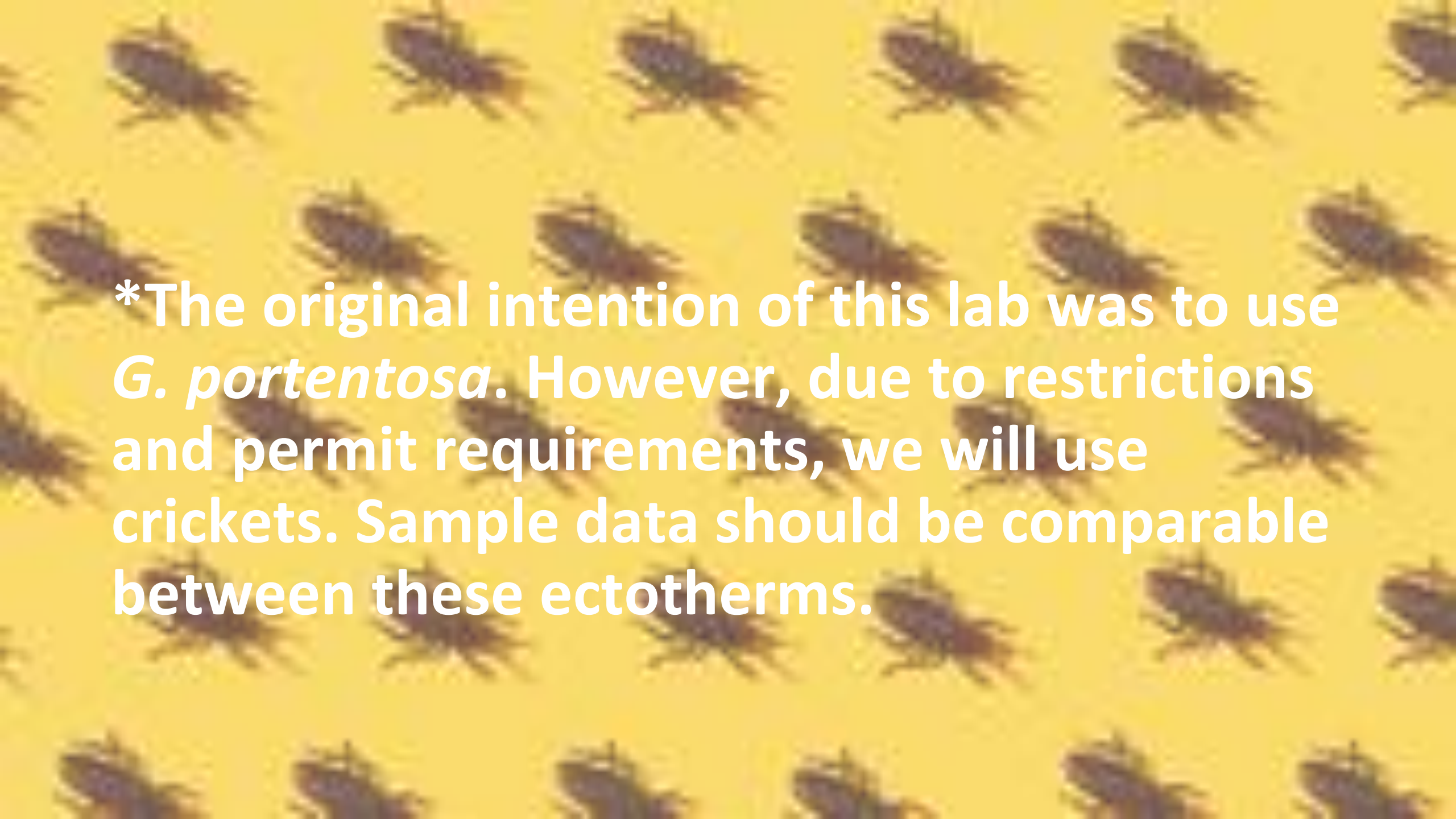
Using *Gromphadorhina portentosa* to teach metabolic and respiratory principles of ectothermic animals

ABLE Conference 2022

University of Victoria


*The original intention of this lab was to use *G. portentosa*. However, due to restrictions and permit requirements, we will use crickets. Sample data should be comparable between these ectotherms.

About the Authors

- Kenneth G. Sossa, Ph.D.
  - Trained neuroscientist with an interest in Animal behavior and physiology
  - Recent funding prompted purchase of gas analyzer and peripherals to explore cockroaches as model organisms in studying respiration and metabolism.
  - The labs proposed here can be simplified or expanded into a semester long research/independent project
- Team of students

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Keywords

- *Gromphadornina portentosa*
- Giant Madagascar Hissing Cockroach
- Respiration
- Insect tracheal system
- Spiracles
- Metabolism
- Metabolic rate
- Respiratory Exchange Ratio (RER)
- Ectotherm

Learning 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.

Target students taking the following courses with lab…

- Intro to Biology (w/systems approach)
- Animal Physiology (3rd-4th yr)
- Entomology (3rd-4th yr)
- Invertebrate Anatomy (3rd-4th yr)

Small Animal Respiratory Exchange Ratio (RER) iWorx Lab

Experiment AMe-1

Animal Metabolism

Equipment

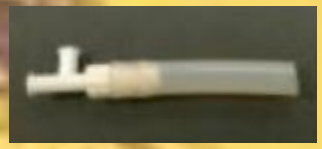
Setup

Calibration

Exercise

Equipment

- PC or Mac Computer
- iWorx IXTA (plus USB cable & power supply)
- Small animal chamber (Li-Cor 9960-093)
- Gas sampling tubing
- iWire gas analyzer
- Calibration kit
- Endotherm Animal (insect)

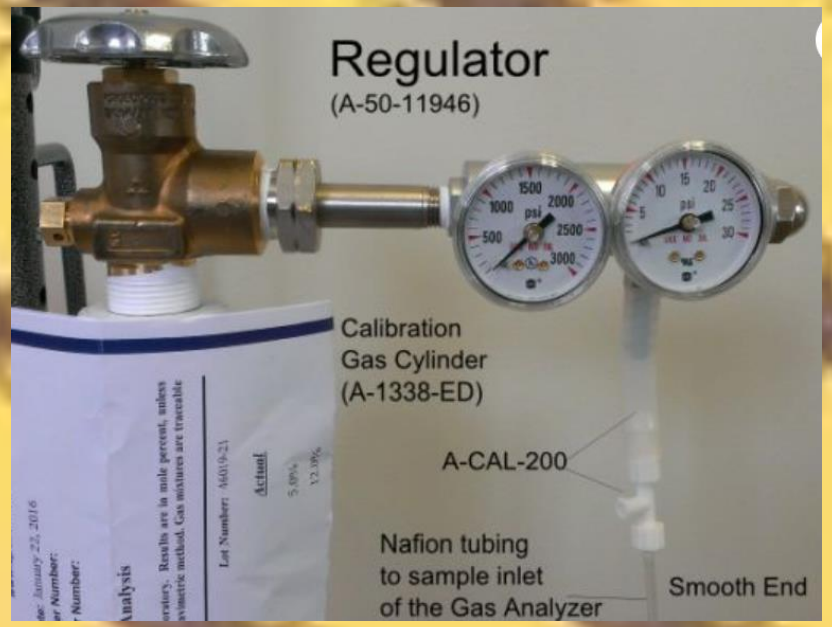


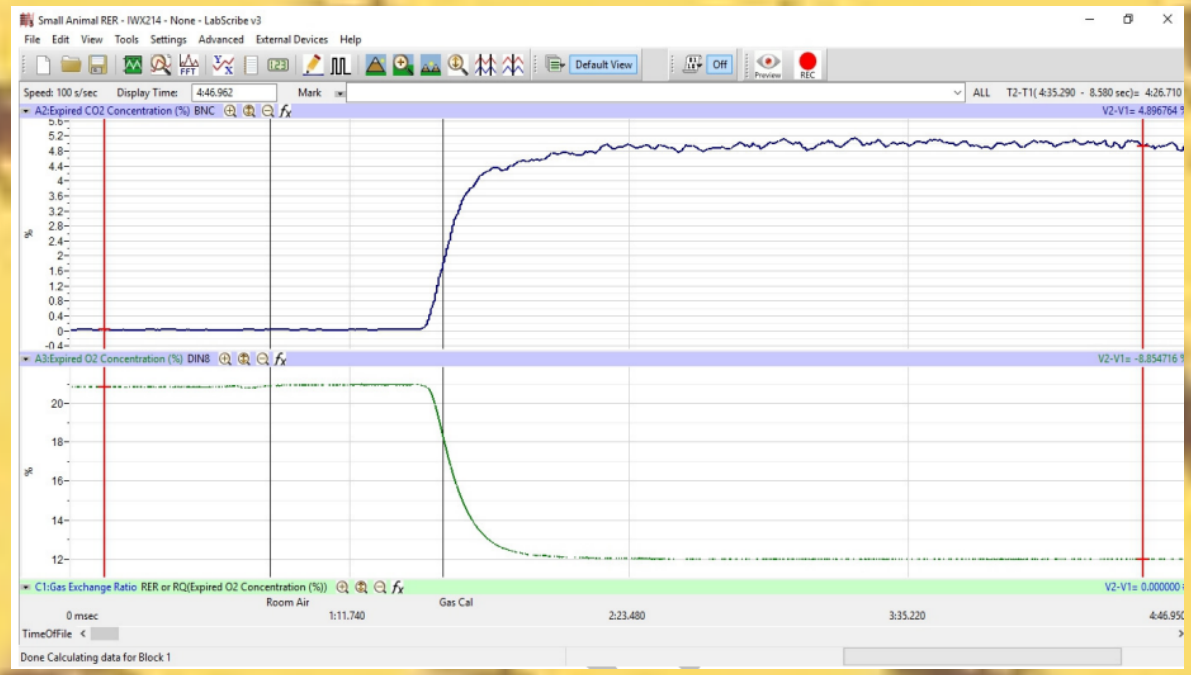
Setup

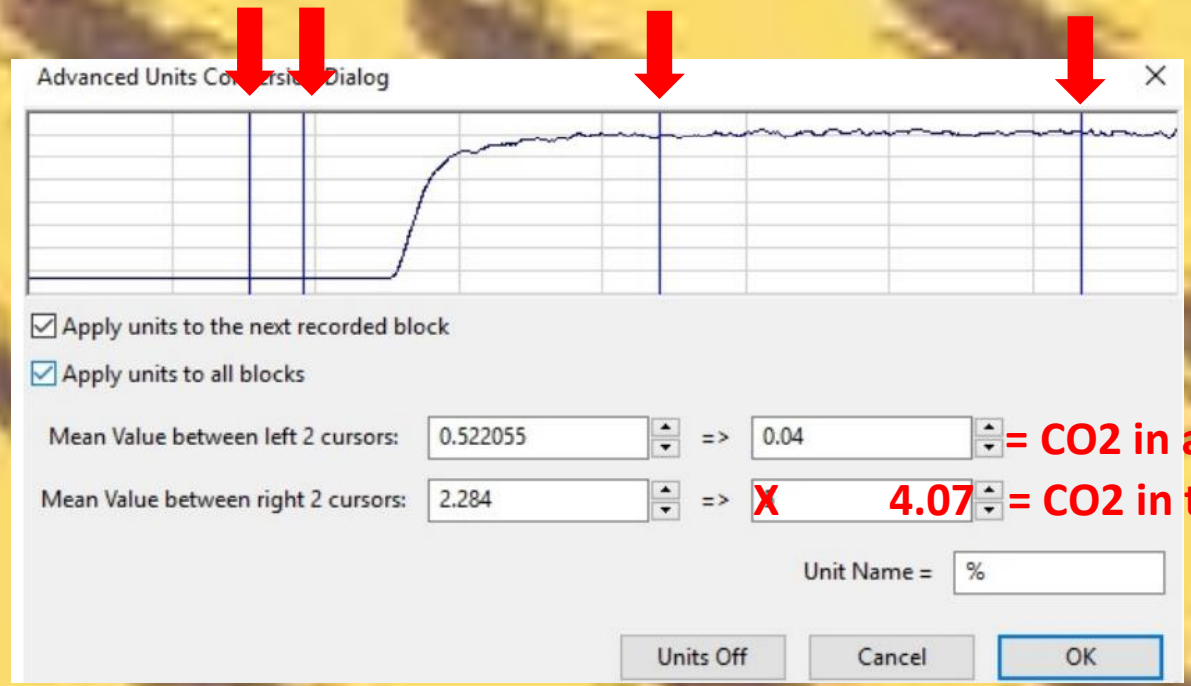
- IXTA & iWire GA
- Follow directions provided in protocol

Calibration

- Follow instructions for calibrating the iWire-GA Gas Analyzer







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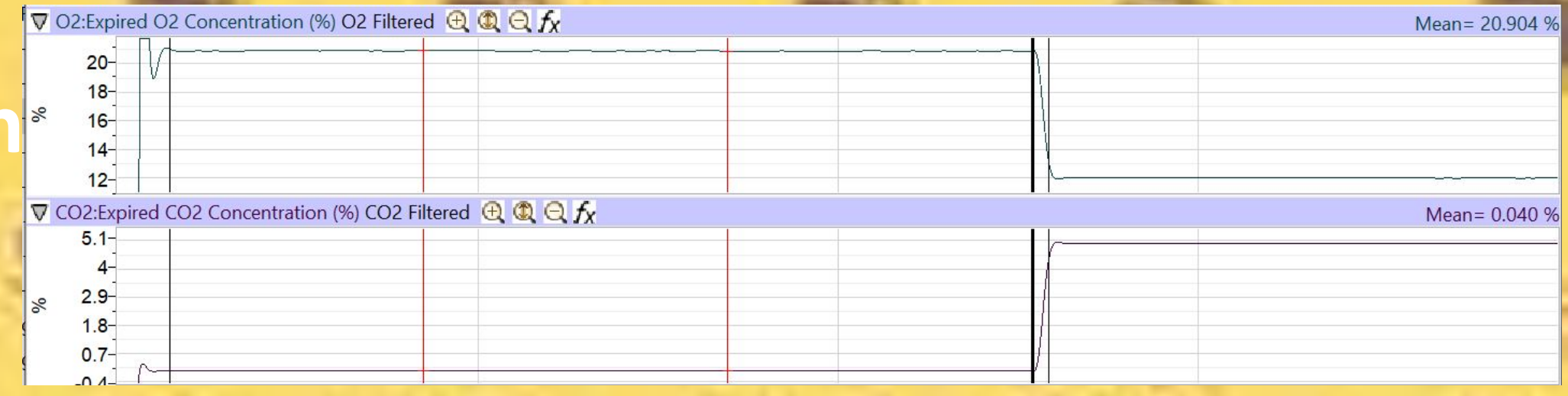
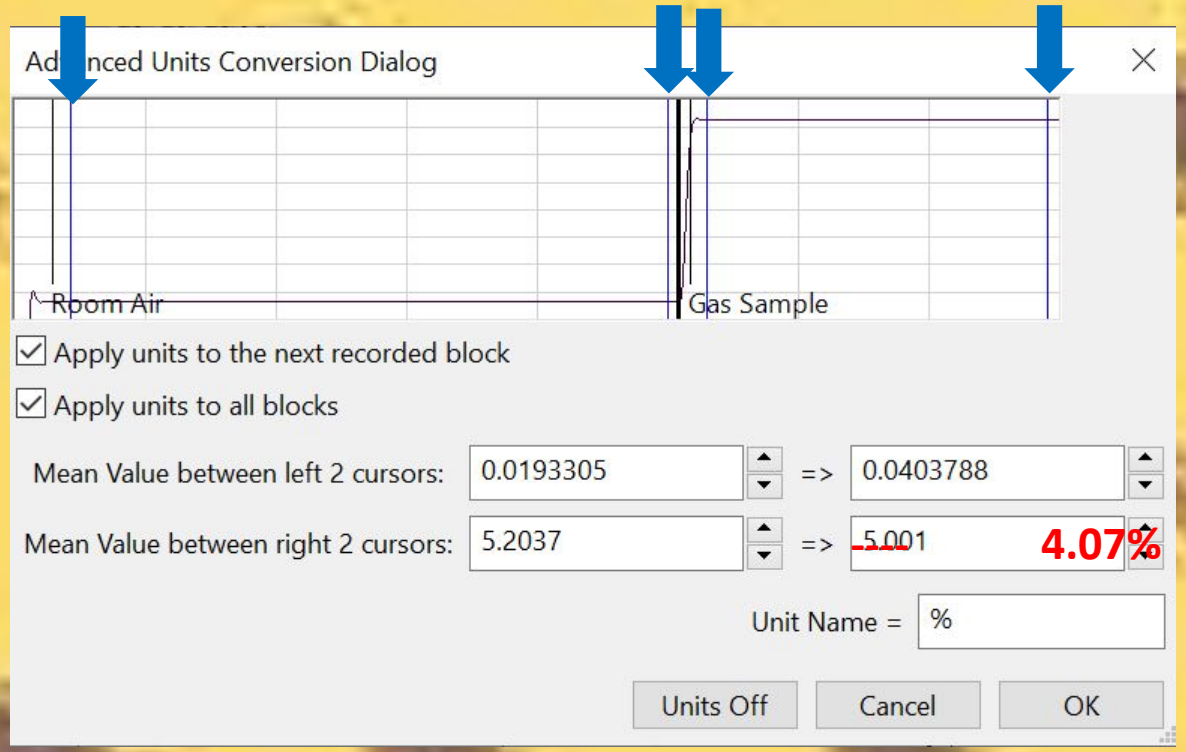
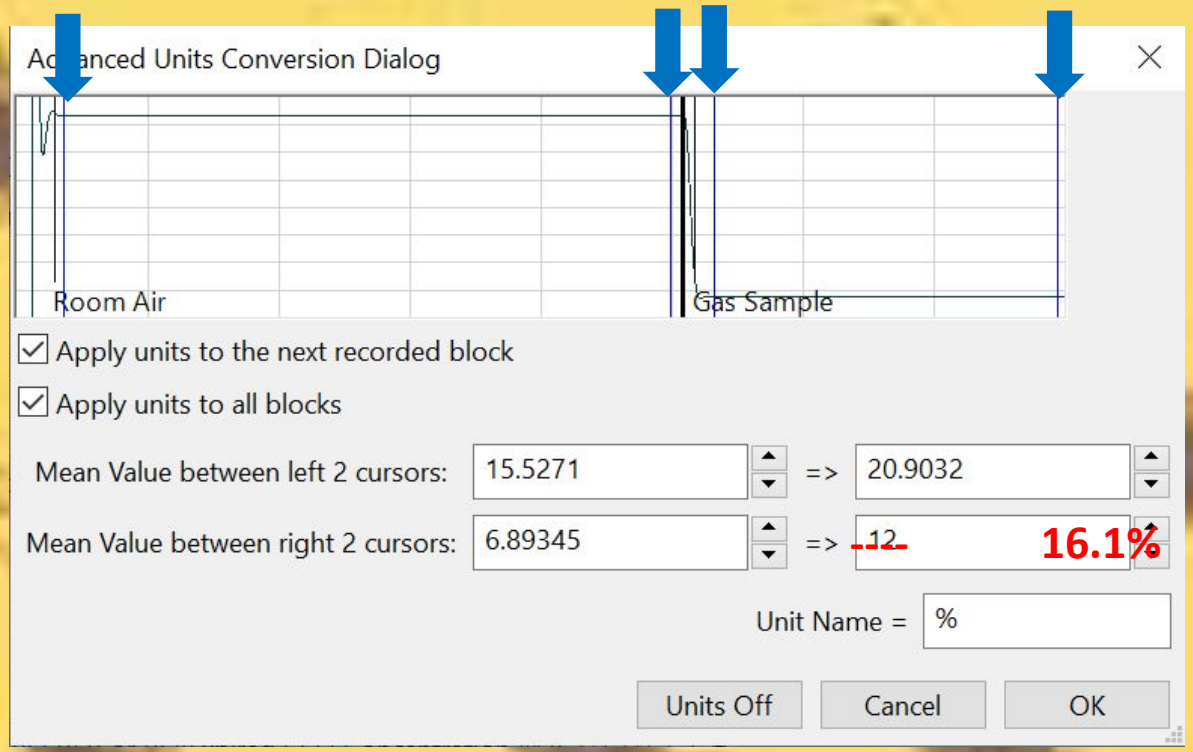


IN AIR

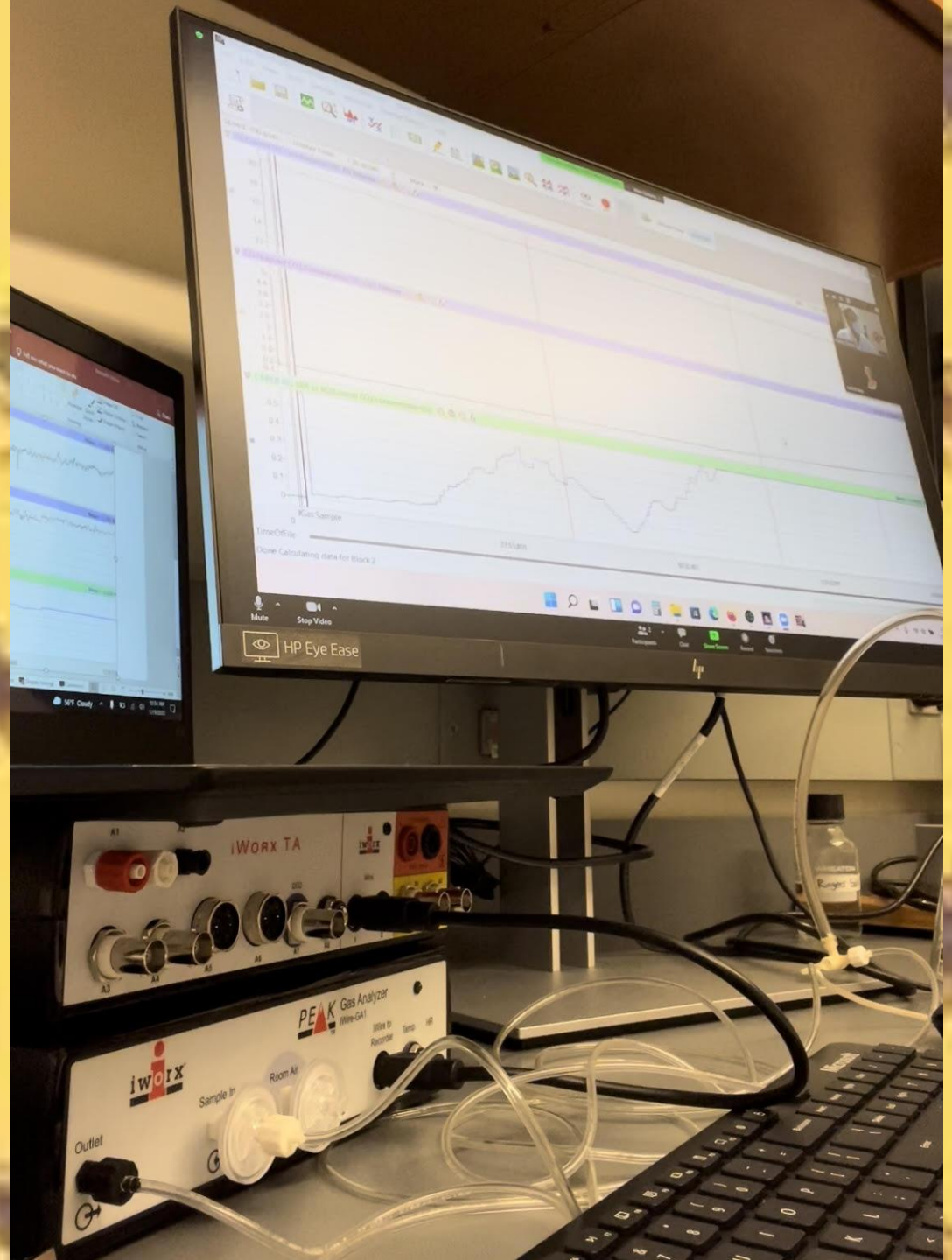


IN TANK

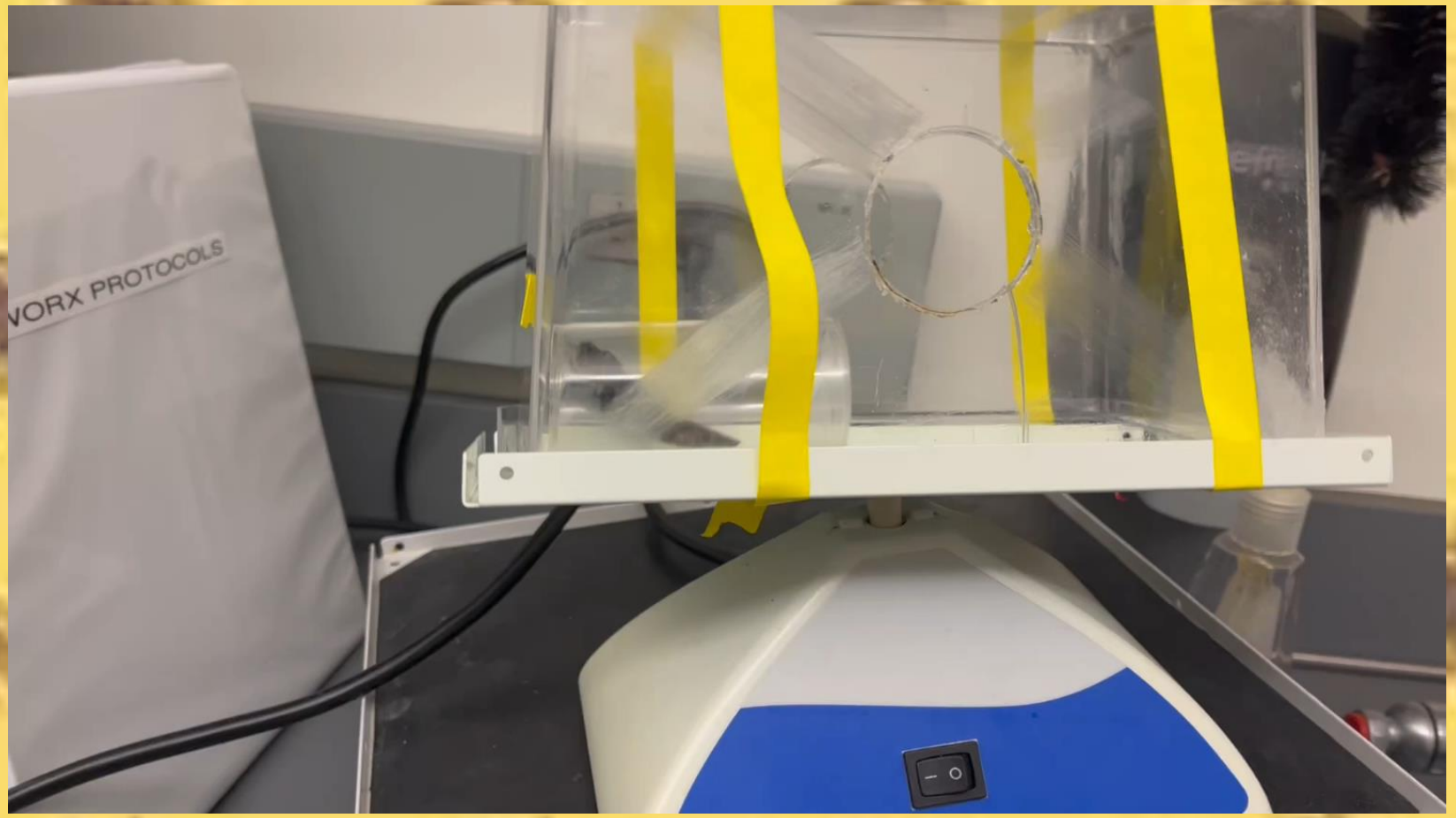


Exercise

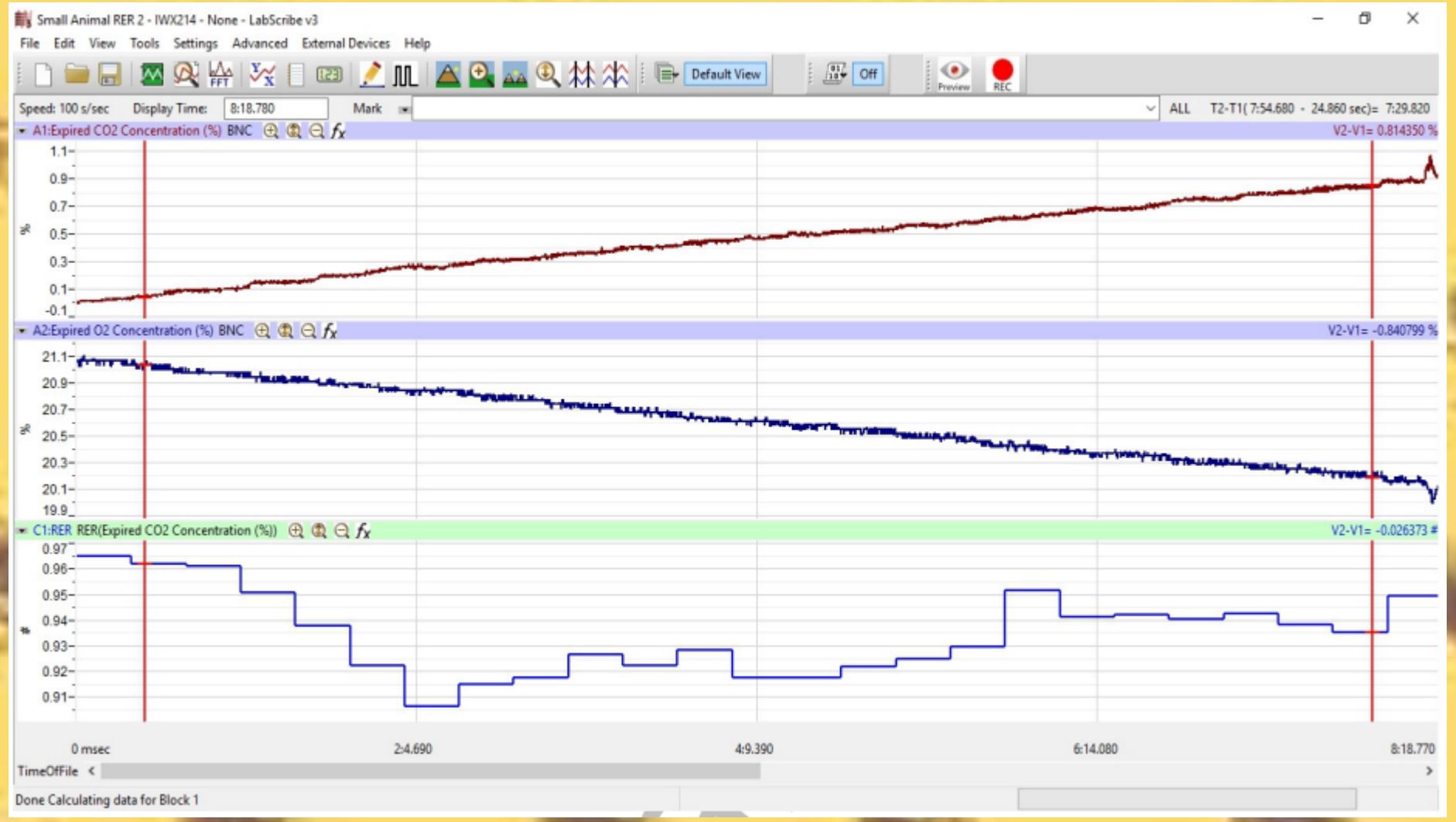
- Changes in CO_2 and O_2 in a closed chamber and RER in an ectothermic animal (cockroach)
- Volume of gases will be measured
- Comparison of measured gases and RER for cockroaches under different
  - Diets (glucose vs high protein)
  - Temperatures (20C vs 30C)
  - Activity levels (rest vs exercised)



“Exercise” video (increased activity cp to rest group)



Example Data



Insect respiratory anatomy & physiology

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Respiration Concepts

- Oxygen and carbon dioxide physiology
- External respiration
- Transport of oxygen and carbon dioxide in body fluids
- Circulation

Respiratory System Functions in Insects

- Area for gas exchange between air ~~and circulation~~
- Ventilation, or moving air to and from exchange surfaces
- Protection and defense of airborne debris and pathogens
- Producing sounds
- ~~Presenting olfactory cues to the nervous system~~

Respiration for energy purposes

- Carbohydrates (CHO) and lipids (fats)
- Products of catabolism include CO_2, water, and energy
  - $6\ O_2 + C_6H_{12}O_6 = 6\ CO_2 + 6\ H_2O + 38\ ATP$
    - Ratio of 1.0
  - $23\ O_2 + C_{16}H_{32}O_2 = 16\ CO_2 + 16\ H_2O + 129\ ATP$
    - Ratio of 0.7

Activity State

- Activity state determines the proportion of carbohydrates and fats used
- At rest body, energy derived from 40% CHO & 60% fats
- Intense activity, energy derived from more and more CHO

Changes in CO_2:O_2 Ratio → Metabolic Shift

- Changes in activity produces change in CHO and fat usage and results in CO_2:O_2 shift
- Reflects cellular metabolism shift

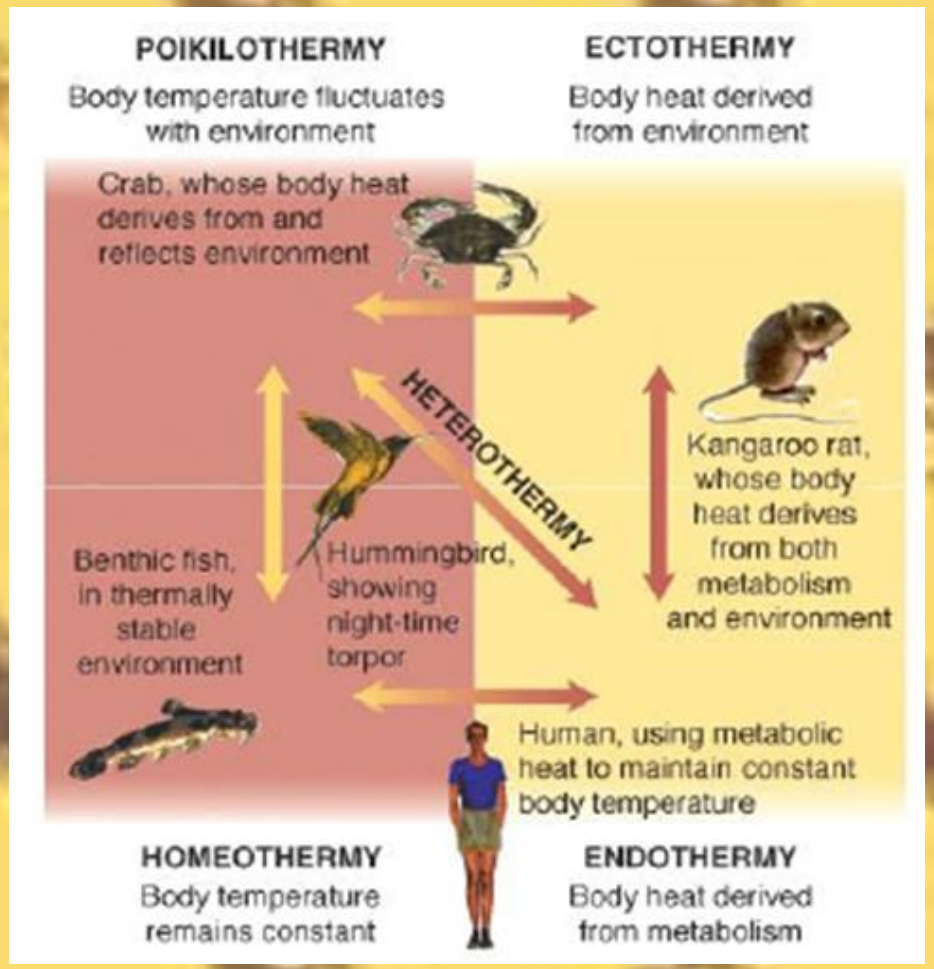
Metabolic rate

- 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

Insects are not as metabolically active as other animals

- 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)

Metabolic status and thus body temperature changes in ecto- and endothermic animals



Factors that affect metabolic rates in animals

- Environmental temperature
- Physical activity
- Body size
- Time of day
- Age
- Gender
- Meal type (protein-rich)

How is metabolic measured?

- Measuring these gasses estimates metabolic rate
- Previously, metabolic rate was measured
  - Direct calorimetry- measures body heat that melts each gram of ice
  - Indirect calorimetry- 2 methods that measure proxy properties
    - Respirometry- measures rate of gas exchange with environment
    - Material-Balance method- measures chemical energy content of organic matter entering and leaving the animal's body-

Respirometry

- Devices used to measure O2 consumption are called respirometers
- Configurations:
  - Closed: animal placed in sealed chamber with a fixed volume of air
  - Open: animal takes O2 from a flowing air stream during measurement

Measuring CO_2 produced & O_2 used

- Gas Analyzer measures gasses of animal in a closed chamber
- Oxygen consumed per minute = VO_2
- Carbon dioxide produced per minute = VCO_2
- Ratio of VCO_2/VO_2 is Respiratory Exchange Ratio (RER)

Insect Respiratory Exchange Ratio (RER)

- Energy expenditure determined from RER used to determine the proportion of CHO and fats used
- RER used to determine the energy expended per liter of O2 used during activity
- Amount of O2 consumed per unit of time, converted to calories or joules, units of energy

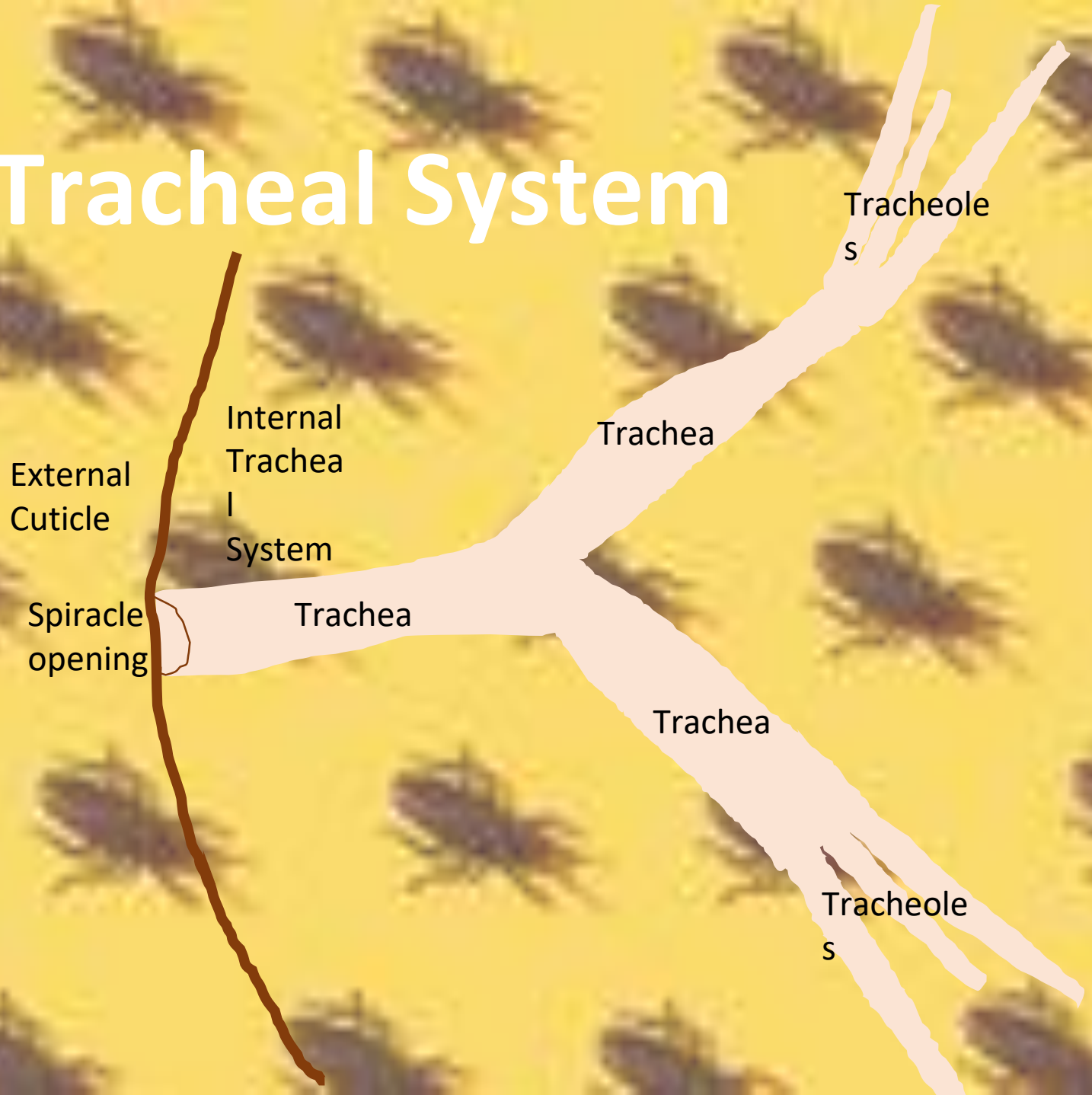
RER	Energy kcal/liter O_2	% Energy from CHO	% Energy from Fats
0.70	4.69	0	100
0.75	4.74	15.6	84.4
0.80	4.80	33.4	66.6
0.85	4.86	50.7	49.3
0.90	4.92	67.5	32.5
0.95	4.99	84.0	16.0
1.00	5.05	100	0

Insect Anatomy & Physiology videos

https://youtu.be/quwhcgkVO3c

https://youtu.be/JQ2zrpBu2ac

Insect Tracheal System



Spiracles

- Air passages through small openings
- Opening cavity called atrium
- Opening and closing apparatus called spiracular valve to control air and water loss
- Usually more than one pair of spiracles present and functional
- Each spiracle surrounded by sclerotized cuticular plate called peritreme

Number and arrangement of spiracles in insects

- 12 pairs of spiracles on thorax & First 9 abdominal
- Most spiracles on 2 pairs on thorax and 8 pairs on abdomen
- Special classification based on number & position of spiracles (e.g. holopneustic has 8 pairs on abdomen)

Tracheae

- Pipe-like, flexible organ
- Spiral sculpture called taenidia allowing for elasticity and compression
- Thick, helical and thread like layer
- When filled with air it appears silvery and shiny in appearance observed during dissection

Tracheoles

- Lattice work of tracheae called tracheoles
- Diameter less than 1 um (0.2-0.3 um)
- Site of gas exchange
- Interconnected network of tracheoles
- Lining not shed during molting but grows and expands

Air Sacs

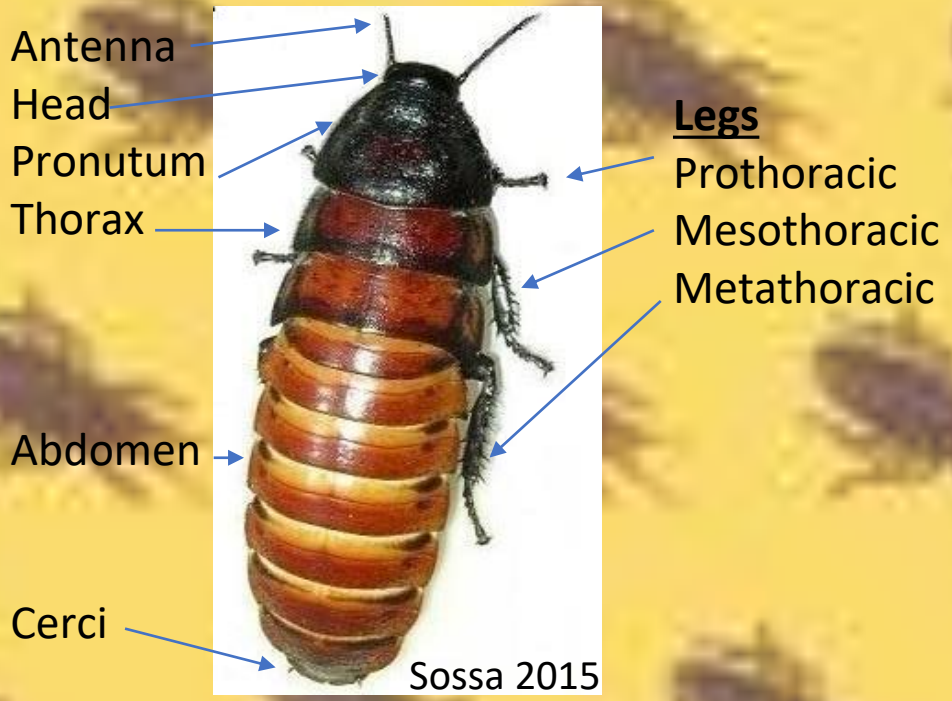
- Tracheoles form thin walled collapsible structures called air sacs
- Air sacs are air reservoirs
- White, silvery vessels filled with air
- Works in sound amplification
- Helps in heat insulation
- Helps in flight by reducing gravity of insects
- Decreases in volume to allow for organ growth & expansion

Activities for students during recording

- Cockroach puzzle of external anatomy
- Play-Do reconstruction of respiratory system (or another system)
- Primary paper analysis (group discussion and presentation)

Cockroach 3D Puzzle

- https://www.pinterest.com/pin/473581717035533878/
- When the pieces have been assembled to look like the image below over white printer paper, label the external anatomy using the example below but Google more to add more anatomy labels



Cockroach (insect) tracheal system

- Using play-do represent the sections of the insect respiratory system on white printer paper
- Label using appropriate nomenclature
- Define function
- Describe the movement of air across these surfaces and potential changes to the metabolic rate

Working with the scientific literature

- Provide instructions on how to read scientific literature
- In-lab assignment can be as general or detailed as instructor desires
- For example- ask students to be able to
  - Summarize study and its significance
  - Identify one figure that relates to work done in the lab
  - Suggest the next experiment

Recommended Papers for Students

- Insect gas exchange and ventilation
  - Heinrich et al 2013 Coordinated ventilation and spiracle activity produce unidirectional airflow in the hissing cockroach, *Gromphadorhina portentosa*
- Diet (Carbohydrate- glucose, protein, vs lipid)
  - Talal et al 2021 High carbohydrate diet ingestion increases post-meal lipid synthesis and drives respiratory exchange ratios above 1
- Temperature (20C vs 30C)
  - Lalouette et al 2011 Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations

Respiratory Exchange Ratio (RER) Example Data

ROACH	RER
Control	0.398
Glucose Fed	0.551
Exercised	0.762
Glucose+Exer	0.724
30°C	0.802

Respiratory Exchange Ratio (RER) Example Data

Cricket	RER
Control	0.440
Exercised	0.471

References

- iWorx.com
- Sossa KG 2015 Using *Gromphadorhina portentosa*, the Giant Madagascar Hissing Cockroach, as a Model Organism in the Biology Laboratory Tested Studies for Laboratory Teaching Proceedings of the Association for Biology Laboratory Education Vol. 36, Article 80