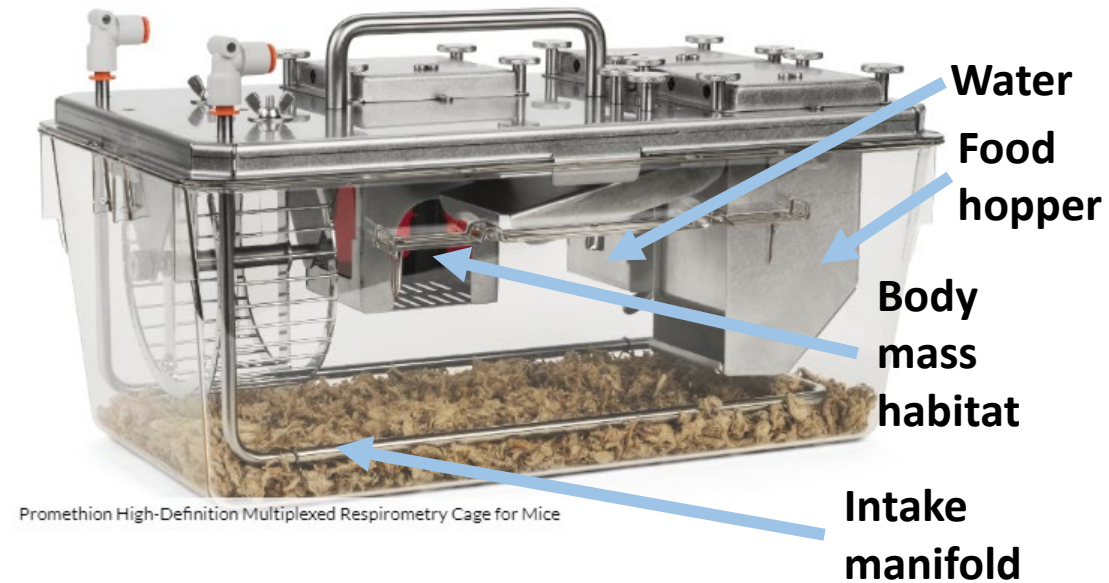


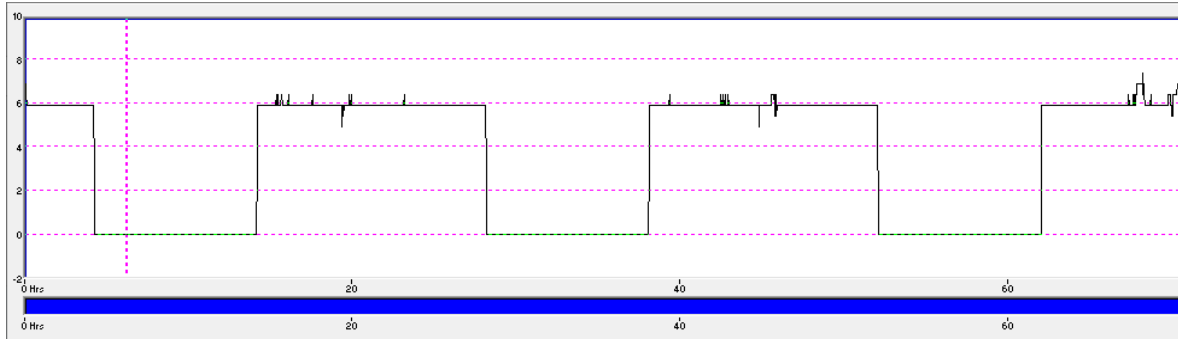
# Sable Promethion

- 16 cages
- Indirect calorimetry at 3 minute intervals
- Continuous food and water quantification
- Continuous activity quant. in X,Y,Z
- Body mass quant. via enrichment habitat
- Optional programmable running wheels
- Optional programmable food restriction
- Temperature cabinet for custom temperatures

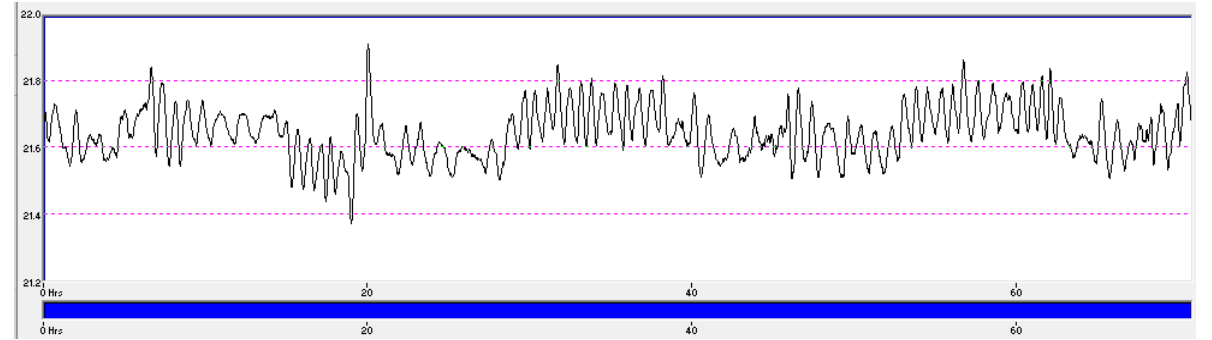


# Environmental Sensing Accessory

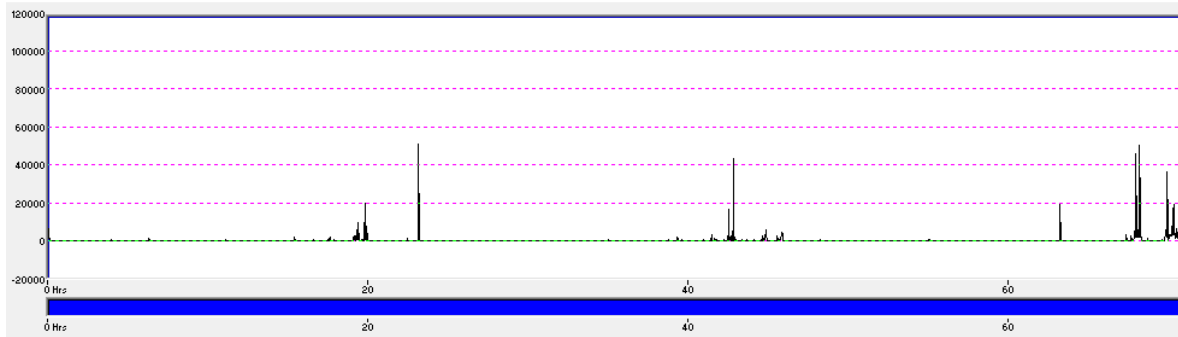
- There are two, one attached to each bank of cages; one is near the wall, one near center of room



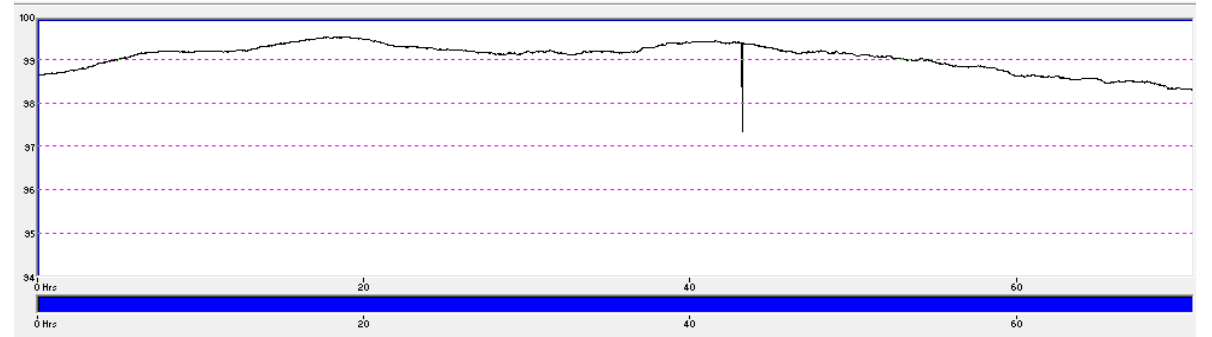
Light



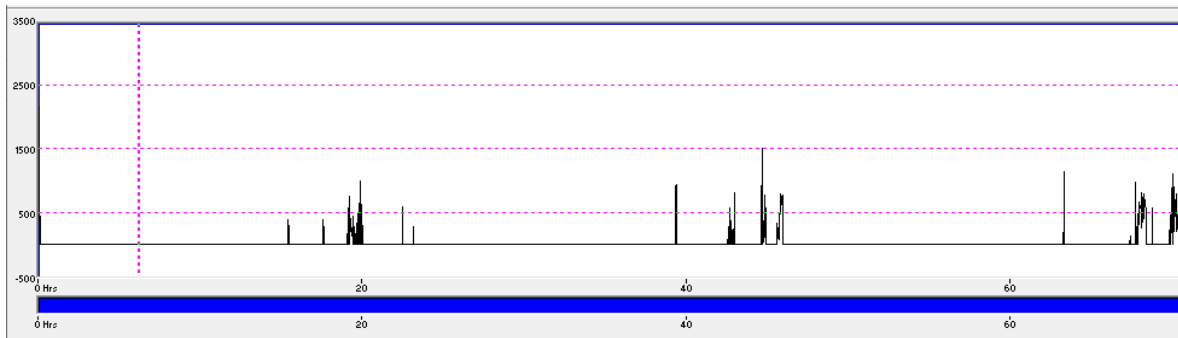
Temperature



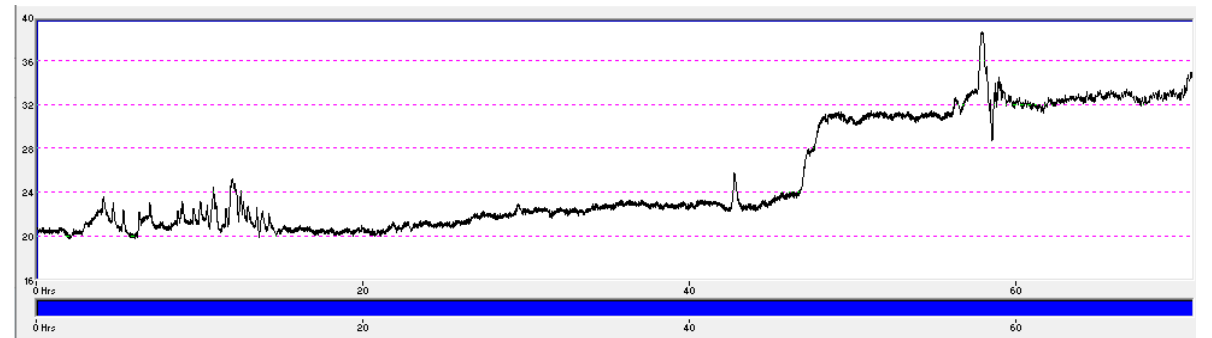
Sound



Barometric pressure



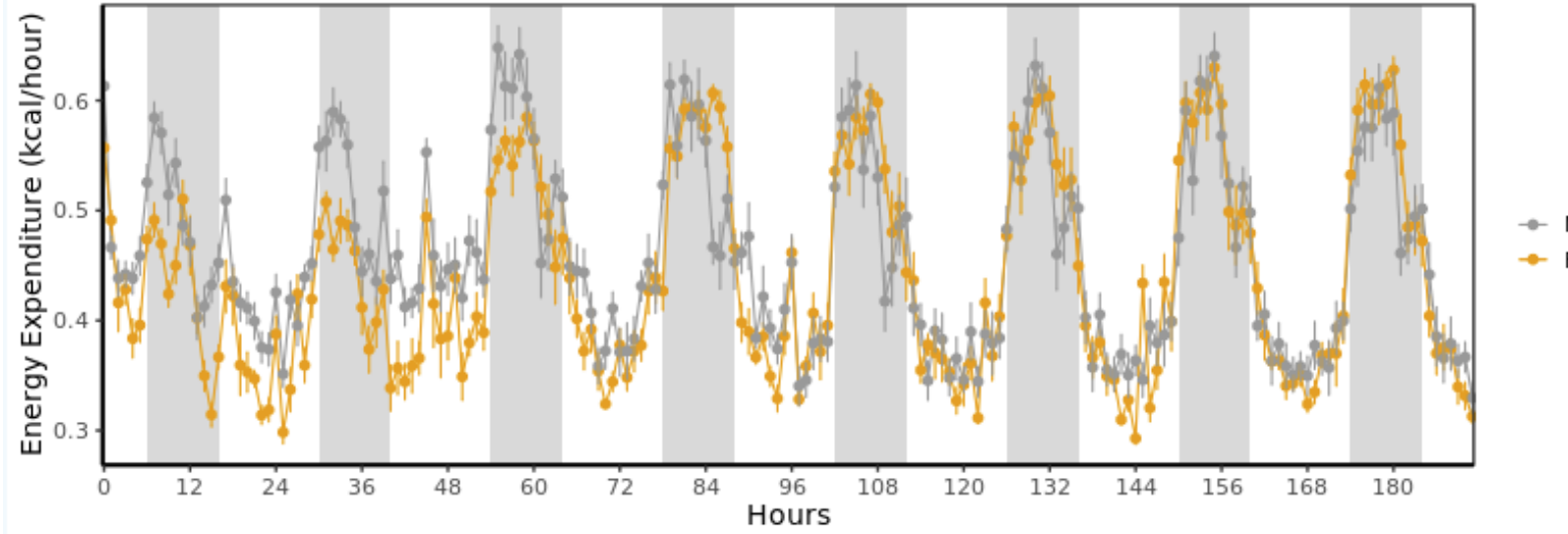
Occupancy



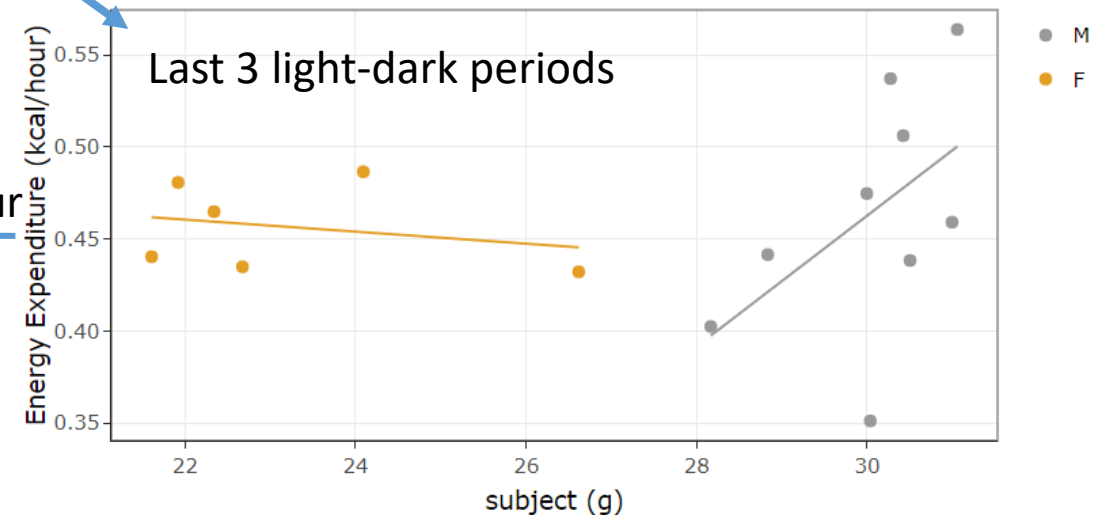
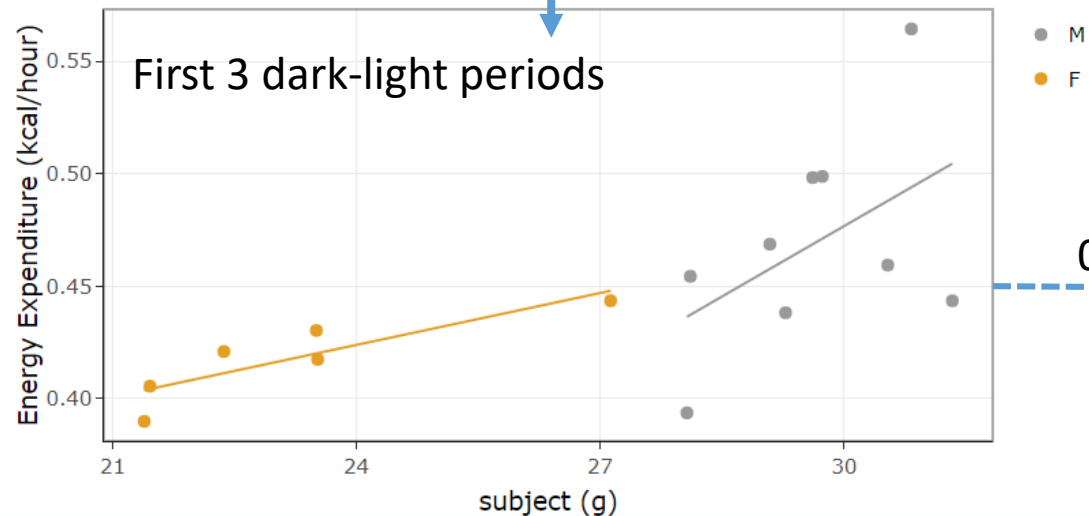
Relative humidity

# First experiment with 16-cage system: BL/6 mice – 10 males, 6 females, age 6 months, on chow diet

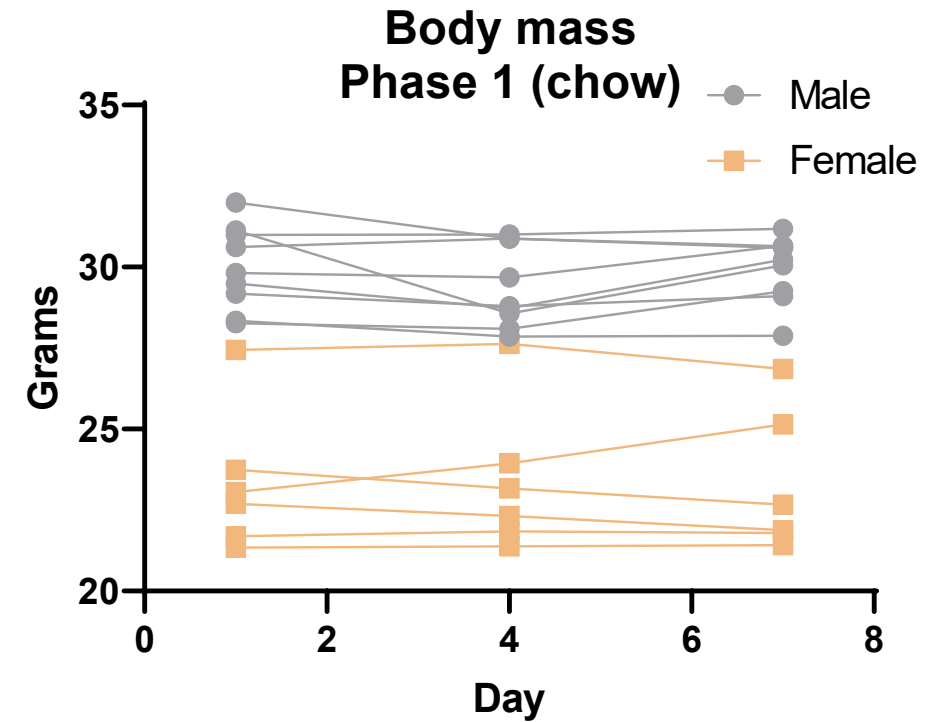
### Hourly Plot



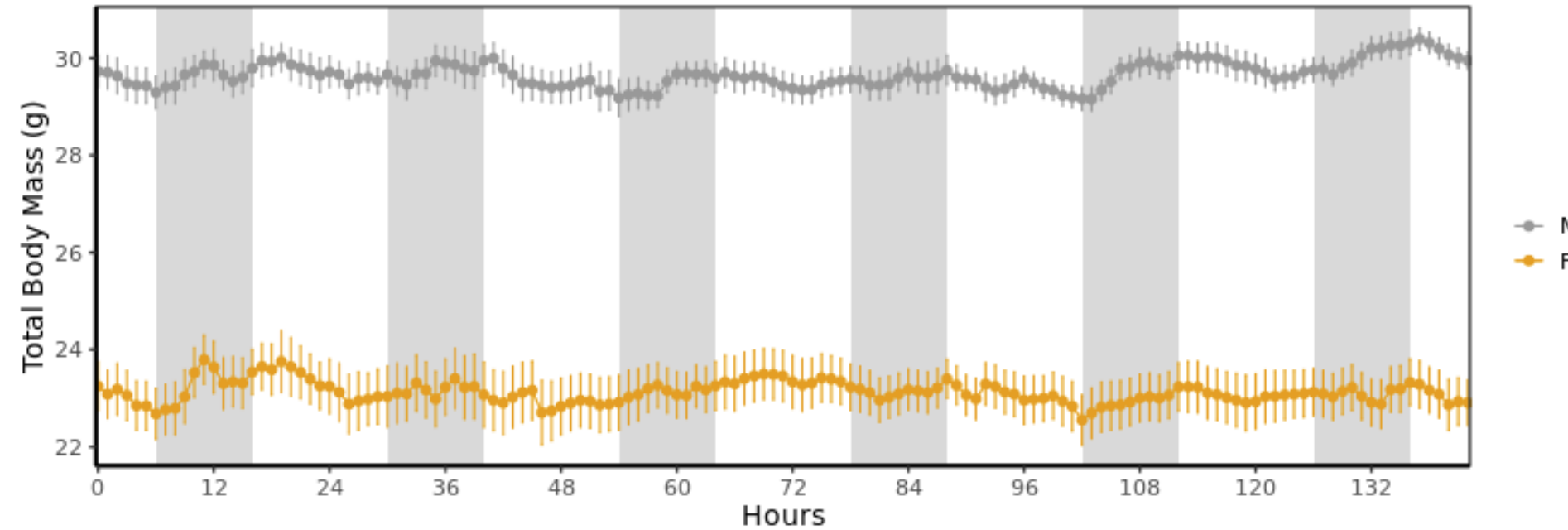
Energy expenditure appeared to stabilize by 3<sup>rd</sup> full light period



Body weight on average was maintained or slightly decreased



### Hourly Plot



# The basic data - metabolism

- Quantified
  - O<sub>2</sub>
  - CO<sub>2</sub>
  - H<sub>2</sub>O vapor (from respiration and evaporated urine)
- Calculated
  - EE in Kcal/hr (from the Weir equation)
  - RQ (RER)

## Other measured values

Food removed from hopper, water removed from hopper

Body mass

Activity

X-breaks (short axis)


Y-breaks (long axis)

Z-breaks (rearing)

# Other calculated values

- Allmeters = distance traveled, calculated from all beam breaks.
  - Beams are 1 cm apart
  - Includes repetitive beam breaks from grooming, feeding and fidgeting
- Pedmeters = distance traveled by walking or running
  - repetitive beam breaks (from grooming, feeding etc) filtered out.
- Wheelmeters = distance traveled on running wheel.

# Vendor-provided Software

- ExpeData: For exploring the raw data  ExpeData Release 1.9.27
- MacroInterpreter: Sorts and compiles the data

Both are freely available to users who wish to have them

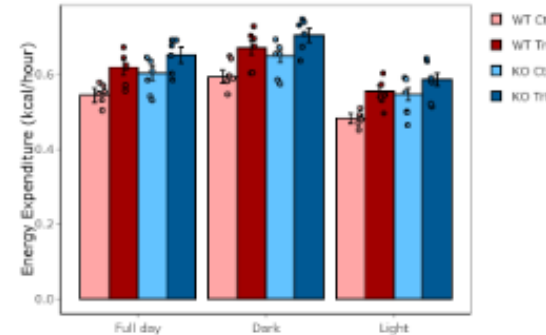
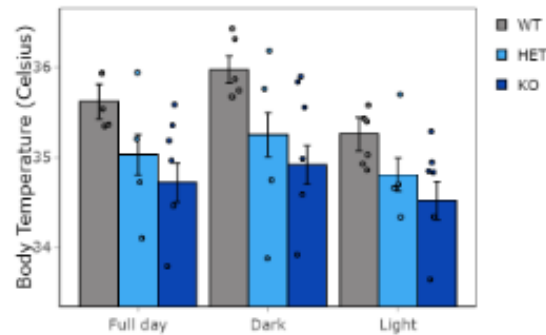
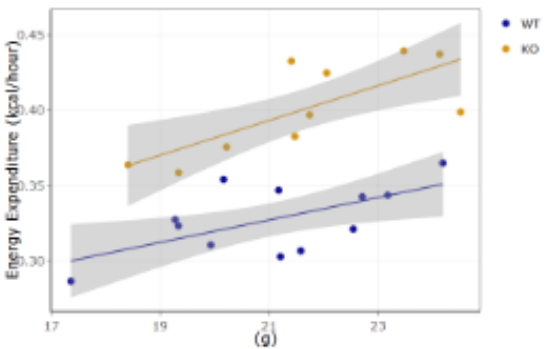
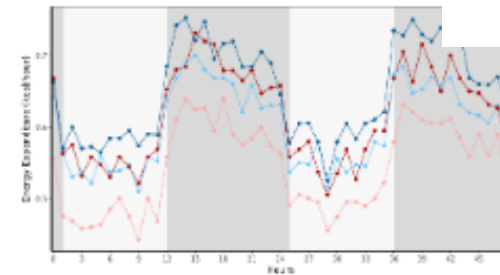
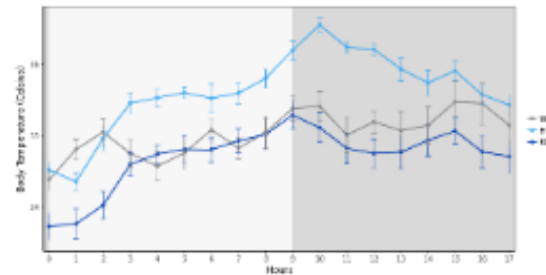
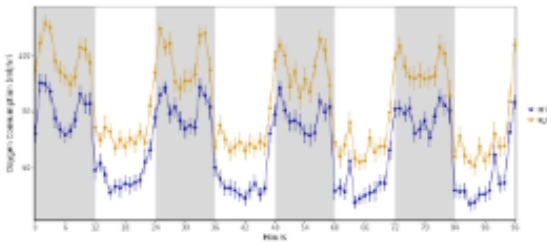
# A free web-based resource for analyzing the basic data:

## CalRapp.com

### A Web Application for Indirect Calorimetry Analysis

CalR quickly generates customizable time, bar and regression plots.

- Two groups
- Two groups acute response
- Three ordered groups
- Three unordered groups
- Four groups
- Two groups crossover
- Combine runs





# ***CalR*: A Web-Based Analysis Tool for Indirect Calorimetry Experiments**

Amir I. Mina,<sup>1</sup> Raymond A. LeClair,<sup>2</sup> Katherine B. LeClair,<sup>1</sup> David E. Cohen,<sup>3</sup> Louise Lantier,<sup>4</sup> and Alexander S. Banks<sup>1,5,\*</sup>

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<sup>2</sup>Springbok LLC, Boston, MA 02109, USA

<sup>3</sup>Division of Gastroenterology & Hepatology, Weill Cornell Medical College, New York, NY 10065, USA

<sup>4</sup>Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37212, USA

<sup>5</sup>Lead Contact

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<https://doi.org/10.1016/j.cmet.2018.06.019>

The supplements to this publication contain tutorials and example data files for each type of analysis performed by CalR.

The tutorials and data examples are also available on the CalR web site.

# CalR setup screen (2 groups format)

The screenshot shows the CalR web interface with the following sections and annotations:

- 1. Upload data file:** Points to the 'IMPORT INDIRECT CALORIMETER DATA' section, specifically the 'BROWSE...' button and the file name '252dwheels5to8201903051557\_r'.
- 2. Define light cycle:** Points to the '3. Designate hours for the light/dark cycle' section, specifically the input fields for 'Light cycle starts at hour' (6) and 'Dark cycle starts at hour' (20).
- 3. (optional) Input Fat Mass and Lean Mass:** Points to the '(OPTIONAL) IMPORT WEIGHT DATA\*' section, specifically the question '1. Will you include lean and fat mass?' and the 'WEIGHT TEMPLATE' download button.
- 4. Define caloric density:** Points to the 'DESIGNATE GROUPS' section, specifically the '1. Designate Caloric Value and Units for Diets of Each Group' section, including the dropdown menus for 'First Group' and 'Second Group' (both set to 'Other') and the table below.
- 5. Define Groups:** Points to the '2. List names of groups separated by a comma' section, specifically the input field containing 'No-wheels, Wheels'.
- 6. Notes:** Points to the 'Notes:' section at the bottom, which contains the text 'Wendy/calor analyses/Sable pilot/2019 demo Raw Data/252dwheels5to8201903051557'.

**DESIGNATE GROUPS**

1. Designate Caloric Value and Units for Diets of Each Group

First Group: Other  
Second Group: Other

\*If the studied diets are not on the list, please choose 'Other' and input the caloric value in 'kcal/g'.

Name	Caloric Value	Name	Caloric value
Envigo-V	4.5	Envigo-V	4.5

2. List names of groups separated by a comma. Please put Wild Type as the first group.

No-wheels, Wheels

2. Select subjects and click arrows to assign groups

1 2 3 4 | 5 6 7 8

GO TO PLOTS

1. Upload data file

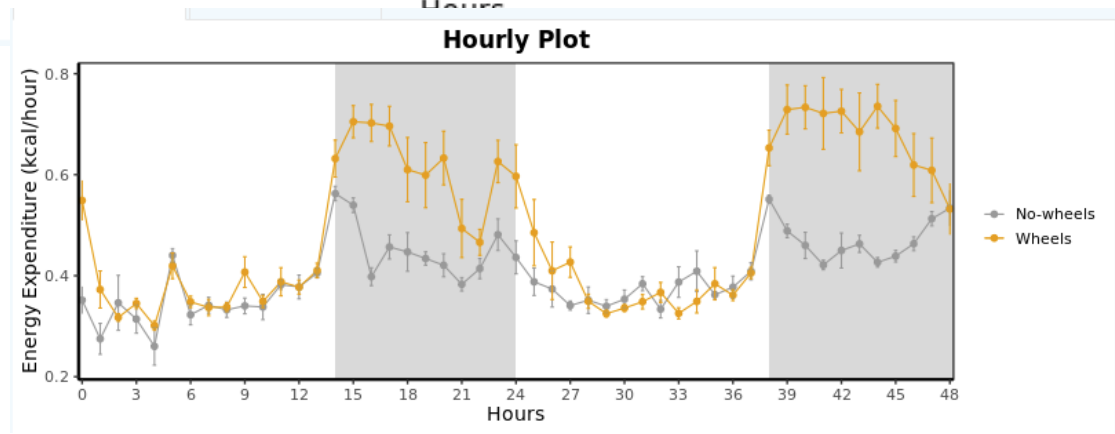
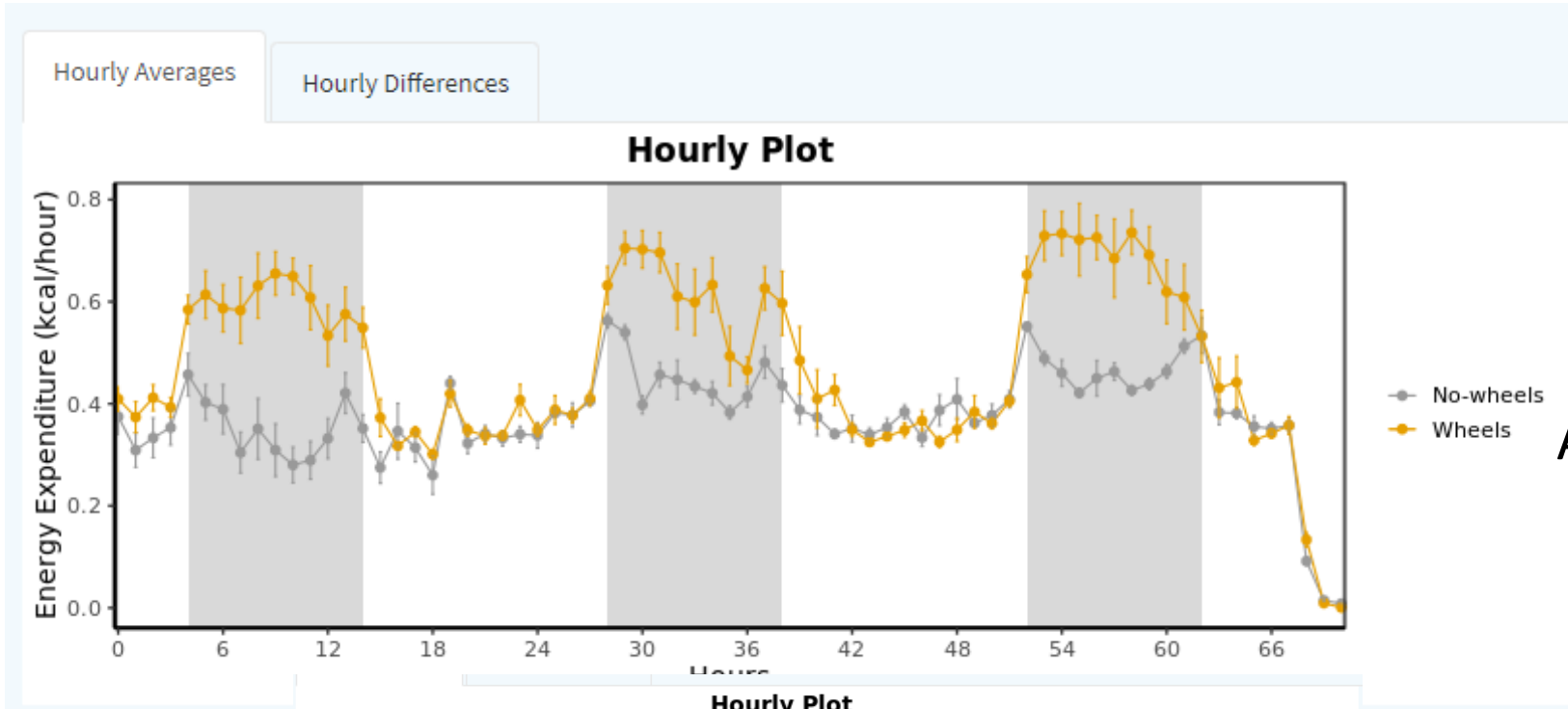
2. Define light cycle

6. Notes

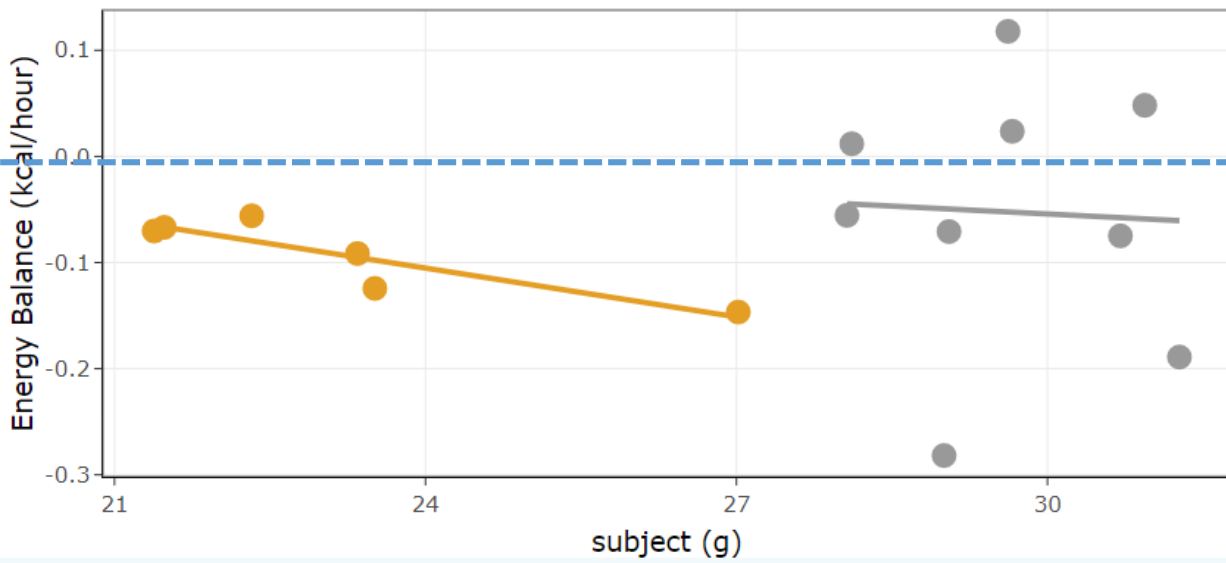
4. Define caloric density

5. Define Groups

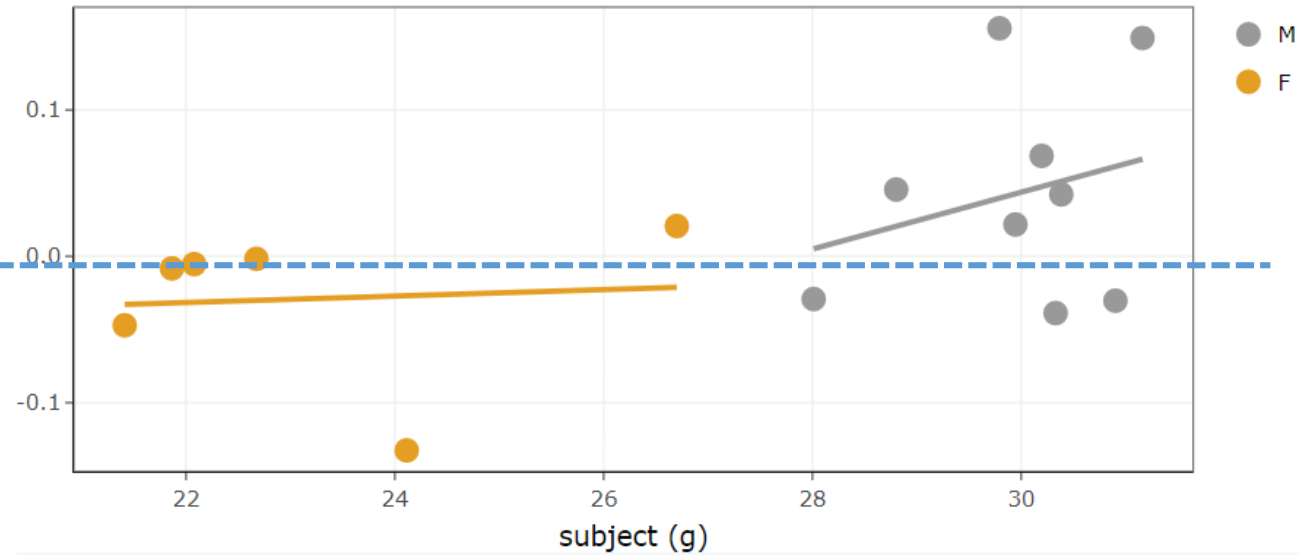
# Data can easily be trimmed in CalR to exclude a period of acclimation and to restrict to complete light & dark periods



# CalR calculates and plots energy balance

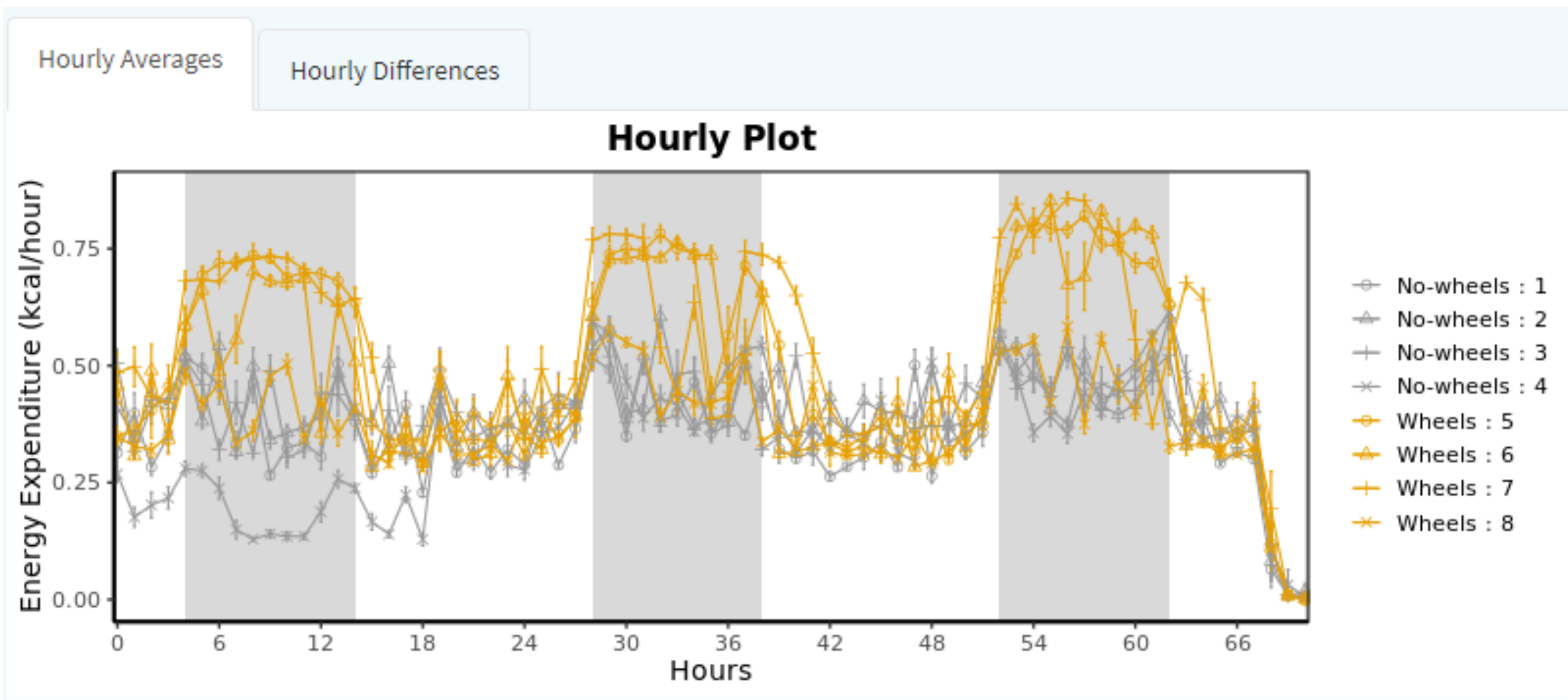


Days 1-3

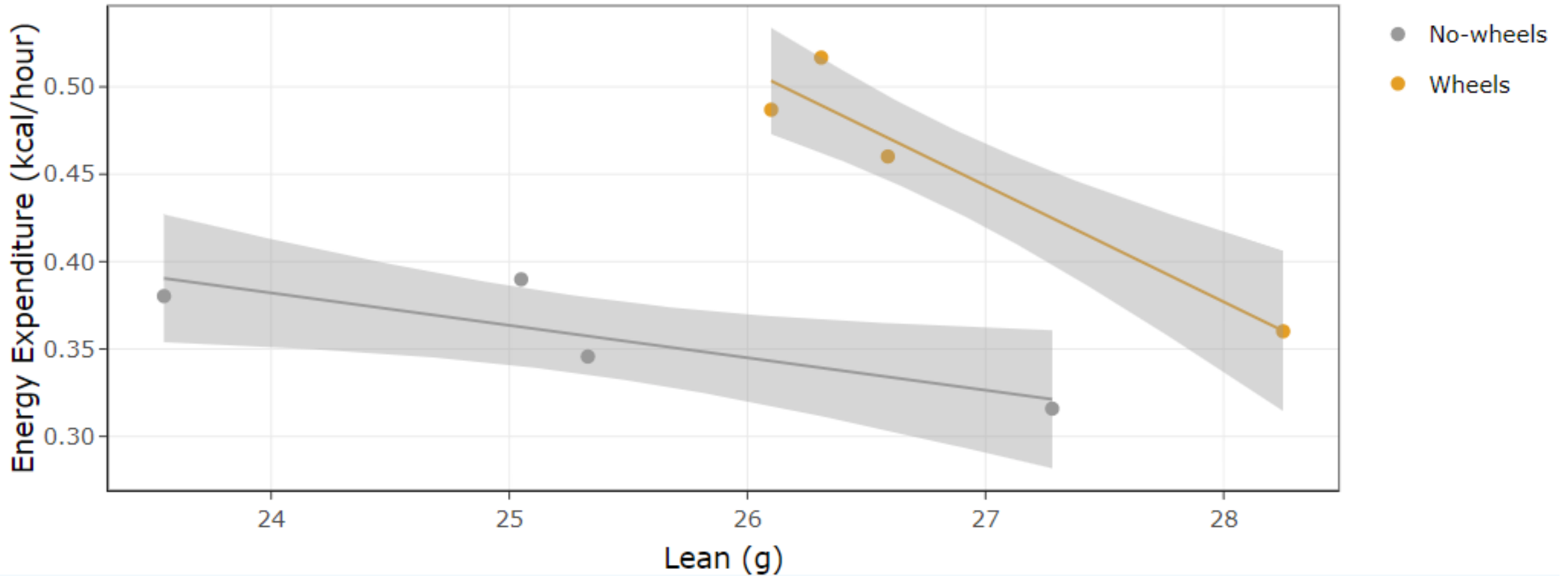


Days 5-7

# Data can be plotted for individuals

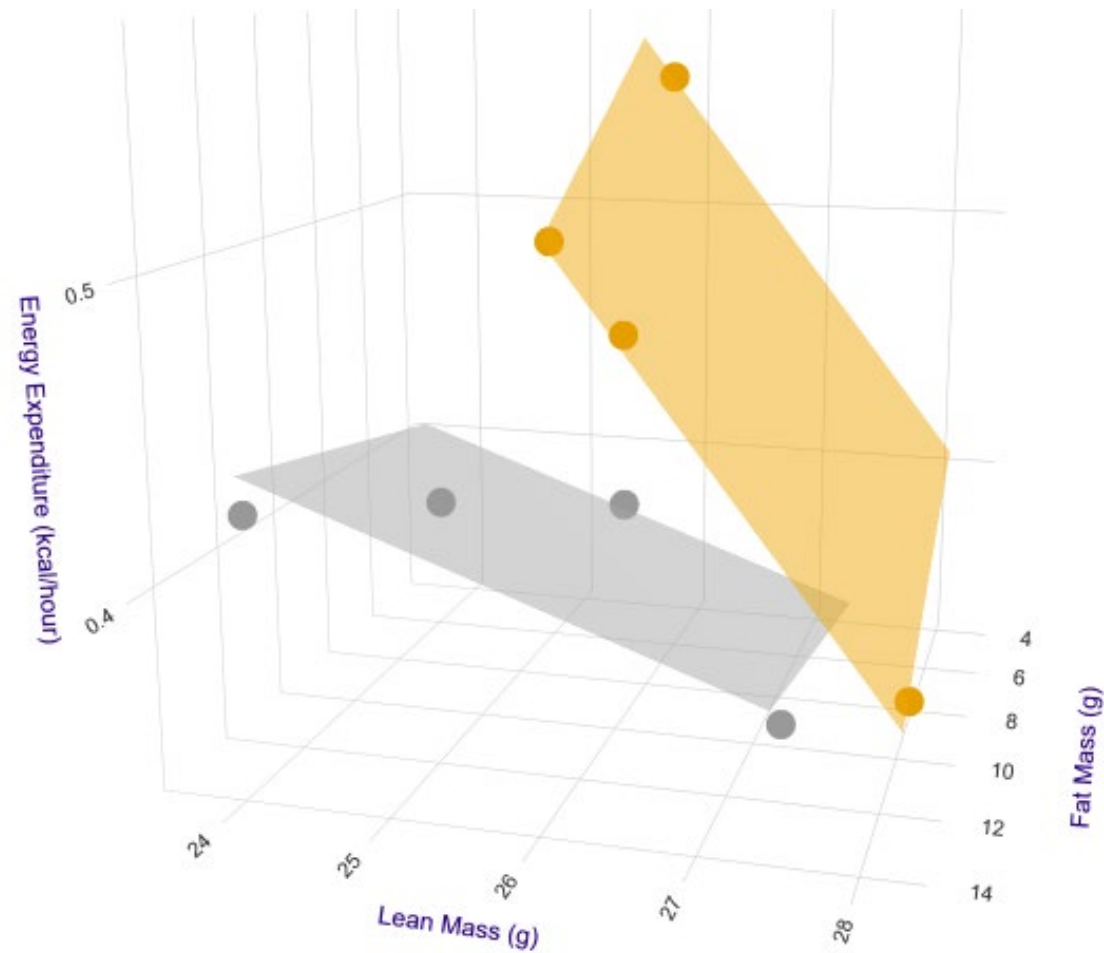


# CalR does standard regression plots



	<b>Mass effect</b>	<b>Group effect</b>	<b>Interaction effect</b>
<i>p-value</i>	0.0851	0.0173 *	0.0245 *

## And rotatable 3-D plots

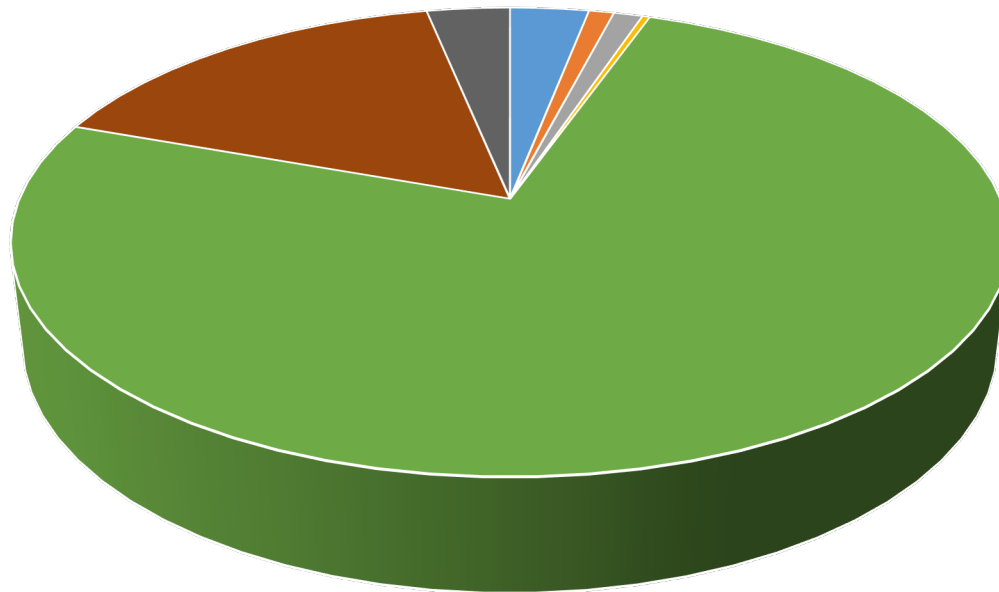


	<b>Mass effect</b>	<b>Group effect</b>	<b>Interaction effect</b>
<i>p-value</i>	0.0796	0.0165 *	0.0235 *

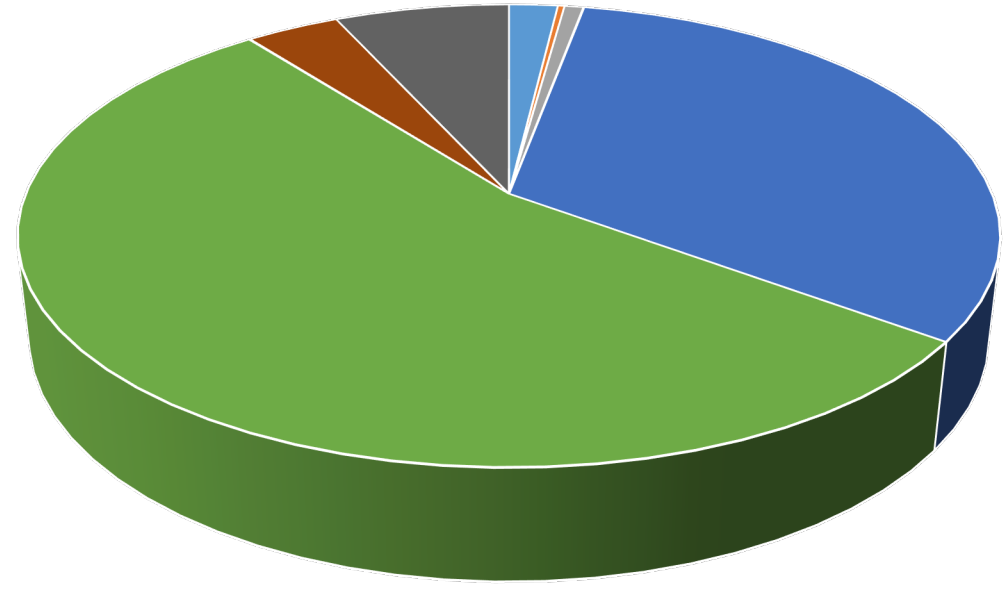
## More data analysis by Promethion: Ethoscan TimeBudget\_% (pie charts made in Excel)

Animal	EfodA	TfodA	DWatr	TWatr	Wheel	IHome	THome	LLnge	SLnge
1	3.05	0.97	1.12	0.29	0	75.03	0.02	16.29	3.22
2	2.99	0.78	0.59	0.1	0	83.59	0.04	9.38	2.53
3	2.95	0.48	0.42	0.05	0	83.54	0.1	9.58	2.87
4	2.78	0.91	0.87	0.12	0	75.37	0.25	15.81	3.9
5	1.91	0.26	0.74	0.03	32.13	54.38	0.05	3.66	6.84
6	2.15	0.35	0.45	0.04	23.47	56.27	0.03	11	6.24
7	2.02	0.26	1.11	0.22	26.6	52.15	0.08	10.8	6.76
8	2.1	0.41	0.77	0.43	8.67	69.79	0.21	11.71	5.89

No Wheels



Wheels

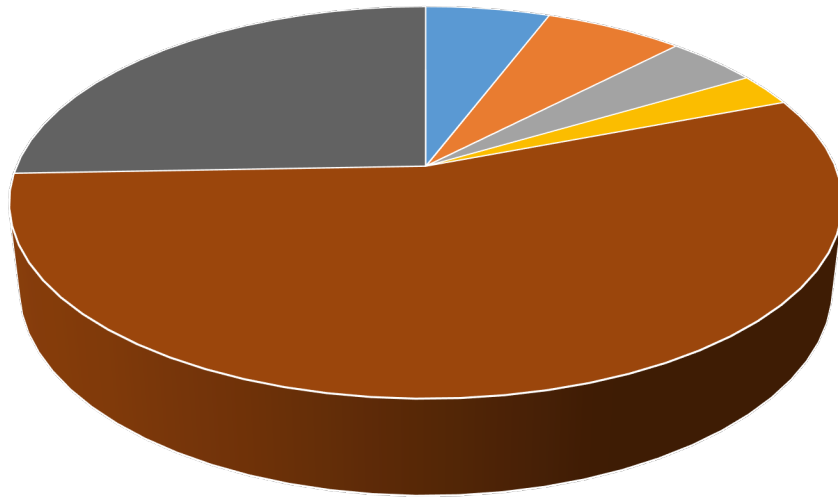




# EthoScan: additional tables

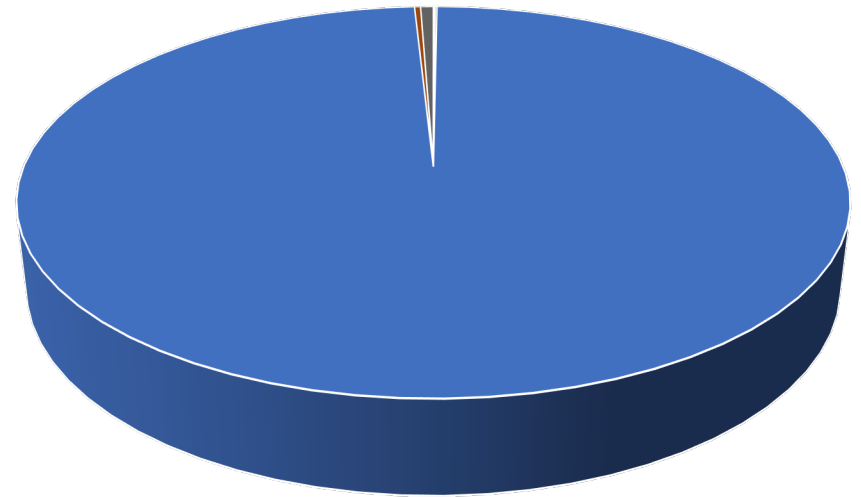
- **Locomotion budget:** percent of locomotor activity spent on each of the behaviors listed above
- **LocoBudget\_meters\_hr** (average distance traveled per hour) for each of the behaviors listed

Locomotion % Budget: No wheels



■ EfodA ■ TfodA ■ DWatr ■ TWatr ■ Wheel ■ IHome ■ THome ■ LLnge ■ SLnge

Locomotion % Budget: Wheels



■ EfodA ■ TfodA ■ DWatr ■ TWatr ■ Wheel ■ IHome ■ THome ■ LLnge ■ SLnge

# EthoScan: additional tables

- **Behavior transitions:** a matrix of percentages describing what the mouse did next after each activity

Example of behavior transitions for mouse #5

Animal	Behav	efoda	tfoda	dwatr	twatr	wheel	ihome	thome	llnge	slnge	
5	efoda	0	0	2.11	1.05	1.05	1.05	0	9.47	85.3	sum:100%
5	tfoda	0	0	3.7	1.23	9.88	0	1.23	3.7	80.3	sum:100%
5	dwatr	0.89	1.79	0	0	0.89	0.89	0	5.36	90.2	sum:100%
5	twatr	0	14.3	0	0	0	0	0	0	85.7	sum:100%
5	wheel	0	0.84	0.21	0	0	0	0	9.26	89.7	sum:100%
5	ihom	0	0	0	0	0	0	0	9.18	90.8	sum:100%
5	thom	0	5.26	0	0	0	0	0	10.5	84.2	sum:100%
5	llnge	16.4	8.22	2.74	1.37	56.2	11	4.11	0	0	sum:100%
5	slnge	10.5	8.55	13.3	0.51	54.1	11.2	1.91	0	0	sum:100%

# Additional data sorting by Promethion: Circadian data tab

- Light period transitions are detected by the light sensor in the ESA
- Calculated data are presented as mean for each period each day
  - Avg VO<sub>2</sub>, Avg VCO<sub>2</sub>
  - Avg EE, Total EE
  - Avg RQ
  - Avg VH<sub>2</sub>O
  
  - R-EE-30 (30 minutes of lowest values)
  - QR-EE-30 (30 lowest minutes when the animal is quiet)
  - A-EE-15 (Mean energy expenditure during the 15 minutes with highest EE)
  - QA-EE-15 (mean of the 15 highest values when the animal is quiet)
  
  - R-RQ-30
  - QR-RQ-30
  - A-RQ-15
  - QA-RQ-15

# More calculations on circadian tab: Food and water uptake

- Food uptake (mean grams per uptake event)
- Number of uptake events per period
- Total uptake per period
- Minutes of food uptake per period
- Minutes between uptake events (mean for period)
  
- Water uptake – same set of calculations
  
- Body mass each period
- Body mass SD (fluctuation during the period)

# Additional data sorting by Promethion: Food Intake Pattern tab: Detailed analysis of food intake events

- Calculated for each animal, each event
  - Start and end times of each intake event
  - Interval since previous event
  - Duration of event
  - Grams taken
  - Grams per minute
  - Cumulative intake
  - Force applied to hopper
  - Statistical calculations on whether the force on the hopper was due to chance

# More calculations on circadian tab: Locomotion

- Beam breaks per period for each of X, Y, Z
- Meters on running wheel
- Wheel speed
- Percent of activity on wheel
- AllMeters (includes fine movement) and PedMeters (traveling) per period
- Ped Speed (based on breaks when the mouse is walking).
- Percent of period walking
- Percent of period still
- Percent of period asleep (still for at least 40 seconds, this duration based on published data).
- Hours per period spent in sleep

# Additional data sorting by Promethion: Detailed analysis of running wheel activity

- Calculated for each animal, each event
  - Start and end time of each event
  - Duration in seconds of each event
  - Wheel revolutions per event
  - Still secs – mouse on the wheel but not running
  - Meters run on the wheel for each event
  - Avg meters per sec - the total distance divided by the total time it is on the wheel including the time that it is still
  - Run meters sec, the speed of the mouse on the wheel only when revolutions are being counted; i.e., ignoring the still\_secs time
  - PrevRun\_sec – the time between the current wheel run and the previous wheel run
  - PostRun\_sec – the time between the current wheel run and the next wheel run

# Promethion's quality control tabs

- One tab for quality control alerts
- One tab of metadata for diagnostic purposes



Questions answered, experiments scheduled, request system documentation, arrange a facility tour:

Wendy Katz

wkatz@uky.edu

