzer since **DRIERITE absorbs some CO_2, and can release it slowly thereby slowing response** time. If unsure of the condition of the Soda Lime in the column, replace it with new Soda Lime or use an alternate Zero CO_2 source such as pure N_2 gas.

Check Calibration

If the CO_2 Span control has been adjusted without following a full calibration procedure, the analyzer must be recalibrated using a zero gas mixture and a gas mixture with a known CO_2 level. See the CO_2 analyzer calibration section of this manual for the correct procedure.

The CO_2 readings on the display of Q-S151 are different from those in Logger Pro

If the CO_2 values displayed on the Q-S151 CO_2 Analyzer are very different from those displayed in the Logger Pro file, check the following. If using the calibration saved in the Logger Pro experiment file, check that the calibration was done at the correct setting selected on the CO_2 analyzer (0-2000ppm should be the default). If the range is selected correctly, check that the LabQuest Mini interfaces have not been reversed (check that values displayed in Logger Pro software for other sensors are correct). If the LabQuest Minis have not been reversed, then the CO_2 Analyzer requires a new calibration. Proceed with the calibration instructions outlined on page 17. If the LabQuest Minis have been reversed follow the instructions below on page 47 on how to fix the problem.

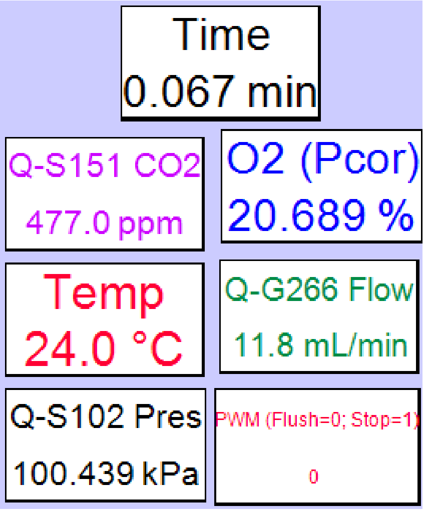
Using the Q-S102 O_2 Analyzer

The Q-S102 O_2 Analyzer contains an O_2 sensor which is a galvanic cell (a lead-oxygen battery) consisting of a lead anode, an O_2 cathode, and an acid electrolyte. It also incorporates an O_2-permeable Teflon FEP membrane with a gold electrode bonded to its surface. Oxygen diffusing through this membrane is reduced electrochemically at the gold electrode. A resistor and a thermistor (for temperature compensating diffusion across the Teflon membrane) are connected between the anode and the cathode. The output of the instrument is proportional to the current flowing through the resistor and thermistor. This current is proportional to the partial pressure of O_2 in contact with the Teflon FEP membrane. The Q-S102 O_2 Analyzer has two analog signal outputs, one for O_2 partial pressure (Q-S102 O2 in kPa) and one for total gas pressure (Q-S102 Pres in kPa) at the O_2 sensor inside the Q-S102 analyzer. The total gas pressure from Q-S102 (essentially atmospheric pressure) is used in correction of the O_2 signal in KPa to O_2 in % (O2 (Pcor)).

The galvanic cell is housed in a brass cylinder for greater temperature stability and more stable O_2 signal. Pressure (Q-S102 Pres) is measured at the O_2 cell and is displayed on Page 1 in a meter and a graph. This pressure is then used in software to convert the partial pressure of O_2 (Q-S102 O2 in kPa) to a concentration (O2 (Pcor) in %) which is independent of pressure.

O2 (Pcor)=((Q-S102 O2)/(Q-S102 Pres))*100

The pressure corrected concentration is displayed in a meter and a graph.



Time
0.067 min

Q-S151 CO2
477.0 ppm

O2 (Pcor)
20.689 %

Temp
24.0 °C

Q-G266 Flow
11.8 mL/min

Q-S102 Pres
100.439 kPa

PWM (Flush=0; Stop=1)
0

O_2 concentration Corrected for Changes in gas Pressure (%)

Total gas pressure at the O_2 sensor (kPa)

The maximum **flow** through the Q-S102 O_2 analyzer **should not exceed 650 ml/min**. The minimum **flow** through the O_2 analyzer **should not decrease below 5mL/min** to avoid local depletion of O_2 at the membrane of the sensor due to O_2 consumption by the electrochemical cell. The analyzer should not be exposed to pressures that are above 20 PSIG or below 10 PSIG, or the damage to the sensor can result. The expected **life** of the O_2 Analyzer's galvanic cell is **3 – 5 years at which point the sensor cell needs to be replaced**. If it is impossible to adjust the O_2 signal amplitude by adjusting the span, a new sensor is necessary. Contact Qubit Systems to obtain a new galvanic cell and instructions for replacement.

The Q-S102 is supplied with its own drying column filled with DRIERITE. When used in series with the QS151 CO_2 Analyzer, only one drying column is necessary (in front of the Q-S151) unless the sample gas is very wet. When using the Q-S102 in a stand-alone mode, gas should be dried before entering the O_2 Analyzer to avoid O_2 dilution by the water vapour. Place the drying column in the bracket mounted on the analyzer and connect it to the "in" port via the blue particulate filter (25µm). Check the filter frequently to ensure that it is not plugged. Plugged filters will result in reduced gas flow to the analyzer and should be replaced.

Troubleshooting Q-S102 O_2 Analyzer

If the O_2 **signal can no longer be adjusted** with the "O_2 Span" and the Analyzer has been used for 3-5 years, the galvanic cell should be replaced. Contact Qubit Systems for instructions on ordering the new cell and replacement procedure.

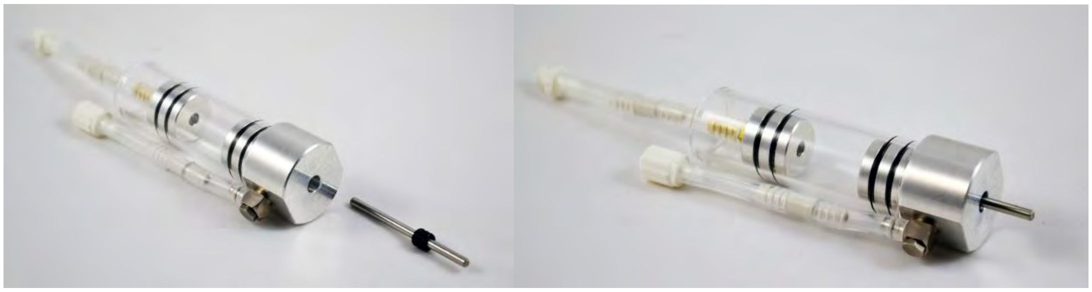
If **significant periodic jumps** (0.5-1%) in the O_2 readings are observed, this may indicate pressure damage to the Teflon membrane inside the sensor cell. If the signal shows unexpected drift, check for other damage to the membrane by removing the Q-S102 O_2 Analyzer from the Q-Box tray by unscrewing the two bolts on the back that hold the analyzer in place. While monitoring the O_2 readings, tilt the analyzer 90 degrees. If there is a large change in the signal following the tilt, the Teflon membrane is likely damaged. In case of damage to the membrane, the galvanic cell should be replaced. Contact Qubit Systems for replacement instructions.

If **small pulses in the O_2 readings** are observed when passing a stable O_2 gas through the analyzer, this may indicate pressure changes in the system resulting from unstable gas delivery. First, check the system for obstructions or restriction in the gas lines. Second, check the O_2 analyzer by unplugging the gas supply line from it. The reading should become stable after detaching the gas supply. A stable reading when no gas is flowing through the analyzer, indicates that gas delivery to the analyzer is the problem.

Unstable gas delivery may be due to the Q-P103 Gas Pump. In an open-flow system, both needle valves on the Q-P103 Gas Pump should be partially restricted (as set at the time of manufacture, about 1/3 closed from the fully open position) and the flow should be adjusted via the needle valve of the Q-G266 Flow Monitor.

Using the S174 Temperature Probe

The temperature sensor is a thermistor. It has an operational range from –40°C to +125°C. The temperature sensor should not require calibration, however this is possible in the Logger Pro software using the menu commands **Experiment>Calibrate**. The sensor is recognized automatically and calibration is loaded when the "Enhanced Q-Box RP1LP Setup" file is opened. The sensor is mounted in a stainless steel tube, which fits through the temperature sensor ports in both types of animal chambers (G117 and G115-E). The temperature sensor port in the chambers must be plugged with a stainless-steel rod provided when the sensor is not in use (see below images).



Using the G122 Gas Bags

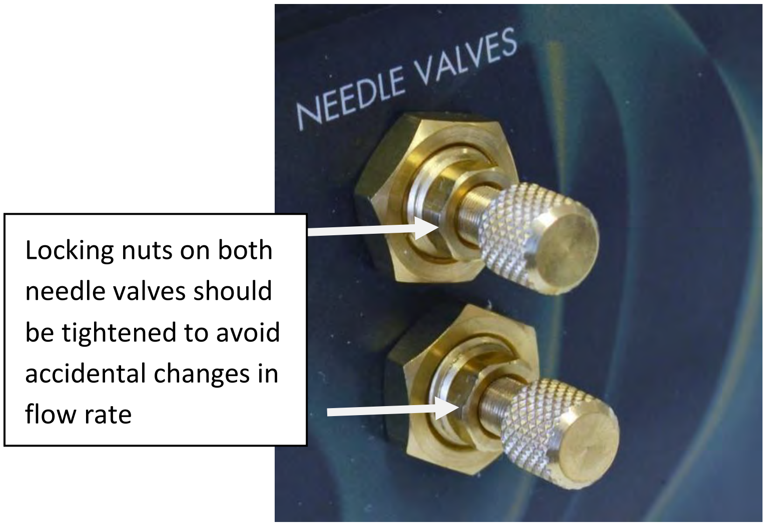
The heat-sealed, 30 L gas bags are made from a gas-impermeable nylon-polyethylene laminate. Tygon tubing is attached to each bag by a luer-lock fitting. The fitting on the other end of the tubing attaches directly to the fittings on the Q-P103 Gas pump. These bags can be filled with air from a compressor (or another gas mixture in a commercial tank) to provide a **constant source of CO_2.** For long experiments, outside air (constant 400 ppm CO_2) should be used since compressor refills may have variable CO_2 concentration if room air is used. **Inside air can vary several hundred ppm in CO_2 due to human breath.** Bags should not be overinflated, as this can cause weakening of the seams and eventual leakage. After use, the bags should be fully deflated, preferably by attachment to a vacuum-line or pump.

Using Q-P103 Gas Pump

The Q-P103 Gas Pump is a 1 L/min (no load) pump. It is used in this Package to pump gas through the system. The Q-P103 Gas Pump is delivered with pre-set pump speed and the flow rate through the system should be adjusted with the needle valve of the Flow monitor. The two needle valves on the back of the pump are locked in position at the time of manufacture.

If the pump is used in a stand-alone mode and the pump speed needs to be adjusted, it can be done with the two needle valves on the back. To set the flow rate, initially fully open both needle valves on the back of the Q-P103 Pump (turn counter-clockwise). Reduce the flow to about twice the required rate using the valve beside the pump outlet (“out”). Second, reduce the flow further to the required rate with the needle valve on the Gas Pump across from the “in” gas port (see photo below).

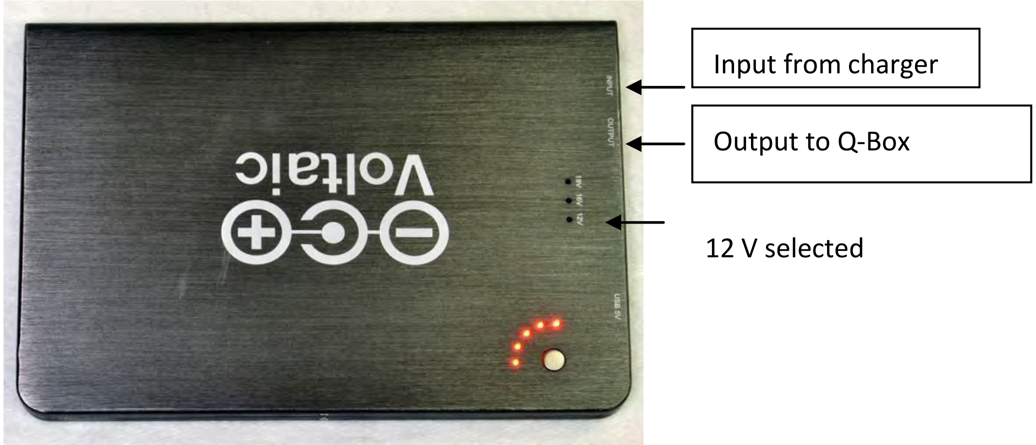
Turn the valves on the gas pump clockwise to reduce the flow or counter-clockwise to increase the flow. Once the flow is adjusted to the desired rate, use the small wrench provided in the accessory pack to lock both valves of the gas pump.



Locking nuts on both needle valves should be tightened to avoid accidental changes in flow rate

A249 Battery Pack (Optional)

The A249 battery pack for the enhanced Q-Box RP1LP package is composed of three Voltaic V88 Universal Batteries. These are Lithium Polymer batteries that can be charged directly via an AC charger or from a car battery via a DC charger (included). The two batteries supply a maximum of 176 Watt hours of power. Both batteries together or one at a time are connected to the Q-Box power outlet via the split cable provided. The third battery is used for the operation of the solenoid valves. Use of A249 Battery Pack allows field operation of the Q-Box RP1LP or any other Q-Box package for up to 11hr (without the lap top computer plugged into the battery pack). Each voltaic battery has one “Out” port for plugging in the Q-Box power cable and an “In” port for connecting the AC or DC charging adaptor. The Voltage output from the battery to the Q-Box must be set to 12V.



The placement of the batteries in the Q-box is shown in the photo below.

Troubleshooting LabQuest Minis

LabQuest Mini 1 and 2 are switched

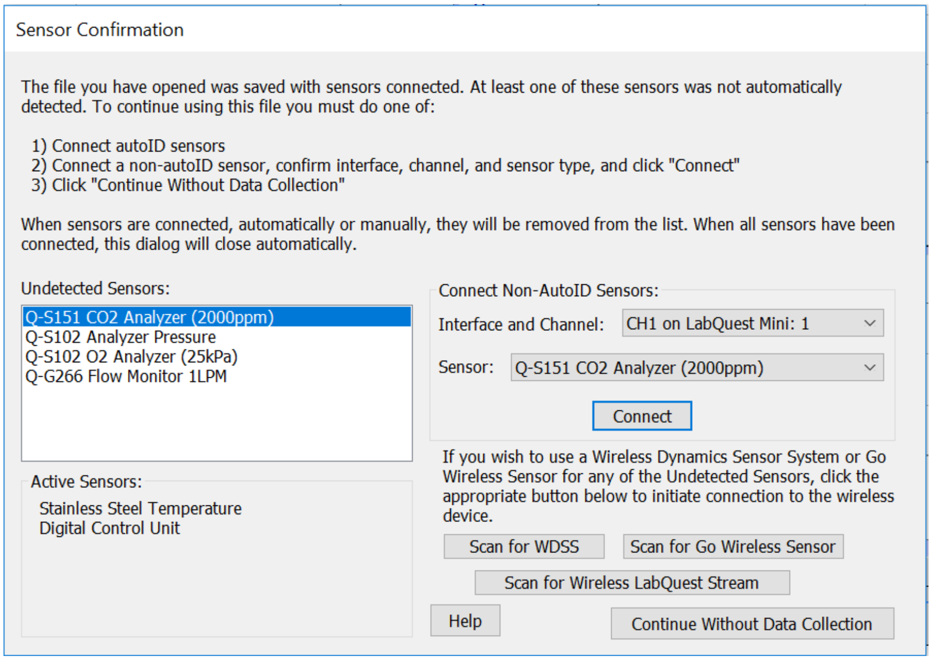
If the sensors show unusual values, Logger Pro software may have confused LabQuest Mini 1 with LabQuest Mini 2. To rectify this situation follow these steps:

Close the "**Enhanced Q-Box RP1LP Setup**" file. Unplug the two USB cables from the frame of the Q-Box.

First, reconnect one cable into the USB 1 outlet on the frame, then plug the second cable into the USB 2 outlet. As each LabQuest Mini is recognized by the drivers, there may be an audible beep and the indicator LED light on the LabQuest Minis will change from red to orange. Reopen the "**Enhanced Q-Box RP1LP Setup**" file and proceed to assigning the sensors to appropriate channels on the two LabQuest Mini interfaces as follows:

a. Ch. 1 LabQuest Mini 1 = Q-S151 CO_2 Analyzer (2000ppm)
b. Ch. 2 LabQuest Mini 1 = Q-S102 Pressure (gas pressure)
c. Ch. 1 LabQuest Mini 2 = Q-S102 O2 Sensor (25KPa)
d. Ch. 2 LabQuest Mini 2 = Q-G266 Flow Monitor 1LPM

Note: The LabQuest Mini 1 automatically recognizes the Stainless Steel Temperature probe (S174) in channel 3. And the digital control unit (DCU) in Dig ch 1 of Mini 2



The file you have opened was saved with sensors connected. At least one of these sensors was not automatically detected. To continue using this file you must do one of:

1) Connect autoID sensors
2) Connect a non-autoID sensor, confirm interface, channel, and sensor type, and click "Connect"
3) Click "Continue Without Data Collection"

When sensors are connected, automatically or manually, they will be removed from the list. When all sensors have been connected, this dialog will close automatically.

Undetected Sensors:
Q-S151 CO2 Analyzer (2000ppm)
Q-S102 Analyzer Pressure
Q-S102 O2 Analyzer (25kPa)
Q-G266 Flow Monitor 1LPM

Active Sensors:
Stainless Steel Temperature
Digital Control Unit

Connect Non-AutoID Sensors:
Interface and Channel: CH1 on LabQuest Mini: 1
Sensor: Q-S151 CO2 Analyzer (2000ppm)

If you wish to use a Wireless Dynamics Sensor System or Go Wireless Sensor for any of the Undetected Sensors, click the appropriate button below to initiate connection to the wireless device.

Note: An alternative method to ensure the minis are recognized in the correct order is to first open the Setup file then plug in min#1, wait until it is recognized (star appears in the top left corner of the menu), then plug in mini#2.

Using Q-G266 Flow Monitor

The Q-G266 Flow Monitor has been factory calibrated and should not require further calibration. However, if after extended use the flow values appear erroneous, the zero may have drifted. To reset zero, adjust “Flow Zero” on the front of the instrument using the small green screwdriver provided with the package. Make this adjustment with no gas running through the system, until a zero flow value is displayed in Logger Pro. **Do not accidentally adjust the “Flow Span”. If “Flow Span” needs adjusting, a calibrated flow standard must be used.**

It is recommended the flow rate through the Q-Box is adjusted using the needle valve on the back of the flow monitor. The flow is increased by turning the valve counter clockwise, and decreased by turning it clockwise. Once the flow is set, lock the ring on the needle valve with the small wrench. The data cable from the analog output labeled “Flow” is plugged into the data interface and the flow data is displayed in the software.

Specifications of Q-S151 CO_2 Analyzer:

- Operating principle - Non-dispersive infrared
- Gas sampling mode - Flowing gas stream, sealed chamber
- Maximum gas flow - 650 mL/min
- Measurement range (LCD display)- 0 – 1999 ppm
- Analog output, , 0 – 2000 ppm or 0-500ppm
- Resolution - ± 1 ppm
- Repeatability (at stable atm press and temp)- Better than ±1 ppm
- Response time (@ 250 mL/min; to 95% of final value) - ca. 25 sec
- Warm up time (@ 22^{o}C) – ca. 5 min
- Output (linear) 0 – 5 VDC
- Calibration adjustments – Zero and Span
- Operating temperature range – 0 to 50^{o}C
- Storage temperature range - -40 to 70^{o}C
- Operating pressure range - ±1.5% local mean pressure
- Humidity range - 5 to 95% RH, non-condensing (recommend drying gas stream)
- Pressure dependence – +0.13% reading per mm Hg
- Power requirements- 12 VDC via 120 VAC/60 Hz adapter
- Current requirements – 125 mA average, 450 mA peak
- Dimensions (cm) – (H x W x D: 5.5 to 9.5 x 9.5 x 17)
- Weight - 1kg
- Warranty - 1 year limited

Specifications of Q-S102 O_2 Analyzer:

- Operating principle - Acid Electrolyte, Teflon Diffusion Membrane
- Detection Range - 0-25% and 0-100 %O2 (Linear)
- Resolution - ±40 ppm
- Accuracy ± 0.21% of Full Scale
- Response Time (90%) - 12 Seconds
- Life Expectancy of sensor - 3-5 years
- Easy sensor replacement
- Output (linear) 0 – 5 VDC
- Influence by Other Gases - Ammonia and Ozone
- min flow - 5 mL/min
- max flow - 650 mL/min
- Built in total gas pressure reading at the sensor (for pressure correction in the software)
- Pressure Range - 0.5 atm to 1.5 atm
- Pressure Effect - Output voltage changes proportionally
- Shock Resistant - to 2.7 G
- Avoid strong vibration
- Operating Temperature - 5 to 40^{o}C (Effective range)
- Passive temperature control of the O2 sensor

- Weight – 1.35 kg
- Dimensions (cm) – (H x W x D: 5.5 to 9.5 x 9.5 x 17)
- Power Supply - 12 Volts
- Warranty: 1 year

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-ondelivery 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:

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 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.