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Using the giant Madagascar hissing cockroach (*Gromphadorhina portentosa)* to teach metabolic and respiratory principles of ectothermic animals

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Understanding the metabolic differences between ectothermic and endothermic animals has fascinated scientists and students alike. Here we propose to study ectothermic metabolism using the Giant Madagascar Hissing Cockroach (*Gromphadorhina portentosa)* in a classroom setting. We describe a laboratory activity in which students quantify carbon dioxide production as well as oxygen usage to better understand the Respiratory Exchange Ratio (RER). Further, students manipulate the insect's activity and conditions to ask how metabolic activity is impacted: students compare metabolic differences during periods of high metabolic demand (i.e. exercise) and diet restrictions (i.e. low carbohydrate). The lab is designed to enhance student understanding of metabolism in ectothermic animals as well as insect respiratory anatomy and physiology.

Keywords: Insect tracheal system, Spiracles, Ectotherm, Respiration, Metabolism, Metabolic Rate, RER

Link to Supplemental Materials: https://doi.org/10.37590/able.v43.sup15

Introduction

Early work in the middle of the last century (Weis-Fogh, 1967) identified the unique air ventilation system and gas exchange patterns that insects employ (Terblanche and Woods, 2018). Unlike the mammalian respiratory system, the insect respiratory system consists of tracheal vessels (a series of air-filled ducts) that terminate at tracheoles (thin-walled cul-de-sacs) where gas exchange occurs. Air enters and exits out of small openings on the side of insect bodies called spiracles. These spiracles open and close to give rise to respiratory patterns aptly named discontinuous, cyclic, and continuous. The respiratory patterns have been shown to correlate with changes in metabolic rates (Contreras and Bradley, 2010). Previous work indicates that the respiratory patterns may be related to oxidative damage, mitochondrial function, and metabolic consumption of oxygen and carbon dioxide (Lalouette et al., 2011; Hetz and Bradley, 2005; Sacktor, 1961).

In this laboratory we use the Giant Madagascar Hissing Cockroach (*Gromphadorhina portentosa)* to explore carbon dioxide production, oxygen usage, and metabolic rate, indicators of energy expended (Lehmann and Schutzner, 2010). Further, the lab activity examines how exercise, diet, and temperature influence metabolic activity in the cockroaches. The giant Madagascar hissing cockroach is an easy-to-use model insect previously introduced at an ABLE conference by the author (Sossa, 2016). It makes for a robust model to study respiratory dynamics and metabolism.

Student Outline

Objectives

- To understand oxygen and carbon dioxide physiology.
- To understand external respiration in insects
- To understand principles of gas exchange and the physiology of ventilation in insects.
- To understand metabolic rate in insects: meaning and measurement
- To understand the basic differences between endotherms and ectotherms.
- To demonstrate proficiency with measuring expired carbon dioxide, consumed oxygen and respiratory exchange ratio in insects.

Introduction

- There are two main sources of energy available for animal metabolism: carbohydrates (CHO) and fats.
- These molecules are broken down, or catabolized, into carbon dioxide, water, and energy.
- As the ratio of energy supplied by fats and carbohydrates shifts during changes in activity, the ratio of carbon dioxide produced to oxygen consumed also shifts.
- This ratio is an estimate of cellular metabolism called the respiratory exchange ratio (RER).
- The RER is measured using an oxygen/carbon dioxide gas analyzer connected to a small animal chamber.

Methods and Data Collection

- Indirect calorimetry- method that measure proxy properties
- Respirometry- measures rate of gas exchange with environment
- Devices used to measure O2 consumption are called respirometers
- Configurations:
- Closed: animal placed in sealed chamber with a fixed volume of air
- Gas Analyzer measures gasses of animal in a closed chamber
- Oxygen consumed per minute = VO2
- Carbon dioxide produced per minute = VCO2
- Ratio of VCO2/VO2 is Respiratory Exchange Ratio (RER)

Discussion

- Metabolic rate is the rate at which animal consumes energy, ie when chemical energy is converted to heat and external work
- Important b/c:
    - Determines how much food animal needs
    - Determines amount of total activity of living animal (e.g. heat produced, etc.)
    - Determines ecological demand of animal on environment
- Lower metabolic rate of insects means less self-generated heat, more dependence of heat from the environment
- Metabolic strategies for thermoregulation important especially for animals, like insects, called ectotherms (poikilotherms).

Materials

Materials and required equipment

- Cockroaches (*Gromphadorhina portentosa*) are the authors' preferred model organism but crickets can be utilized (see example data). Purchased on Amazon or local pet stores. Number depends on class size but estimate 4 per student group.
- 4 PC or Mac computers
- 4 barometers (Aowutus Outdoor Barometer Thermometer Hygrometer)
- 4 thermometers
- Balance or scale (iBalance table top precision scale)
- Rotating tube rack or shaking incubator (to stimulate roach exercise inside a 50-mL conical tube),
- 10 gallon tank (to maintain cockroaches)
- iWorx IXTA data acquisition unit with a power supply and USB cable
- Small animal chamber
- Gas sample tubing (Tygon)
- iWire-GA gas analyzer
- gas sample tank, and calibration kit are purchased from iWorx and may be included as part of a kit.

Computer needs

- PC or Mac
- internet access
- LabScribe (available from **https://iworx.com/labscribe-software-download/**)
- LCD projector for lecture presentation

A computer with internet access and the LabScribe program (described here- https://iworx.com/labscribe/) is required for each student pair. Configuration and number of recording stations is dependent on the instructor, however, a typical setup will include four iWorx stations with 3-4 students per station.

Cockroach husbandry

Cockroaches are housed in a 10-gallon tank and fed a diet of dog kibble and water ad libitum in a 12 hr:12 hr light: dark cycle, at 22-24°C.

Cockroach experimentation

This lab exercise details only resting conditions and cockroaches reared at 30°C, but experimental groups are only limited by available resources and the creativity of students and instructor. For example, an experimental design can consist of four groups with one control (does not experience exercise or diet-restrictions or temperature fluctuations) and the remainder of the groups receiving experimental treatments: group #1 run on a treadmill or rotating tube rack for at least 30 min, group #2 is fed a low carbohydrate diet (high protein dog kibble) for 48 hours; group #3 placement on ice or on a heating pad (e.g., Ziploc with hot water) for 30 min prior to recording. Due to the use of invertebrates in this experiment an IACUC protocol is not required.

Respirometry

The gas analyzer is first calibrated before beginning the recording (see student handout for "Calibrating the iWire-GA Gas Analyzer**"** instructions). The procedure described in the student handout involves the iWorx data acquisition device, gas analyzer, and small animal chamber. The recording is performed with 30°C reared cockroaches (or condition of choice, e.g. post-exercise) in the small animal chamber measuring oxygen and carbon dioxide levels. The data on oxygen and carbon dioxide are utilized to calculate the respiratory exchange ratio (RER) which indicates metabolic rate and energy expended.

Notes for the Instructor

The purpose of this lab activity is to familiarize students with invertebrate animal physiology and equip them with an understanding of the value of the model organism *G. portentosa*. Additional skills gained through these labs include a familiarity with metabolic testing equipment such as the iWorx data acquisition device and utilizing the scientific literature.

The formal protocol for this lab (by iWorx) calls for students to compare endotherms and ectotherms. This proposal takes the protocol a step further for ectotherms exploring how exercise, diet, and temperature influence carbon dioxide production, oxygen usage, and metabolic rate while eliminating the need for an endotherm. Instructors may choose to perform the iWorx lab experiment as written but here we focused on ectotherms. The key to having a quality recording is the calibration of the gas analyzer done using room air and gas tank air. Following calibration it will be important to maintain a tight seal of the animal chamber and prevent breathing into tubes or chamber when changing animals. The data acquisition device and gas analyzer do the work while students visually evaluate changes in gas levels and RER. Students will formulate predictions before beginning the experimental groups. This is also a good opportunity to introduce primary literature for biology majors.

Problems that could arise include the following: lab instructors and students alike may encounter a software learning curve and need familiarity/practice with the equipment. The gas analyzer and iWorx data acquisition device equipment are expensive and may need funding. Also, with some long recording periods instructors may need to find an effective use of time during data collecting, but this can be overcome by using shorter tubes, smaller chambers, and/or larger animals. The accompanying PowerPoint presentation includes ideas to enhance the learning experience of students during longer recording periods. Canada highly restricts the availability of hissing cockroaches which means alternative ectotherms are needed. The cricket is a good option for a substitute model organism.

Benefits of the lab include, but are not limited to, teaching important lab techniques, directed investigative lab experience, encouraging collaboration between students, extending student thinking beyond content knowledge, introducing up to date concepts in the biological sciences, reinforcing data analysis/scientific method, being an open investigative lab, and allowing for visualization of complex concepts that may be difficult to understand in other methods of teaching. This lab can be a directed or open investigative lab depending on the needs of the instructor.

Description of how the lab could be presented

To start, a PowerPoint will be presented that features ectothermic physiology compared to endothermic physiology. The value of the cockroach as a model organism will then be introduced and discussed. We explore the respiratory system of the cockroach as there are more than a few nuances compared to mammals. Following this presentation, each group will be given four cockroaches which they weigh, lab equipment will be explained and set up. First, students calibrate the gas analyzer. The procedure and rationale are explained step by step. Cockroaches are then placed inside the animal chambers to begin recording baseline oxygen and carbon dioxide levels.

As the recording proceeds, the PowerPoint presentation will continue with principles of metabolism. A short historical perspective is delivered in order to mention calorimetry and changes in instrumentation. Next, the instructor discusses the relative advantages and disadvantages of old versus new instrumentation and highlights the advantages of the iWorx system.

After visualizing a change in carbon dioxide utilization for at least 30 minutes, the recording is stopped in order to switch out for cockroaches that were diet-restricted (i.e. low carbohydrate for 48 hours). The recording proceeds with these diet-restricted cockroaches for 30 minutes. A review of the effects of diet on metabolism is presented. During this time students formulate predictions and find primary scientific literature to support their predictions.

Once this part of the lab is complete, diet-restricted cockroaches are swapped for “exercised” cockroaches. Exercise is mimicked by placing a cockroach in a conical tube that rotates on a tube rack. This 30-minute treatment (begun after the previous diet-restricted recording was started) forces cockroaches to flare out their limbs continuously. This recording begins once the exercised cockroaches are inside the sealed animal chambers. A review of the effects of exercise on metabolism is presented. This is also another opportunity to formulate predictions and use the primary literature.

A similar exercise can be repeated given time by manipulating cockroach environmental temperature with ice or hot water in Ziploc bags. Next, students calculate weight specific RER and graph and tabulate results.

The exercises or variables can be scaled down or up depending on the time allotted and number of students. The more time and students, the likelier that a class average of control, diet-restricted, and exercised RER can be obtained. As such simple statistical tests (student's t test, or ANOVA) can be applied. Results and their implications are discussed. This lab will serve to clarify ectothermal and metabolic concepts in insects and is appropriate for 200-, 300-, or 400-level Biology majors including Gen/Intro Biology, Animal Physiology, Entomology, Comparative Physiology.

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Appendix A. Student Handout

The material below is adapted from the iWorx Physiology Lab Experiment (https://www.iworx.com/documents/LabExercises/SmallAnimalRER.pdf) with a focus on ectotherms, particularly *G. portentosa* and crickets.

Experiment AMe-1: Insect Respiratory Exchange Ratio (RER)

Background

There are two main sources of energy available for animal metabolism: carbohydrates (CHO) and fats. These molecules are broken down, or catabolized, into carbon dioxide, water, and energy. However, the oxidation of fats requires more oxygen than the oxidation of carbohydrates.

The oxidation of a molecule of carbohydrate is expressed by the following equation:

$$6\ O_2 + C_6H_{12}O_6 = 6\ CO_2 + 6\ H_2O + 38\ ATP$$

As shown in this equation, 6 molecules of carbon dioxide are produced for every 6 molecules of oxygen consumed during the oxidation of carbohydrates, a ratio of 1.0.

The oxidation of a molecule of fatty acid is expressed by this equation:

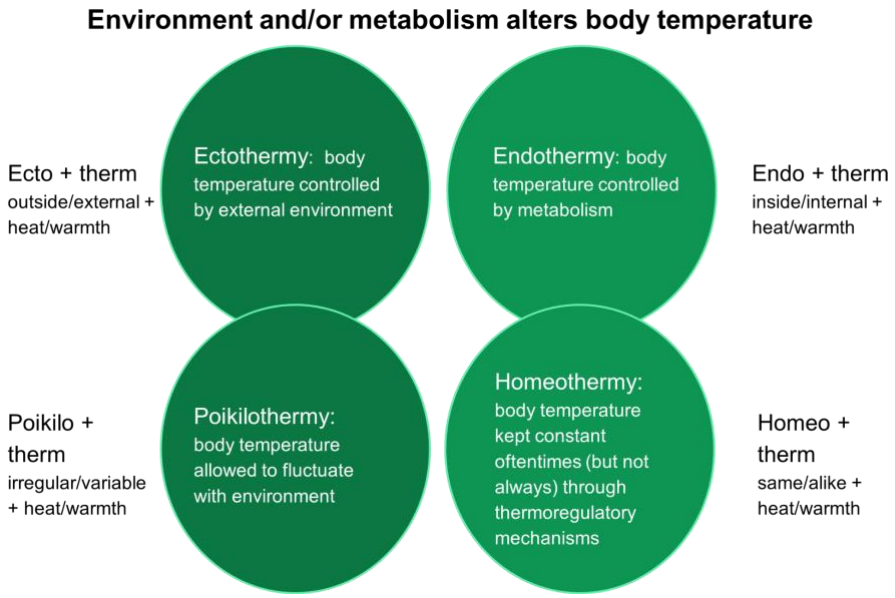
$$23\ O_2 + C_{16}H_{32}O_2 = 16\ CO_2 + 16\ H_2O + 129\ ATP$$

As shown in this equation, 16 molecules of CO_2 are produced for every 23 molecules of O_2 consumed during the oxidation of fatty acids, a ratio of 0.7.

The energy requirements of the animal are met with a mixture of energy derived from carbohydrates and fats. The activity being performed determines the proportion of carbohydrates and fats being utilized. At rest, a body derives about 40% of its energy from carbohydrates and 60% from fats. As the intensity of activity increases, the demand for energy increases, and a greater proportion of this demand is met by the oxidation of carbohydrates because the catabolism of fat is too slow to supply the amount of energy required.

As the ratio of energy supplied by fats and carbohydrates shifts during changes in activity, the ratio of carbon dioxide produced to oxygen consumed also shifts. The ratio of carbon dioxide produced to oxygen consumed during cellular metabolism can be measured by determining the changes in the concentrations of oxygen and carbon dioxide in the air that passes into and out of the organism being studied.

Homeothermic or endothermic (warm-blooded) animals and poikilothermic or ectothermic (cold-blooded) animals have a metabolic rate related to body mass at a slope of 0.75. These organisms will show very different metabolic rates based on the way they maintain their body temperatures. Endotherms have a constant body temperature and maintain this elevated temperature by endogenous heat production. They tend to have a high VO_2, high heat production, low thermal conductivity (good insulation) and a high metabolism - up to 5 times the metabolism of ectotherms. Ectotherms or poikilotherms base their internal body temperature on the thermal condition outside their bodies. Their body temperatures are high in warm environments, but low in cool environments. They adjust body temperature by means other than heat production and heat loss - thus having a lower overall metabolism than endotherms. Ectothermic animals tend to have a low VO_2, low heat production and are poorly insulated.



In these animals, the amounts of oxygen consumed and carbon dioxide produced are measured using an oxygen/carbon dioxide gas analyzer connected to a small animal chamber. The gas analyzer measures the concentration of oxygen consumed and carbon dioxide produced by the organism in the chamber. When the concentrations and volumes are brought together in a series of equations, the volume of oxygen consumed per minute, known as VO2, and the volume of carbon dioxide produced per minute, known as VCO2 are determined. The ratio of VCO2/VO2 is the Respiratory Exchange Ratio (RER), which can be used to determine the proportion of carbohydrates and fats utilized, and the energy expended per liter of oxygen consumed, during an activity (Table 1).

The fat and carbohydrate percentages utilized during an activity are determined using the following equations:

$$((1.00\text{-RER})/(1.00\text{-}0.70)) \times 100 = \%\text{Fat utilized}$$

$$100\% - \%\text{Fat utilized} = \%\text{CHO utilized}$$

The energy expended during an activity is calculated from the RER and the volume of oxygen consumed. For example, if the RER is 0.90, the energy expended is 4.92kcal/liter O2.

If 2.5 liters of oxygen are consumed per minute for 20 minutes, a total of 246 kcal are expended during the activity:

$$(2.5\text{LO2/minute})(20\text{min})(4.92\text{kcal/liter O2}) = 246\text{kcal}$$

At less intense activity levels, the rates of energy expenditure and RER values are lower. To expend the same amount of energy at a less intense level of activity, the duration of activity must be longer. For example, if the RER is 0.80, the energy expended is 4.80kcal/liter O2. If 1.7 liters of oxygen are consumed per minute, 8.16kcal are expended per minute:

$$(1.7\text{LO2/minute})(4.80\text{kcal/liter O2}) = 8.16\text{kcal/min}$$

To expend 246 kcal at a rate 8.16kcal/min would require 30 minutes, 9 seconds:

246 kcal/(8.16 kcal/min) = 30.15 minutes.

In this experiment, students will measure the expired CO2 and consumed O2 Values, RER, and proportion of fat and carbohydrates utilized while an endotherm (mouse or rat) is in the small animal chamber. They will repeat the experiment with an ectothermic organism (frog, snake or lizard) and make a comparison between the RER values of these two types of animals. Students will then be able to make an accurate assessment of the metabolic capability of these organisms. These measurements will be performed quickly and easily using an iWorx gas analyzer.

As an additional calculation, students can adjust the values for CO2 and O2 using Body Weight Normalization and Effective Mass Correction. CO2 and O2 values are normalized with respect to the animal's body weight and corrected according to an effective mass value. Using a value of 1.0 will eliminate any effective mass correction, if desired.

Table AMe-1-B1: Respiratory Exchange Ratio (RER) as a Function of the Proportions of Energy Sources.

RER	Energy kcal/liter O2	% Energy from CHO	% Energy from Fats
0.70	4.69	0	100
0.75	4.74	15.6	84.4
0.80	4.80	33.4	66.6
0.85	4.86	50.7	49.3
0.90	4.92	67.5	32.5
0.95	4.99	84.0	16.0
1.00	5.05	100	0

Experiment AMe-1: Insect Respiratory Exchange Ratio (RER)_Setup Instructions

NOTE: The iWire-GA must be plugged into the IXTA prior to turning both machines on.

Setup the IXTA and iWire-GA

1. Connect the iWire-GA to the iWire1 port on the front of the IX-TA, and plug it into the wall using the power supply.

2. Plug the IX-TA into the wall and, using the USB cable, to the computer.

3. Turn on the iWire-GA and then the IX-TA.

Setup the Metabolic Cart

1. Locate the small animal chamber, the iWire-GA gas analyzer, the gas analyzer power supply, 2 inlet filters, room air sampling tube, and a 6ft long Nafion sampling tubing.

2. Position the gas analyzer on the desktop, so that the analyzer can be connected to the data recording unit and the small animal chamber at the same time.

3. Make sure to connect the iWire-GA to the iWire 1 port on the front of the IXTA. Turn both units on after connecting this cable.

4. On the iWire-GA, place one filter on the “Room Air” port, place a second filter on the “Sample In” port. Attach the braided end of the Nafion sampling tube to the filter on the “Sample In” port.

5. Place the other end of the Nafion sampling tube on the gas sampling port near the outlet of the small animal chamber.

6. Connect the Room Air filter port to the tubing with the black leur lock, and connect the other end to the inlet port of the small animal chamber.

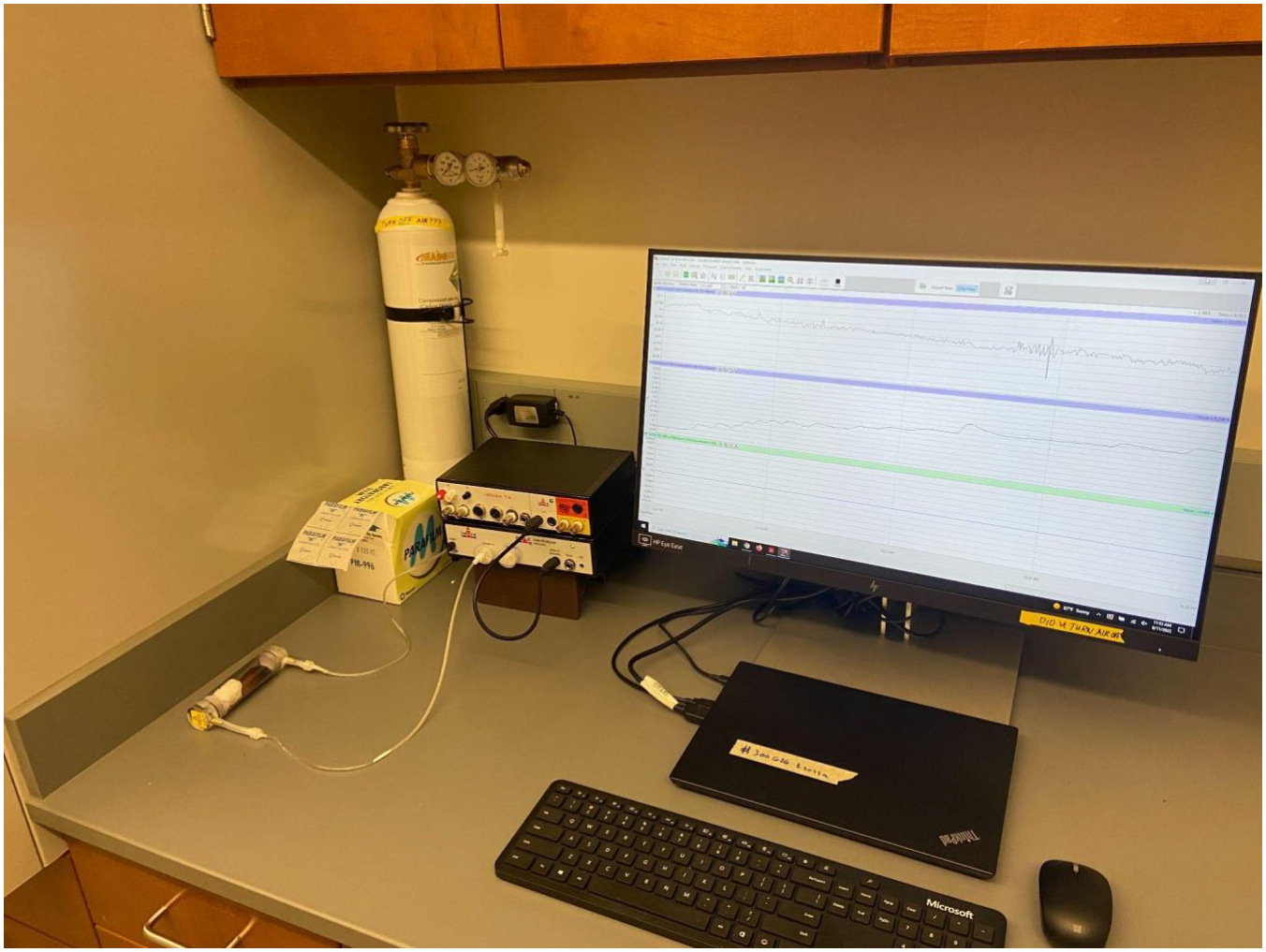


Figure AMe-1-S1: Image showing basic setup of the IXTA, iWire-GA, and animal chamber with the G. portentosa inside. The anchored compressed gas tank is also shown.

Calibrating the iWire-GA Gas Analyzer

Note: *Warm up the gas analyzer for at least 15 minutes prior to use. Make sure the calibration gas tank is located close to the gas analyzer.*

Prior to use users must perform a two-point calibration of the oxygen and carbon dioxide sensors.

The recommended gas mixtures include:

- 12% O2 and 5% CO2 with the balance of the mixture being N2; or,
- 16% O2 and 4% CO2 with the balance of the mixture being N2.

Room air can provide both the high concentration of O2 at 20.90%, and the low concentration of CO2, 0.04%.

This procedure will calibrate both the O2 and CO2 channels.

Connect the gas sample tubing of the A-CAL-200 Calibration Kit to the Luer-Lock connector on the output of the regulator.

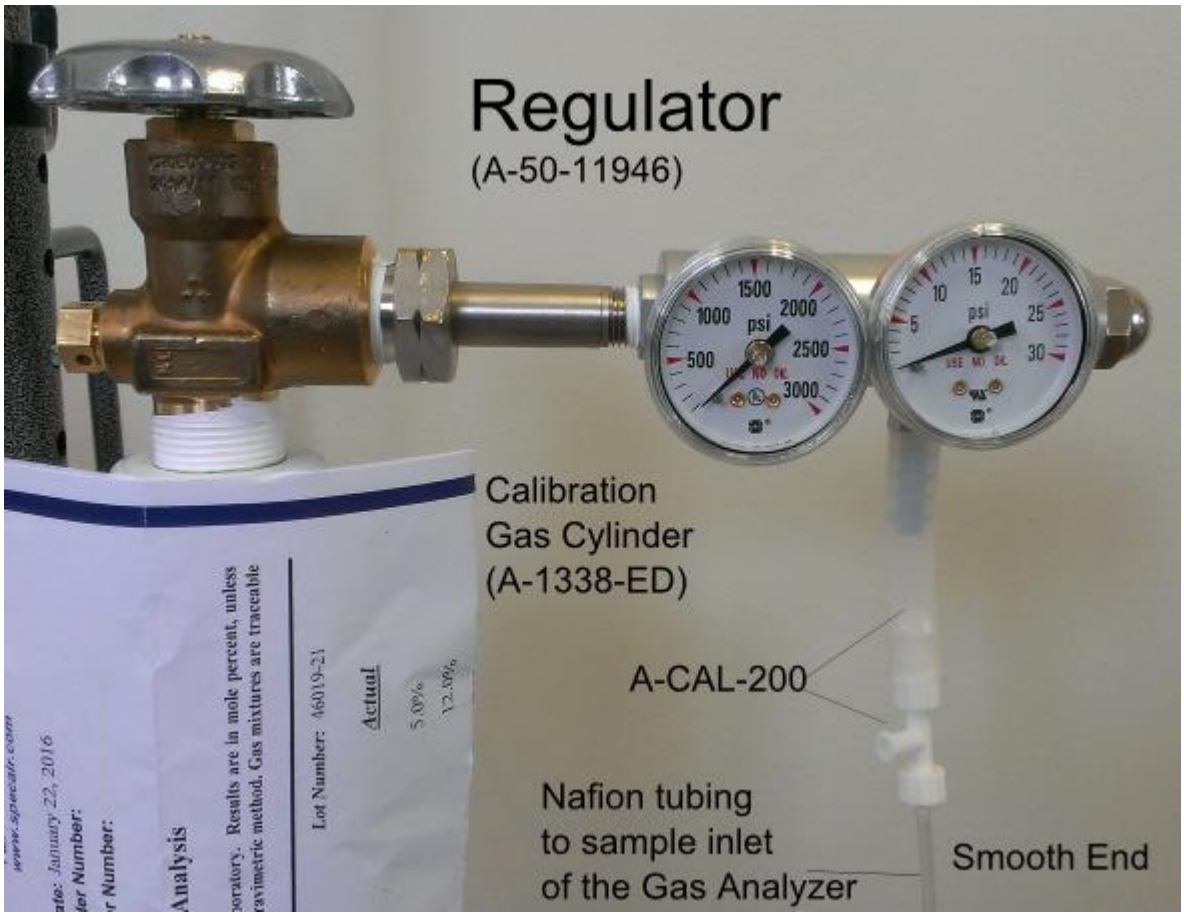


Figure AMe-1-S2: Calibration Kit (A-CAL-200).

Record the Voltage Outputs of the Gas Sensors

1. Turn on the gas analyzer for at least 15 minutes before performing a calibration.

2. Prepare the equipment that will deliver any gas samples, other than room air, to the iWire-GA:

    - Clamp and secure any gas cylinders that will be used to provide gas samples near the gas analyzer.

    - Attach the regulator to the gas cylinder with a wrench making sure there are no leaks in the system.

3. Disconnect the nafion tubing from the small animal chamber.

4. Measure the voltage outputs of the oxygen and carbon dioxide sensors when measuring a sample of room air.

- Place the gas sampling tubing away from the users to prevent the sampling of exhaled air. Allow room air to be pumped through the gas analyzer for 10 seconds before recording the outputs of the sensors.
- Type **Room Air** in the Mark box to the right of the Mark button.
- Click on the Record button. The recording should scroll across the screen.
- While recording, press the Mark button to mark the recording with information about the room air gas sample.
- Record the outputs of the two gas sensors for about ten seconds. The recording which should be like the first segment of data shown.
- Continue to record while moving to the next series of steps.

5. Measure the voltage outputs of the oxygen and carbon dioxide sensors when measuring a second sample of a gas mixture containing known concentrations of oxygen and carbon dioxide.

- Open the valve on the regulator all the way.
- While the gas sample is flowing from the regulator, connect the gas sample tubing of the A-CAL-200 Calibration Kit to the Luer-Lock connector on the output of the regulator.
- Connect the outlet from the A-CAL-200 Calibration Kit to the inlet filter port on the gas analyzer. The gas analyzer will pull the air in from the calibration kit.
- Type **Gas Sample** in the Mark box.
- While continuing to record with the sample gas flowing into the gas analyzer, press the Mark button to mark the recording with information about the second gas sample.

6. Once the recordings of the gas concentrations reach a steady level, record for another ten seconds. This may take a minute or so to fully level out.

7. Click the Stop button.

8. Turn off the regulator and reconnect the nafion tubing to the small animal chamber.

9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

Convert the Units on Gas Concentration Channels

1. Use the Display Time icons to adjust the Display Time of the Main window to show the complete calibration data on the Main window at the same time. The required data can also be selected by:

- Placing the cursors on either side of data required.

- Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the entire segment of data to the width of the Main window.

2. Click the 2-Cursor icon so that two blue cursors appear on the Main window. Place one cursor on the section of data recorded when the gas analyzer was collecting a sample of room air and the second cursor on the section of data recorded when the second sample was collected.

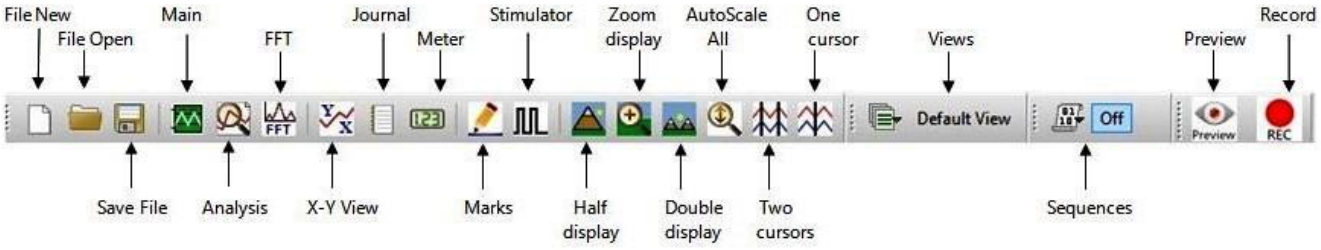


Figure AMe-1-S3: The LabScribe toolbar.

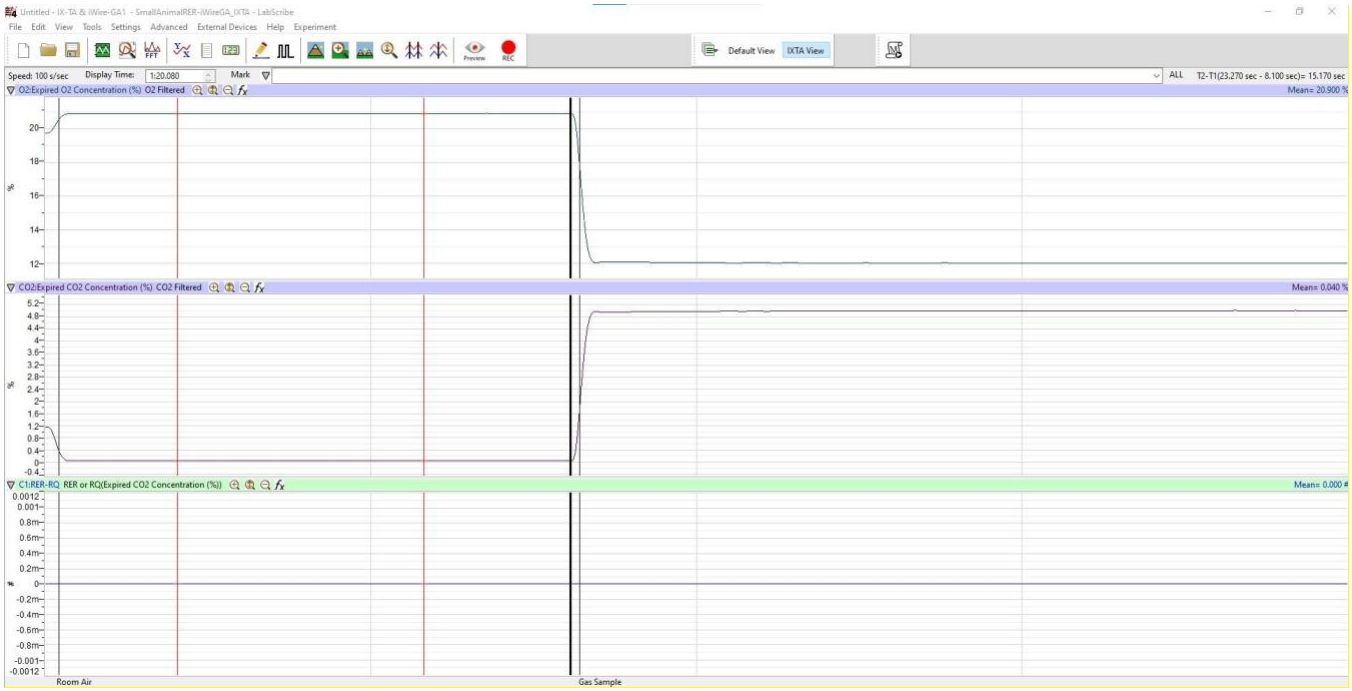
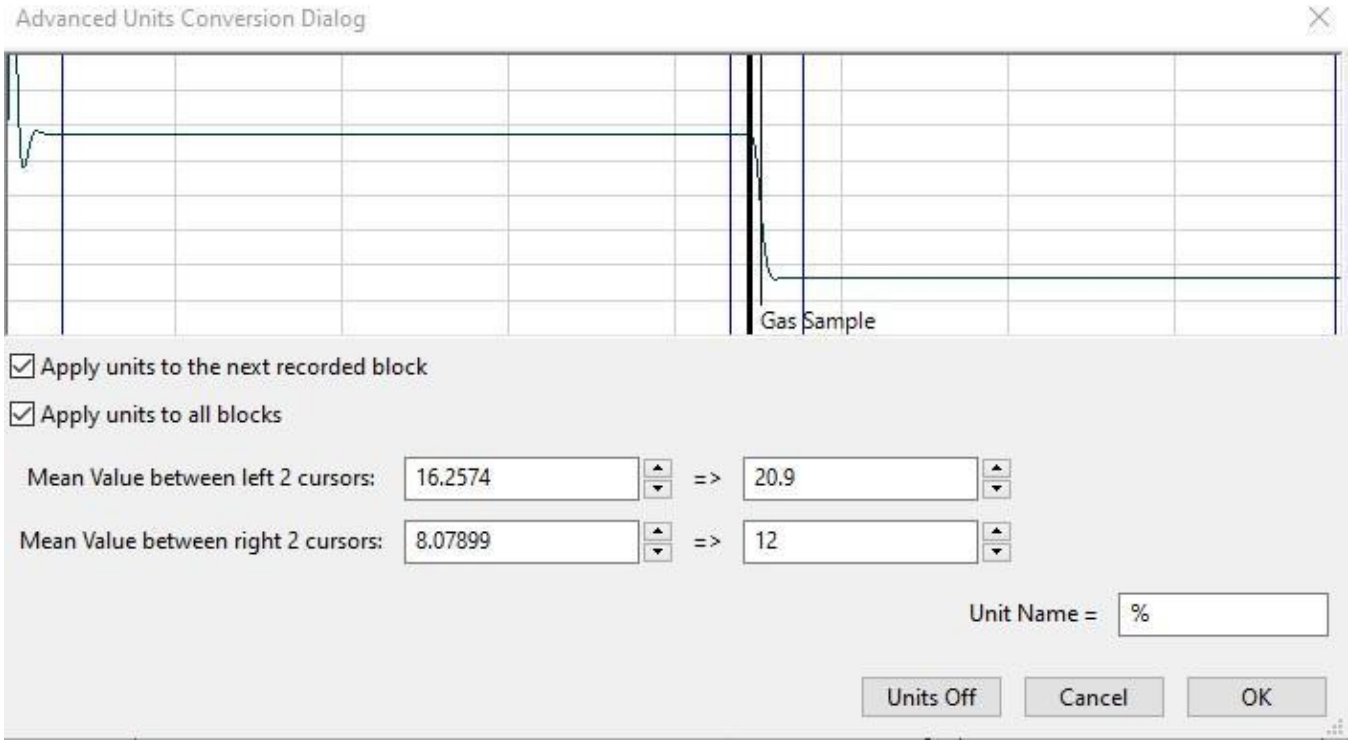


Figure AMe-1-S4: The mean percentage of the two sensors in the gas analyzer, oxygen on the top and carbon dioxide on the bottom. The third recording window shows mean RER value; given that this is a calibration no RER value is calculated.

4. Convert the voltages at the positions of the cursors to concentrations using the Advanced Units Conversion dialogue window.

5. To convert the voltages on the Expired CO2 Concentration (%) channel, click on the arrow to the left of the channel title to open the channel menu. Select Units from the channel menu, and select Advanced from the Units submenu.



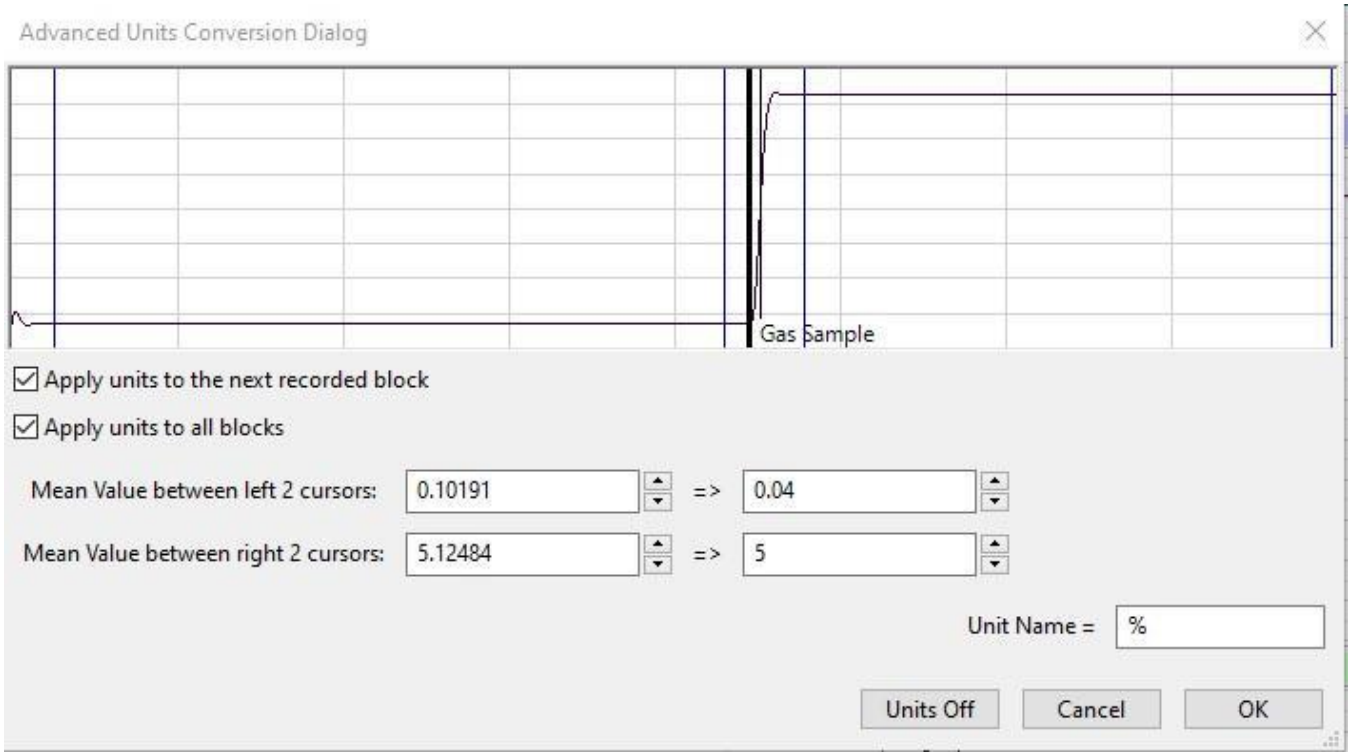


Figure AMe-1-S5: The Advanced Units Conversion dialogue window with the percentages between the cursors set to equal the concentrations used in calibration. Top image shows Advanced Units Conversion dialogue for oxygen while the bottom image is for carbon dioxide.

6. On the Units Conversion window:

    - Make sure Apply units to the next recorded block and Apply units to all blocks are selected in the menu under the displayed graph on the left side of the window by putting a check mark in the boxes next to each statement.

    - Click on and move the cursors so that they are in position such that:

        - The first 2 cursors are on the area where room air values were recorded. Leave a space between the cursors so that you have an average value being calculated while room air was moving into the iWire-GA gas analyzer.

        - The second 2 cursors are on the area where the gas sample values were recorded. Leave a space between the cursors so that you have an average value being calculated while the gas sample was moving into the iWire-GA gas analyzer.

    - Notice that the voltages from the positions between the cursors are automatically entered into the value equations. Enter the two concentrations of carbon dioxide measured from the two samples in the corresponding boxes on the right side of the conversion equations.

    - Using room air - the concentration of CO_2 = 0.04

- The second gas concentration will be the one from the gas cylinder. Generally a 5% CO_2 concentration is recommended.
- Enter the name of the units, %, in the box below the concentrations.
- Click the OK button in the lower right corner of the window.

7. Repeat Steps 4 and 5 on the Expired O_2 Concentration (%) channel.
    - Room air = 20.9
    - Second gas concentration will be the one from the gas cylinder. Generally a 12% O_2 concentration is recommended.
8. Click on the Save button.

Experiment AMe-1: Insect Respiratory Exchange Ratio (RER)_Exercises

(Adapted for ectothermic animals from original iWorx lab for endothermic and ectothermic animals)

Exercise 1: Changes in CO_2 and O_2 in a closed chamber, and RER in an ectothermic animal.

Aim: To determine mean RER of an ectotherm at rest.

Approximate Time: 45-90 minutes or longer depending on the animal

Procedure

1. Place the animal in the chamber and close the lid securely.
2. Click on the Record button. Type **Roach/Cricket** in the Mark box to the right of the Mark button. Press the Mark button to mark the recording.
3. Click the AutoScale All button.
4. On the Expired CO_2 Concentration (%) channel, notice that the CO_2 concentration shows a continuous steady rise in level throughout the recording.

Note: *During the first few minutes or so of the recording, the small animal chamber fills with expired air from the organism inside. Since this is a closed system, the concentration of CO2 continues to rise as the animal exhales into the chamber.*

- The time that it takes the chamber to be filled with expired air and a steady increase in the level of carbon dioxide will depend on the volume and respiration rate of the organism and the volume of the small animal chamber. It will take longer to fill the chamber if the organism's respiration rate and tidal volume are low, or the animal is very small.

Warning: The CO2 concentration will continue to rise throughout the experiment and levels can become toxic to the animal in the chamber. Endothermic animals should be immediately removed if the CO2 levels reach 3.5%. Ectothermic animals, however, may endure high CO2 levels for an undetermined period of time. These exercises are conducted until the CO2 value changes by 1%, e.g. if the recording started at 0.03% CO2 then the recording continued until the CO2 percentage reaches 1.03%.

5. On the Expired O2 Concentration (%) channel, notice that the O2 concentration will show a steady decrease as the animal is using the O2 in the chamber. As pointed out in the previous step, the size of the chamber, the tidal volume, and respiration rate of the organism, determine the time it takes for the concentration of oxygen to decrease significantly.

6. On the RER channel, an equation programmed in the software determines RER based on the relative levels of CO2 (which is increasing) and O2 (which is decreasing).

7. Record until the concentration of carbon dioxide in the chamber changes by 1%.

Note: Recording at 1-3% change did not show significant difference in respiratory exchange ratio. Recordings to 3% change, however, required significantly more time.

8. Once the appropriate data is recorded, click Stop to halt the recording. Your recording should be similar to the data shown below.

9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

10. Remove the animal from the chamber and place it back into its container.

11. Clean and dry the small animal chamber if necessary.

RER Channel Set Up

1. Display the complete data recording in the Main window. Use the Display Time icons to adjust the Display Time of the Main window to show the complete recording on the Main window.

2. Select and display a section of data that shows the change in CO2 concentration at 1%.

3. This can be done by:

    • Clicking on and dragging the cursors to either side of the data, and looking at the V2-V1 value in the upper right corner of the Expired CO2 channel.

    • When V2-V1 value is equal to 1%, look at the T2-T1 value in the upper right to determine the time it took for the CO2 concentration to change by 1%.

4. Click on RER Expired CO2 Concentration (%) on the RER channel. Choose Setup Function from the drop down list. This will open the RER Calculation Dialog window.

5. Setup the RER calculations:

    • Choose the appropriate RER Type by clicking on the down arrow and choosing Closed Small Animal Chamber.

    • Check the O2 and CO2 channel information so that they are being calculated from the correct channels.

    • Change the Time(s) to average to 30 seconds

    • Change the Delta Time(min) to be the T2-T1 value noted in Step 2. This is the time it took for the CO2 concentration in the small animal chamber to change by 1%. (Time(s) to average (sec) value will be less than Delta Time (min).)

    • Click OK.

6. The recording on the main window will now have a histogram on the RER channel.

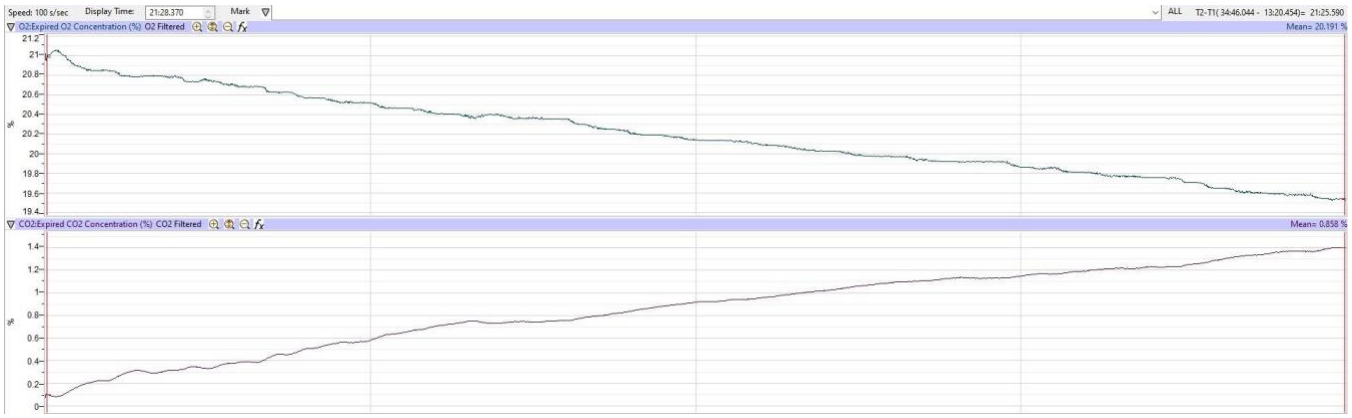


Figure AMe-1-L1: The O2 and CO2 recording over a period of 23 minutes showing a change in concentration by 1%.

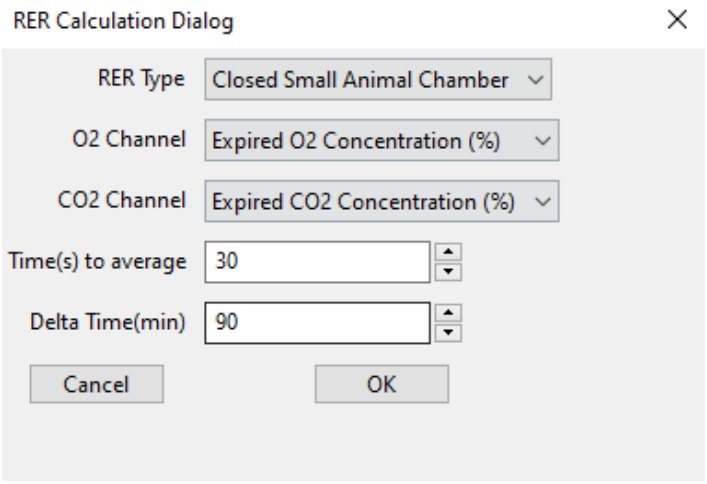


Figure AMe-1-L2: RER Calculation Dialog window.

Data Analysis

1. Scroll to the beginning of the recording where the histogram is not a straight line. You should be able to see a step-like histogram.

2. Click and drag the cursors to either side of the step-like histogram.

3. Click the Zoom between Cursors button on the toolbar to expand this section of data to fill the window.

4. Click AutoScale on all channels. Your data should look like the data seen in the image below.

Figure AMe-1-L3: The RER channel showing the histogram for the ratio of CO2 to O2 in the ectothermic organism, G. portentosa.

5. Click on the Analysis window icon.

6. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The functions, Mean, T2-T1, and V2-V1 should appear in this table. Values for these parameters on each channel are seen in the table across the top margin of each channel.

7. Once the cursors are placed in the correct positions for determining the CO2 and O2 concentrations, the values for these parameters can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal.

8. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of these parameters in the Journal. To use them:

- Place the cursors at the locations used to measure the concentrations and RER.
- Transfer the names of the mathematical functions used to determine these data to the Journal using the Add Title to Journal function in the Expired CO2 Channel pull-down menu.

9. Transfer the values for the data to the Journal using the Add All Data to Journal function in the Expired CO2 Channel pull-down menu.

10. Repeat this procedure for the Expired O2 Concentration (%) channel.

11. The values for the following parameters are determined when the cursors are positioned as directed:

- Mean concentration of CO2 in expired air, which is the value for Mean on the Expired CO2 Concentration channel.
- Mean concentration of O2 in expired air, which is the value for Mean on the Expired O2 Concentration channel.

12. Mean RER, which is the value for Mean on the RER channel. This is calculated by dividing the expired CO2 value by the expired O2 value to get the RER ratio as set up previously.

13. Record the T2-T1 value that is used to measure the mean values of CO2, O2 and RER.

14. Record the values in the Journal using one of the techniques described in Steps 6 or 7.

15. Record the values for the Mean CO2 and O2 concentrations in expired air in Table 1. Also record the RER and T2-T1 values.

Exercise 2: Changes in CO2 and O2 in a closed chamber, and RER in ectothermic animal reared at 30ºC

Aim: To determine the changes in CO2 and O2 volumes of an ectotherm and make a comparison with the values obtained from an ectotherm at rest.

Approximate Time: 30-45 minutes, depending on the animal.

Procedure

1. Use the same procedures used in Exercise 1 to record the CO2, O2 and RER values from an ectotherm.

2. Place the ectotherm (cockroach or cricket) into the clean, dry small animal chamber and close the lid securely.

3. Mark the recording with comments that indicate the organism in the chamber.

4. Click Record to begin the recording.

5. Record until a concentration of 1% carbon dioxide is reached (3% for ectothermic vertebrates, e.g. lizard, frog, snakes).

6. Click Stop to halt the recording and Save your data file.

7. Remove the animal from the chamber and place it back in its container.

RER Setup and Data Analysis

1. Use the same procedures used in Exercise 1 to set up the RER channel showing a 1% change in CO_2 value.

2. Make any necessary changes on the RER channel by opening the RER Calculation dialog window.

3. Follow the procedures in Exercise 1 for data analysis of the beginning section of the recording for the ectotherm showing the step-like histogram.

4. Determine the oxygen (O_2), carbon dioxide (CO_2), and respiratory exchange ratio (RER) values. Note the T2-T1 value for the section of data with the step-like histogram.

5. Record the values for the Mean CO_2, O_2 concentrations, RER, and the T2-T1 value in Table 1.

Questions

1. During which experimental period was the ectotherm's expired CO_2 the highest? In which period was it the lowest?

2. During which period was the ectotherm's inspired O_2 the highest? In which period was it the lowest?

3. During which period did the ectotherm have the highest RER? In which period was the RER the lowest?

4. How do the values obtained from the ectothermic animal at rest compare to those from the ectothermic animal reared at 30^{o}C?

5. How does the RER values from these organisms relate to their overall metabolic rate?

6. How does ambient temperature influence the overall metabolism?

7. What would happen to the RER values for the ectotherm if you lowered the ambient temperature? What would happen to the RER once the ectotherm returns to normal body temperature or to room temperature? Would the RER values differ if using an endothermic animal?

8. For what reason should the animal not have eaten within an hour of performing these experiments? Evaluate the diet of these animals. How does diet correlate to the RER values?

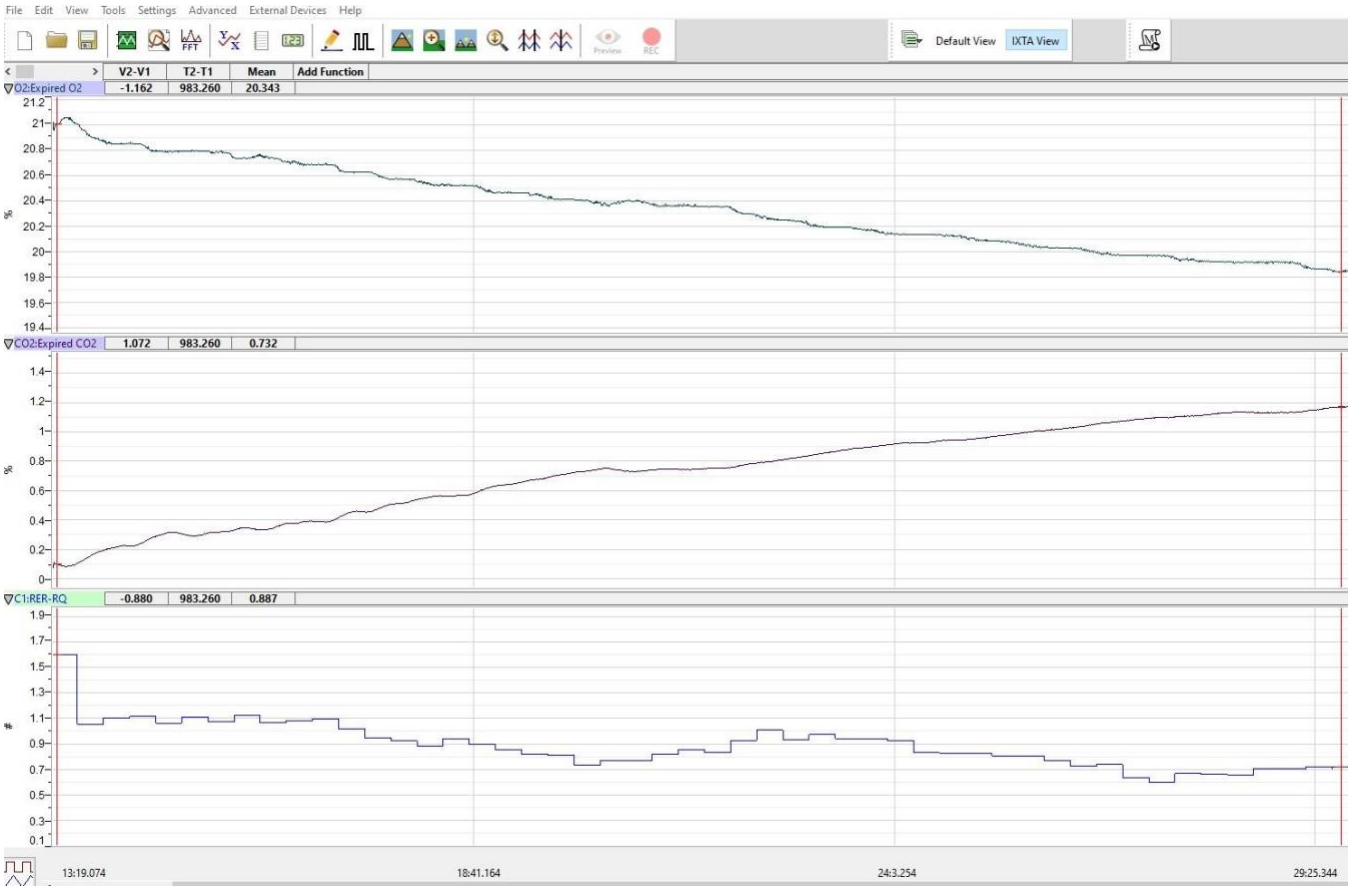


Figure AMe-1-L4: The oxygen (channel 1) and carbon dioxide (channel 2) values and RER (mean value of channel 3) of a small animal as displayed in the Analysis windows for each channel respectively. The red cursors are in position to measure the mean values of the selected interval.

Optional Exercise

RER is affected by metabolic changes. As an optional activity, the animals can be exercised and RER can be calculated as they return to rest. Other experiments can be designed by students and instructor(s) given available resources and equipment.

Table for recording Mean CO2, O2 and RER values for the endothermic and ectothermic organisms. T2-T1 values are also recorded.

Environmental Conditions		Organism	Mean Concentration (%) in the Chamber		Mean RER
Temperature (ºC)	T2-T1 (min/sec) for RER calculation	*Genus* *species*	CO2	O2	Usu. <1.0
		Ectotherm (at rest)			
		Ectotherm (at 30ºC)			

Weight Normalization Calculation

Normalized Expired O2

O2 / [(weight(g) / mass unit)]*effective mass

Example: O2 = 0.83 ml/min, weight = 25 grams,

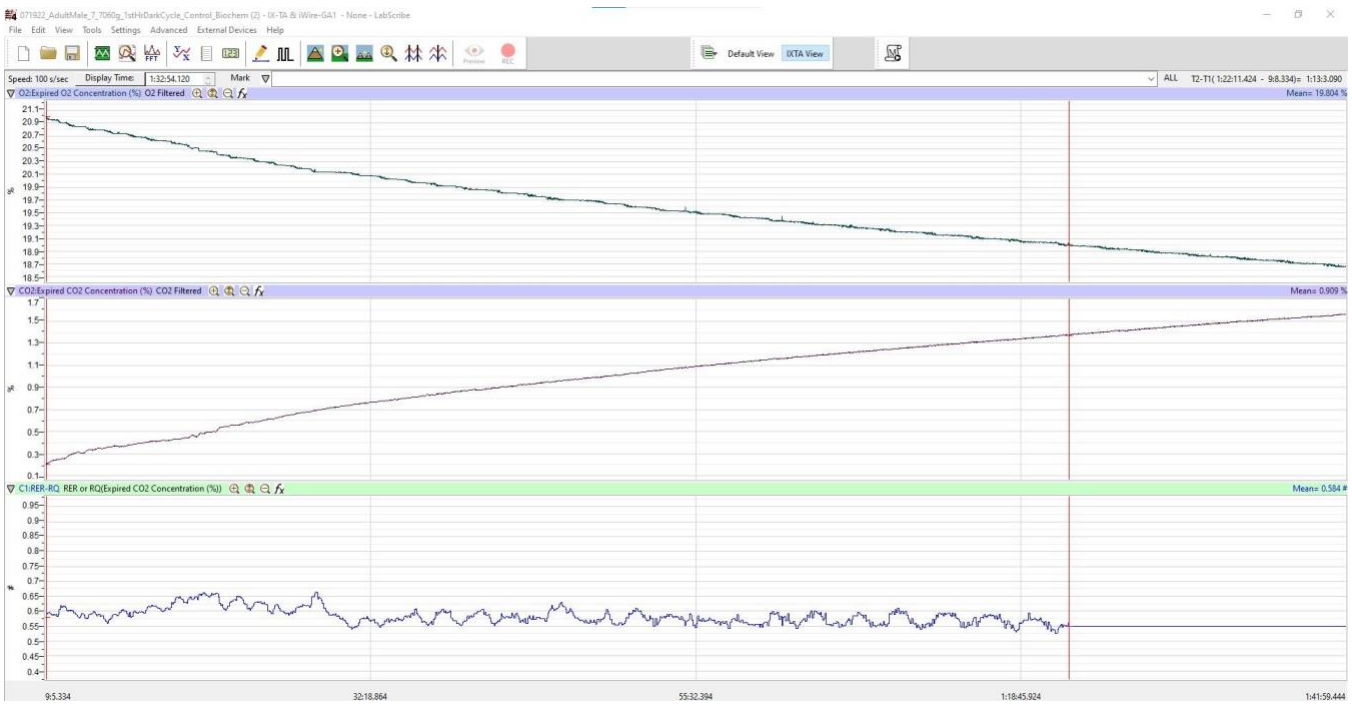
mass unit = KG, effective mass factor (slope) = 0.75

O2norm = 0.83 / (25/1000)0.75 = 13.2 ml/KG/min

Using an effective mass factor of 1 eliminates any effective mass correction.

This can be repeated for the CO2 values. When RER is calculated using normalized values, the weight of the organism is taken into account.

Example Data (*G. portentosa*)



Exercise 1: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) measured at 0.584 in an *G. portentosa* (control).

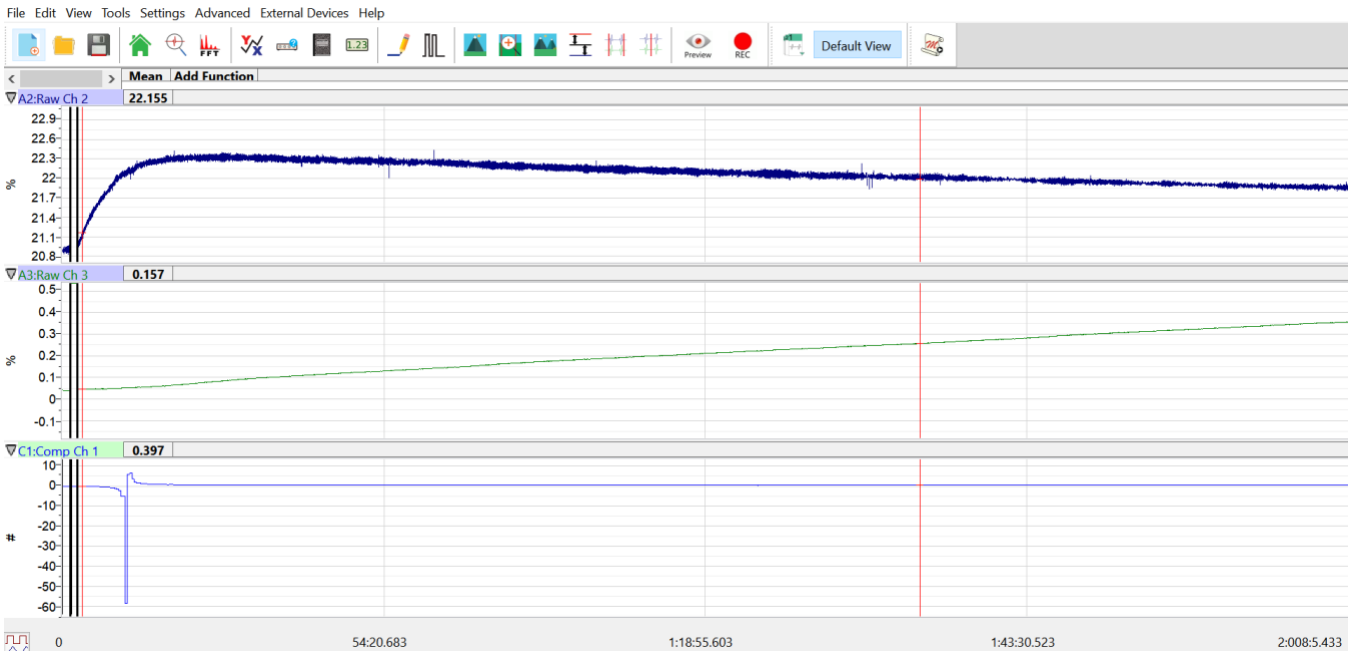
Exercise 2: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) recorded as 0.659 in an exercise induced *G. portentosa* (experimental 1: rolling on rocking platform).

Exercise 3: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) recorded as 0.682 in a diet-restricted *G. portentosa* (experimental 2: glucose).

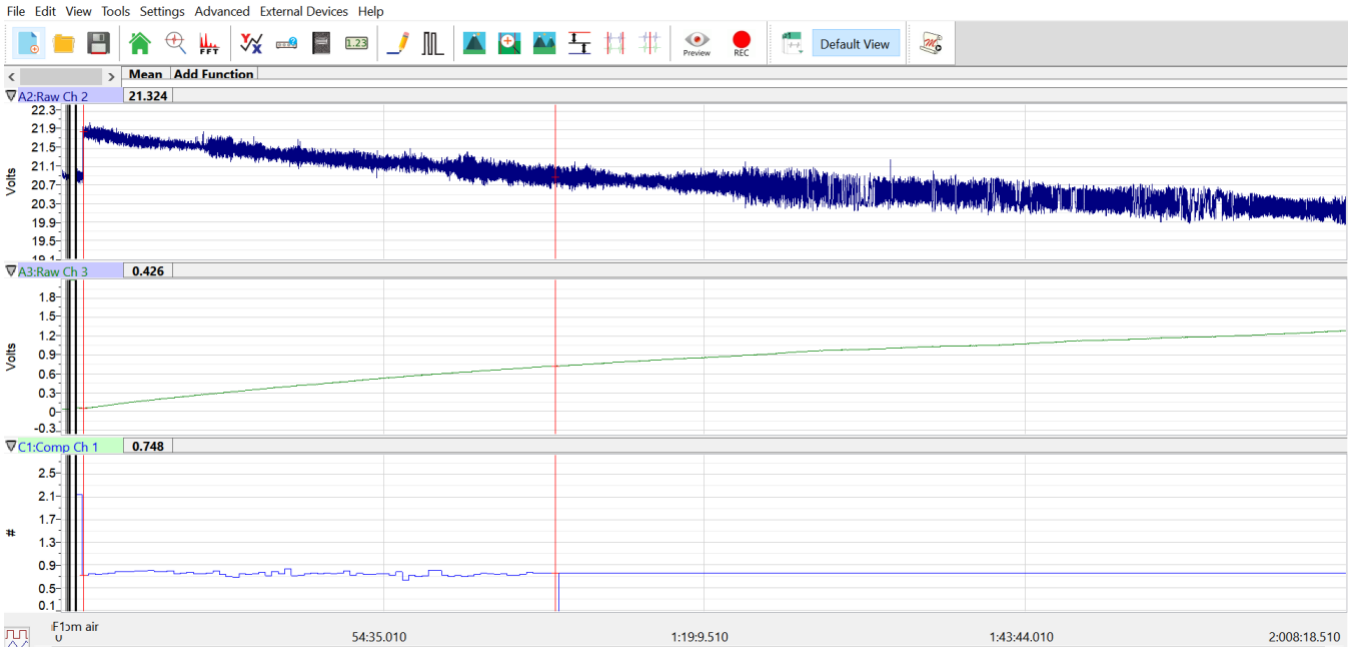
Exercise 4: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) recorded as 0.876 in a *G. portentosa* experiencing temperature extremes (experimental 3: 30^{o}C for at least 30 minutes).

Example Data (Cricket)

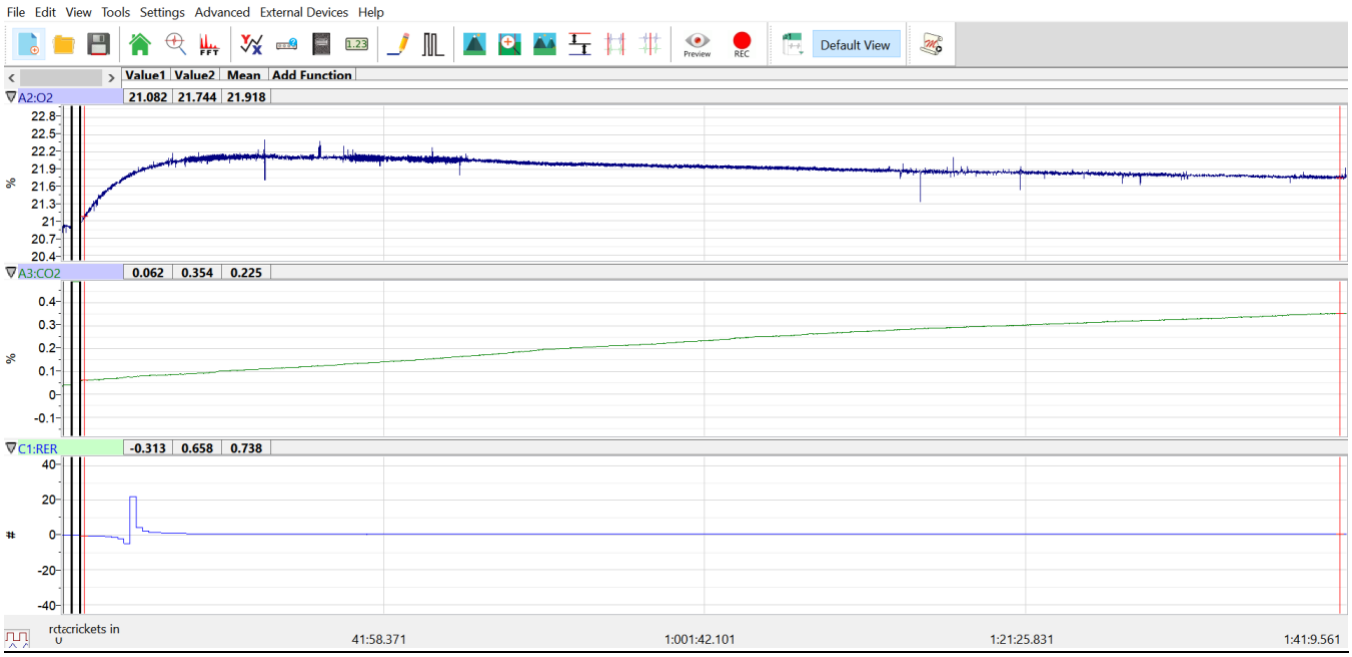
Example 1A: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) measured at 0.347 in an cricket (at rest).



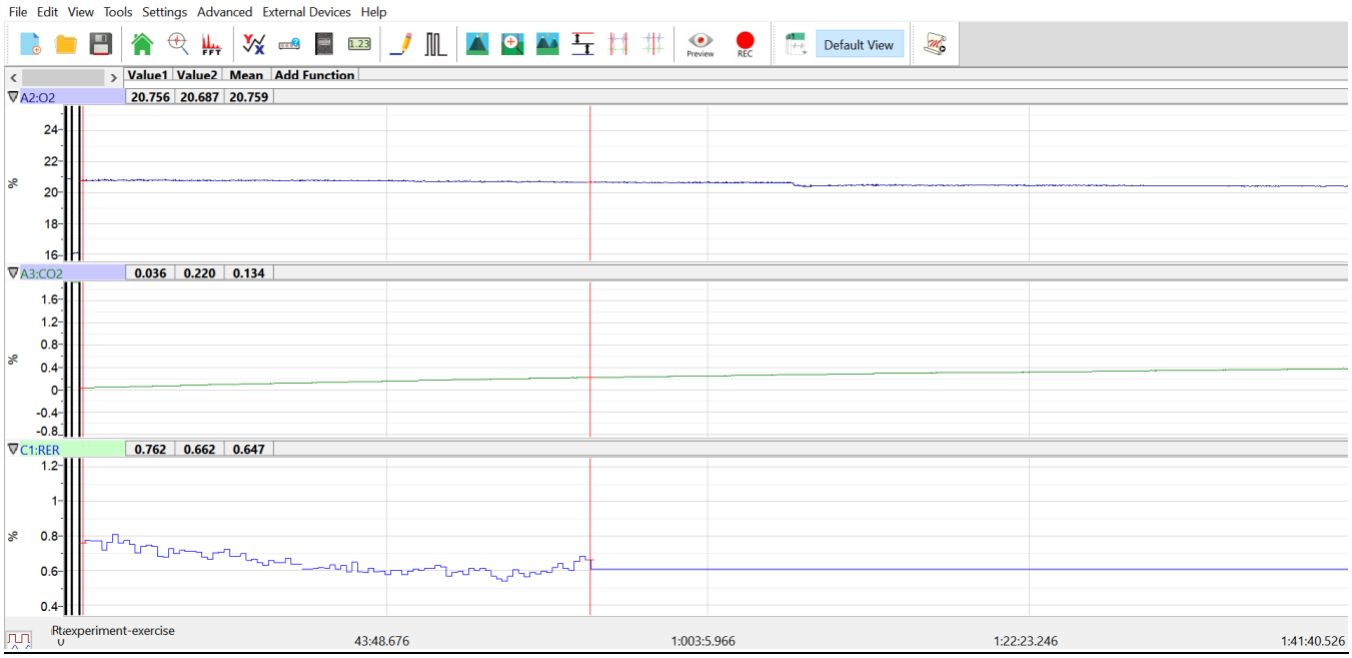
Example 1B: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) measured at 0.397 in an cricket (at rest). Analysis window shows the means of each channel.



Example 2A: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) recorded as 0.748 in a diet-restricted *cricket* (glucose for 1 week). Analysis window shows the means of each channel. Mean RER is less than expected perhaps a longer period of diet-restriction is required to ensure no proteins are consumed.



Example 2B: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) recorded as 0.738 in a diet-restricted cricket (high protein for 1 week). Analysis window shows the means of each channel. Mean RER is greater than expected perhaps a longer period of diet-restriction is required to ensure no carbohydrates are consumed.



Example 3: Changes in O2 (Channel 1) and CO2 (Channel 2) in a closed chamber with mean RER (Channel 3) measured at 0.647 in an cricket (exercised). Analysis window shows the means of each channel. Mean RER is less than expected perhaps due to the smaller size of experimental cricket in this case.

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