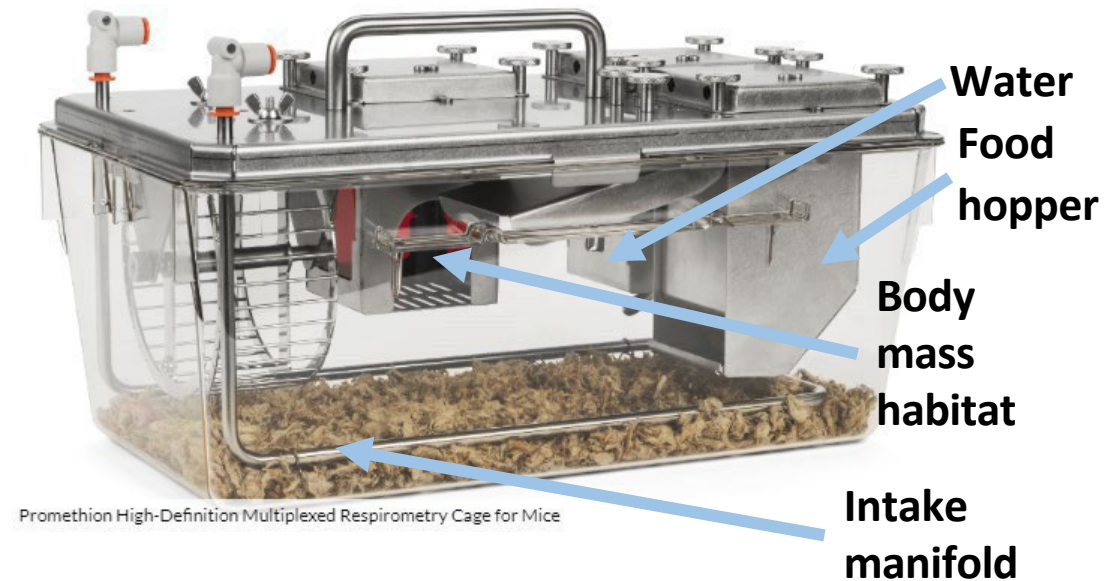# Sable Promethion

- 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

Water
Food hopper
Body mass habitat
Intake manifold

Promethion High-Definition Multiplexed Respirometry Cage for Mice
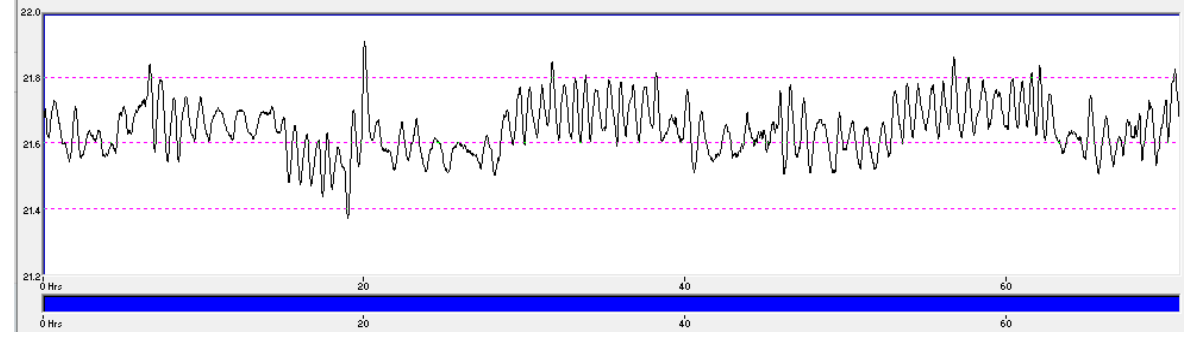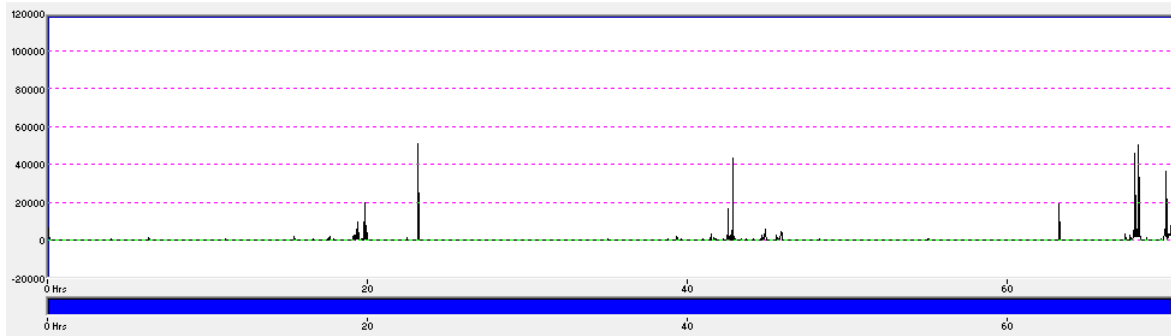
Environmental Sensing Accessory

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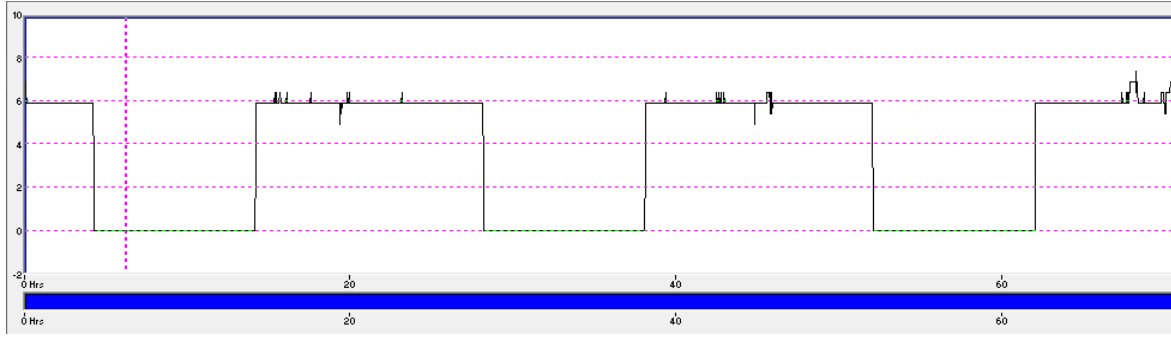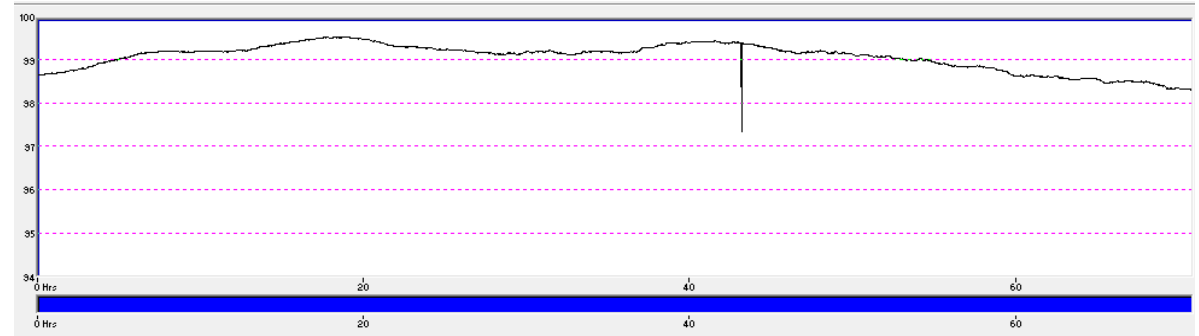

Light

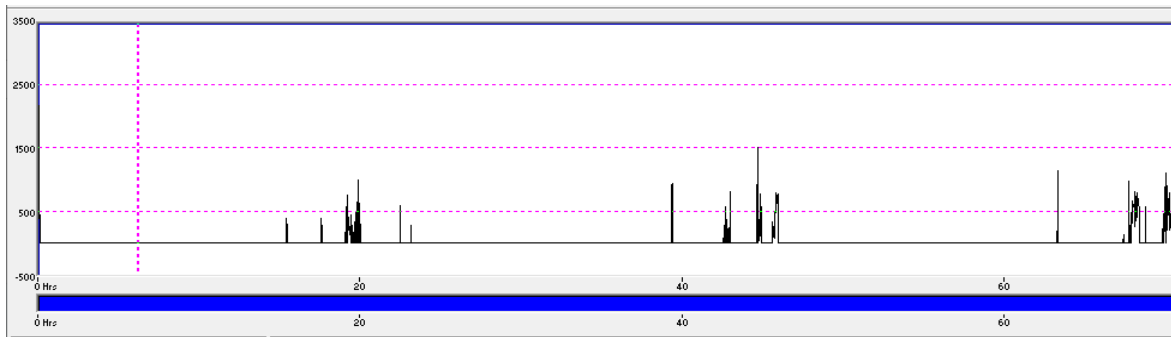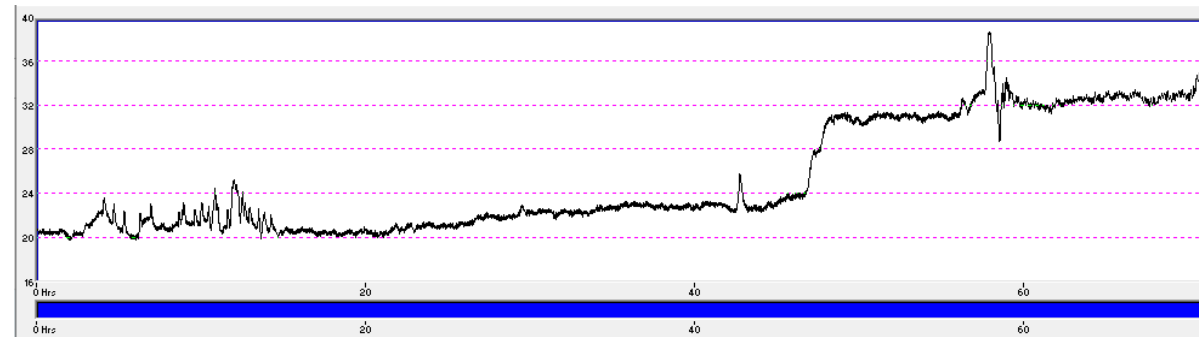Temperature

Sound

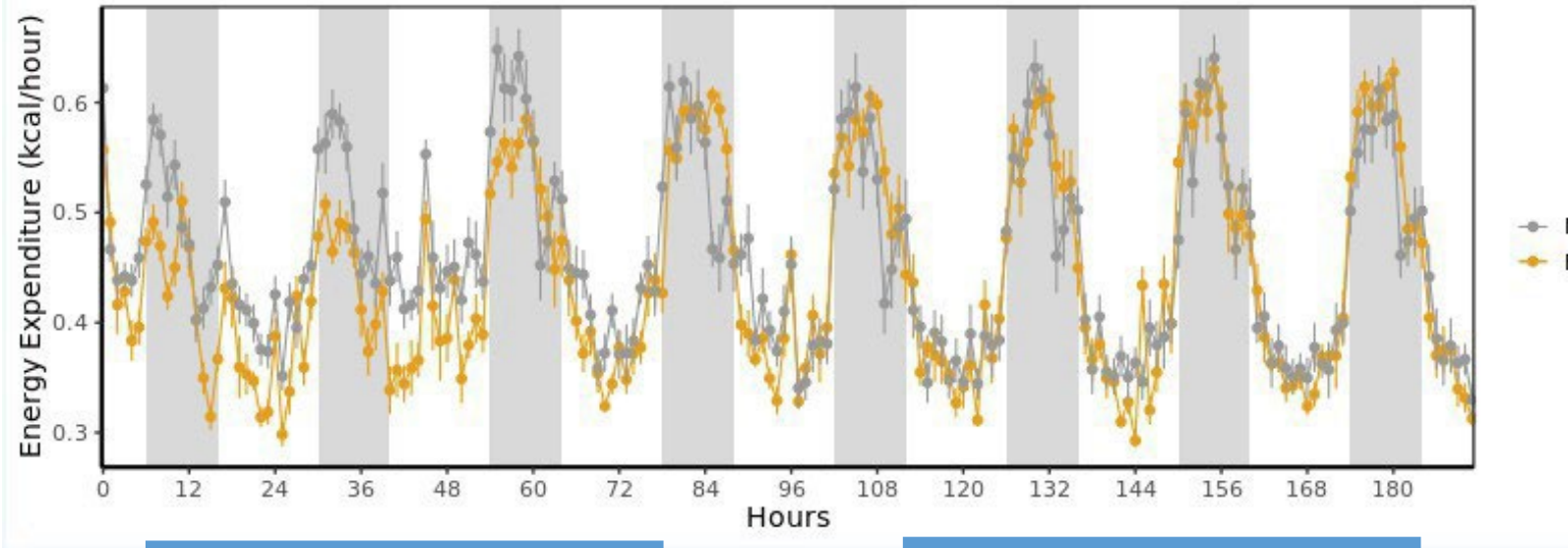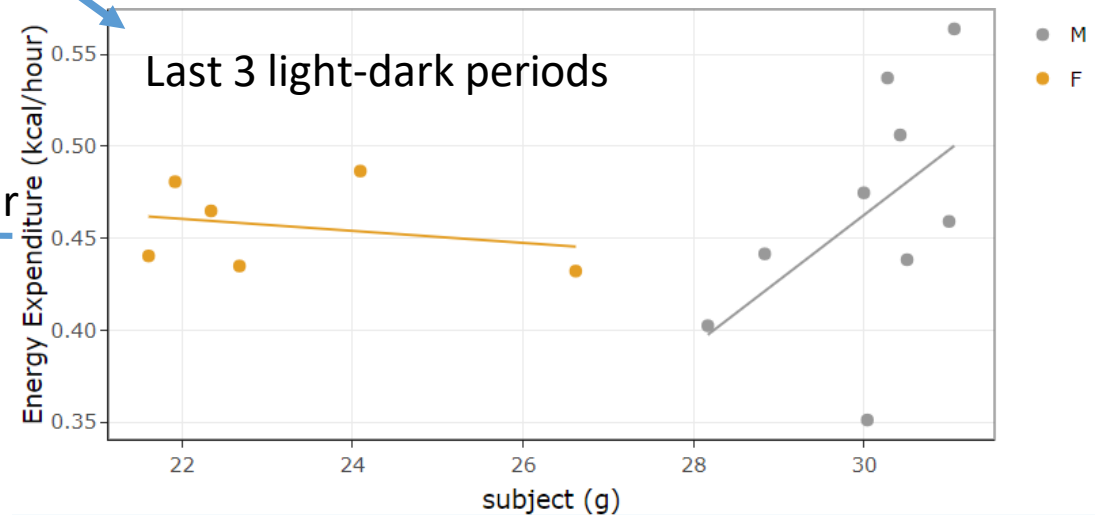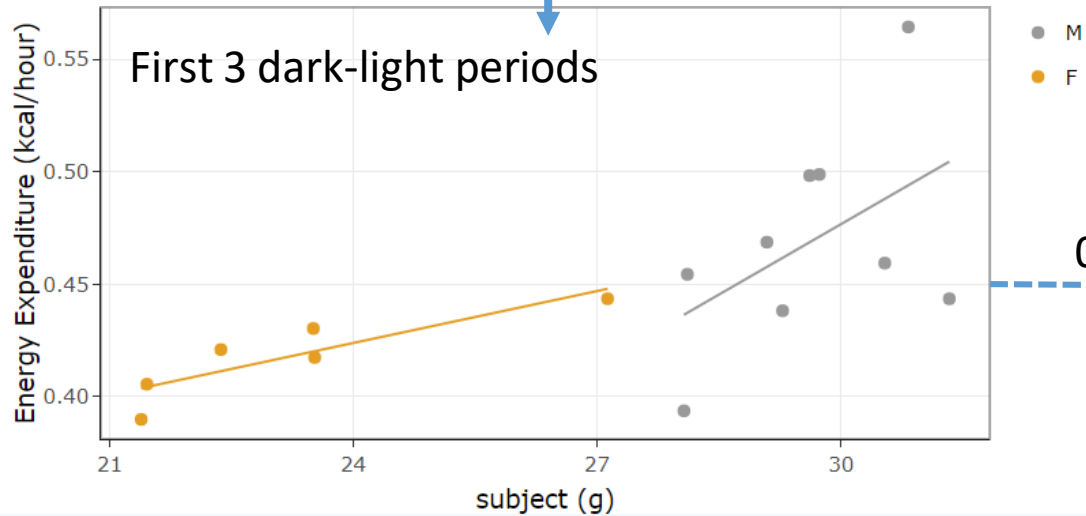Barometric pressure

Occupancy

Relative humidity

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

Energy expenditure appeared to stabilize by 3rd full light period

Body weight on average was maintained or slightly decreased

Body mass Phase 1 (chow)

Grams vs Day. Male, Female.

Hourly Plot

Total Body Mass (g) vs Hours. M, F.
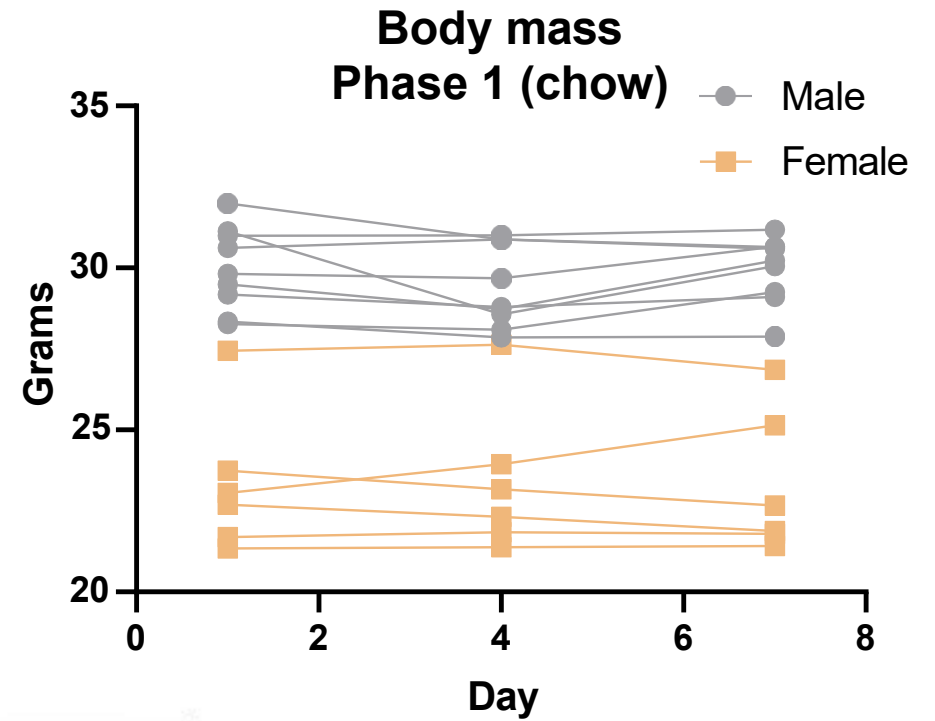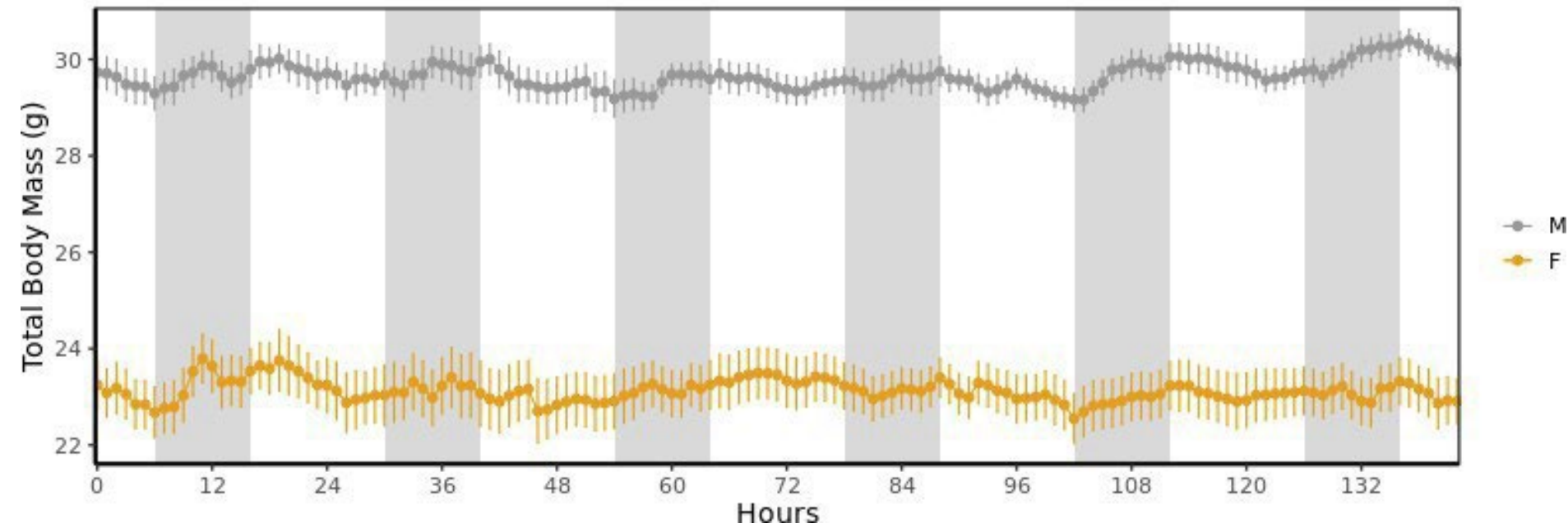
The basic data - metabolism

- Quantified
  - O2
  - CO2
  - H2O 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

Vignettes   Help   Templates ▾

Two groups

Two groups acute response

Three ordered groups

Three unordered groups

Four groups

Two groups crossover

Combine runs

A Web Application for Indirect Calorimetry Analysis

CalR quickly generates customizable time, bar and regression plots.
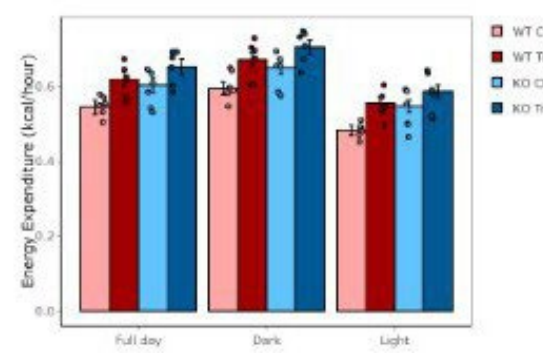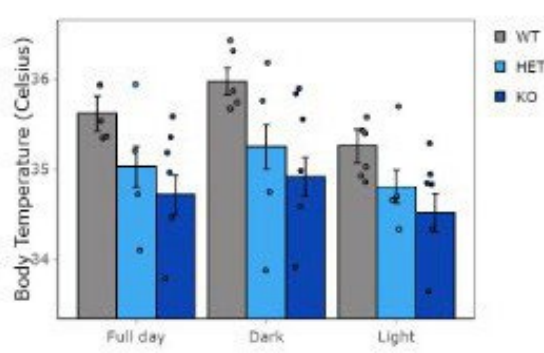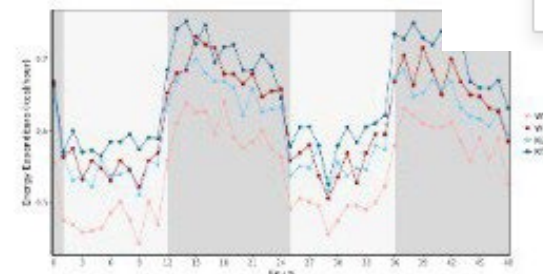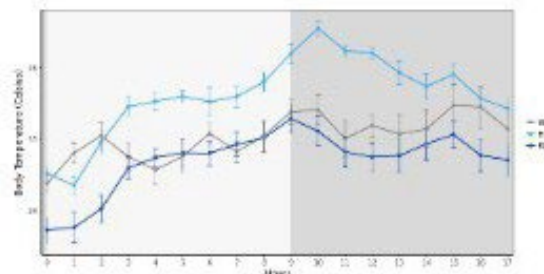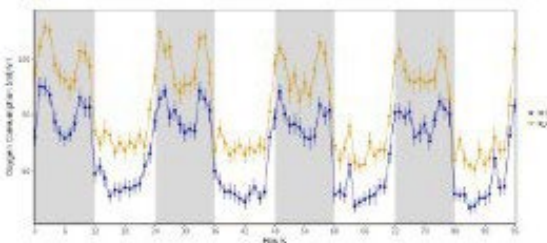
CalR: A Web-Based Analysis Tool for Indirect Calorimetry Experiments

Amir I. Mina,[1] Raymond A. LeClair,[2] Katherine B. LeClair,[1] David E. Cohen,[3] Louise Lantier,[4] and Alexander S. Banks[1,5,*]

[1]Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115, USA
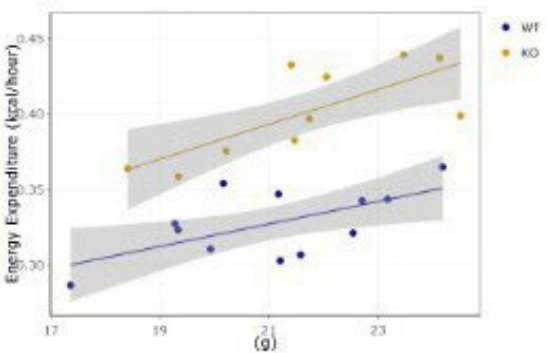
[2]Springbok LLC, Boston, MA 02109, USA

[3]Division of Gastroenterology & Hepatology, Weill Cornell Medical College, New York, NY 10065, USA

[4]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37212, USA

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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 avaliable on the CalR web site.

CalR setup screen (2 groups format)

1. Upload data file

2. Define light cycle

3. (optional) Input Fat Mass and Lean Mass

4. Define caloric density

5. Define Groups

6. Notes

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

All time points

Trimmed to two 24-hour periods
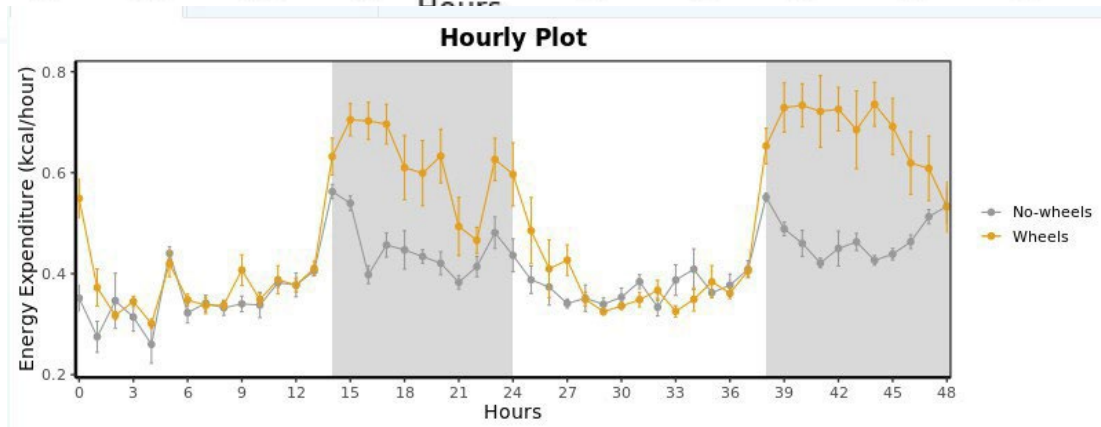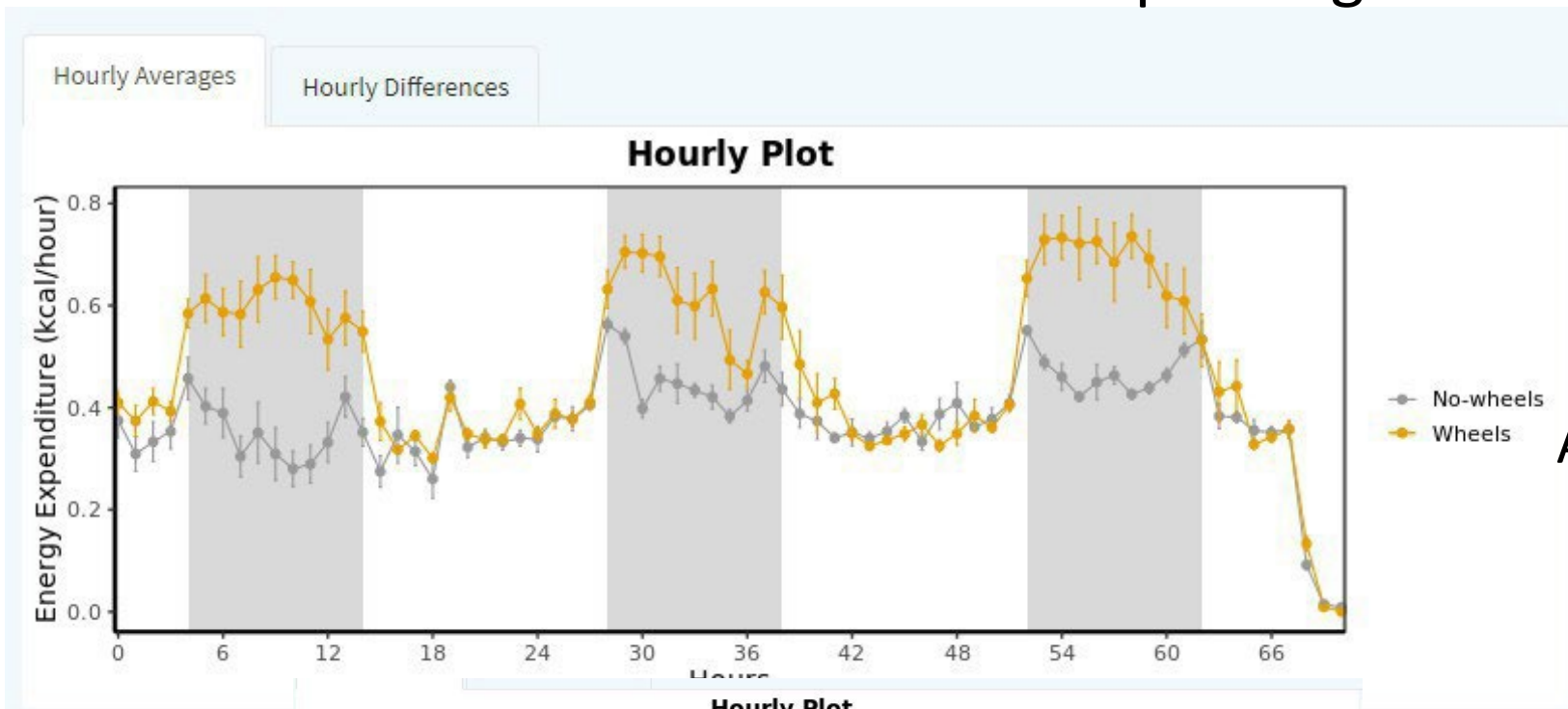
CalR calculates and plots energy balance
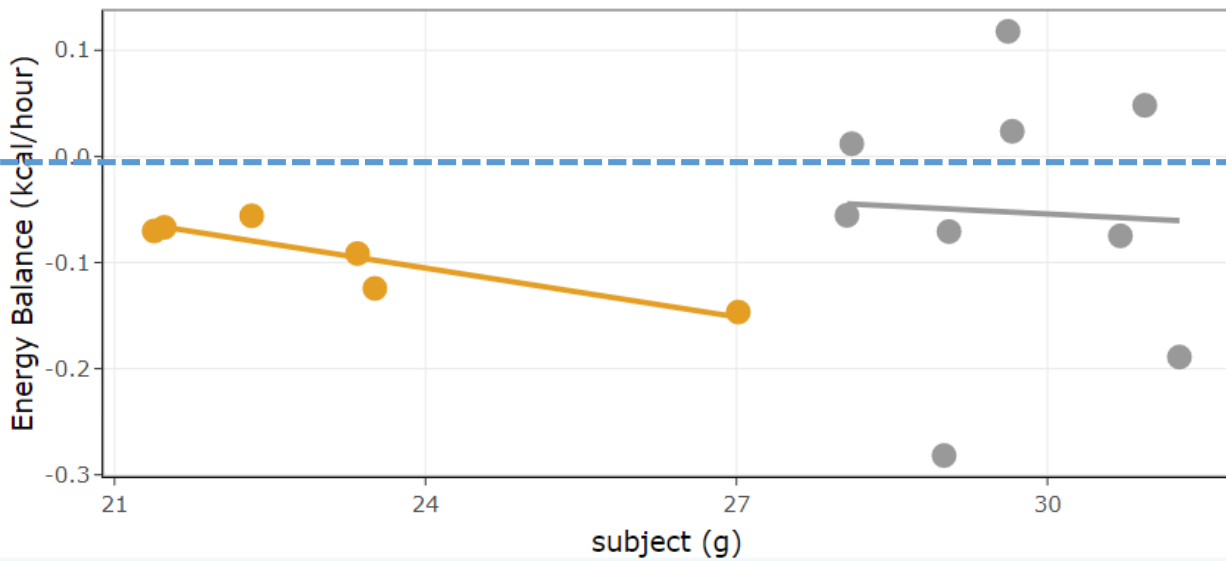
Days 1-3
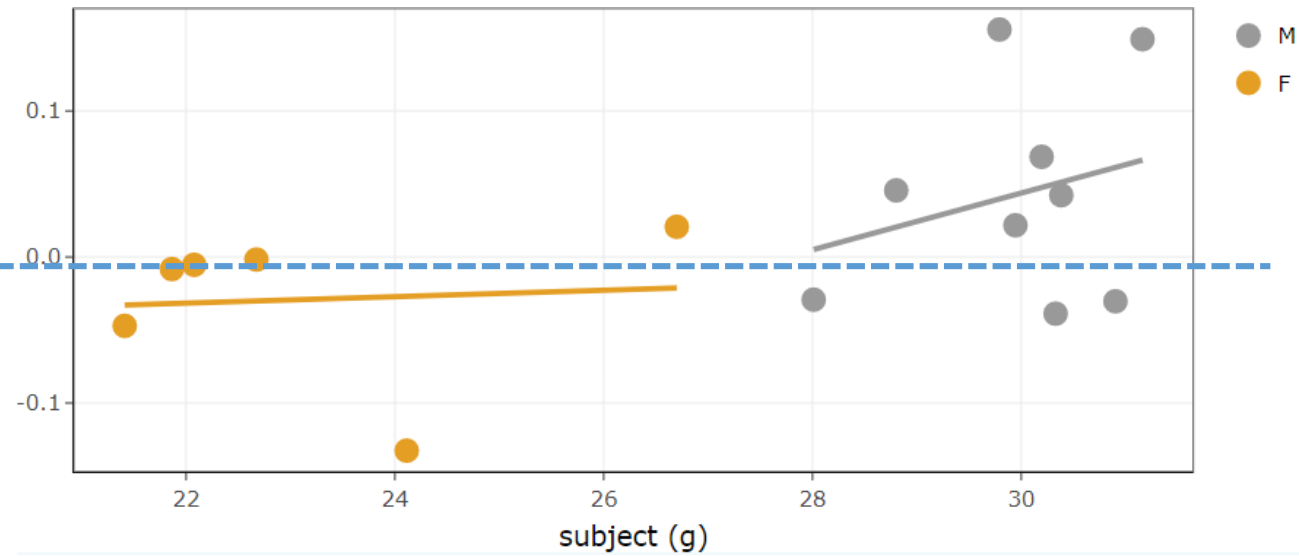
Days 5-7

Data can be plotted for individuals

Hourly Averages | Hourly Differences

Hourly Plot

Legend:
- No-wheels : 1
- No-wheels : 2
- No-wheels : 3
- No-wheels : 4
- Wheels : 5
- Wheels : 6
- Wheels : 7
- Wheels : 8

Y-axis: Energy Expenditure (kcal/hour) — 0.00, 0.25, 0.50, 0.75

X-axis: Hours — 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66
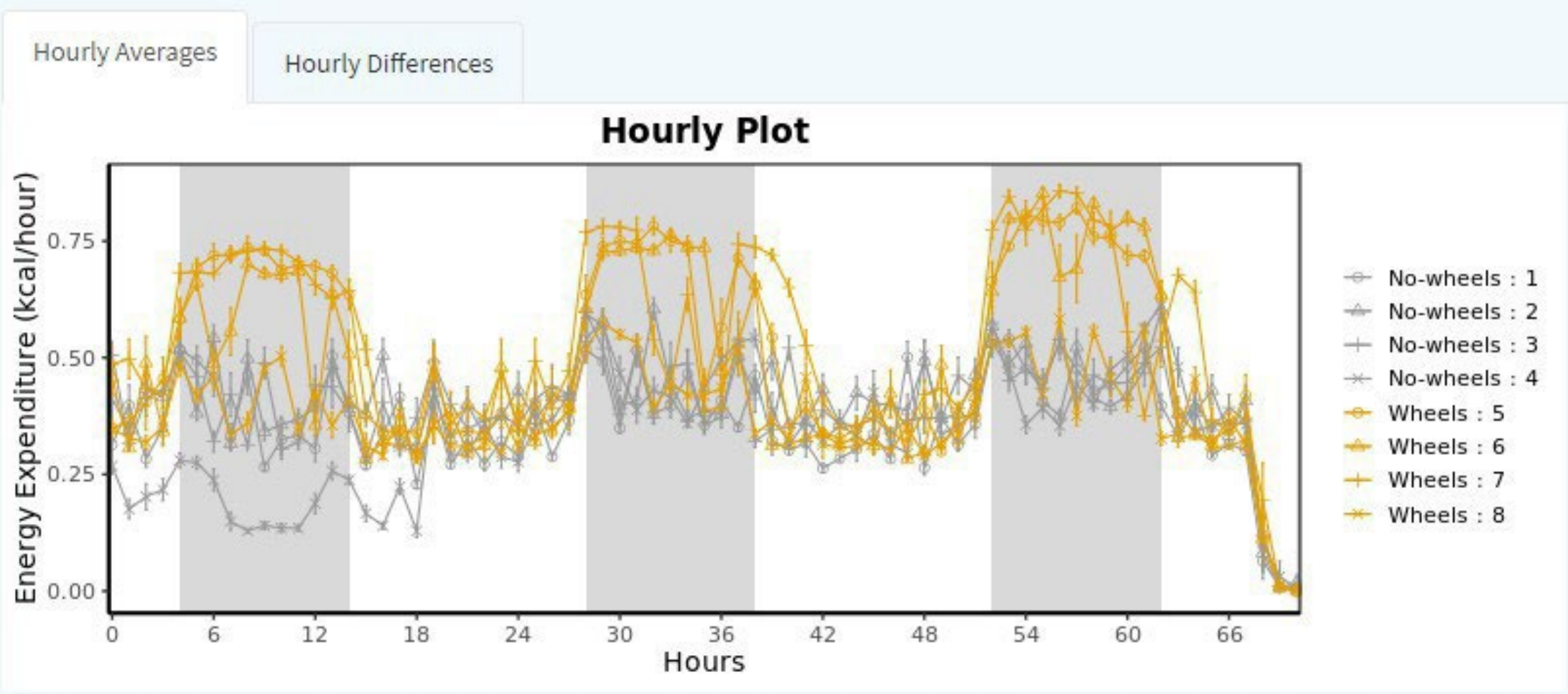
CalR does standard regression plots
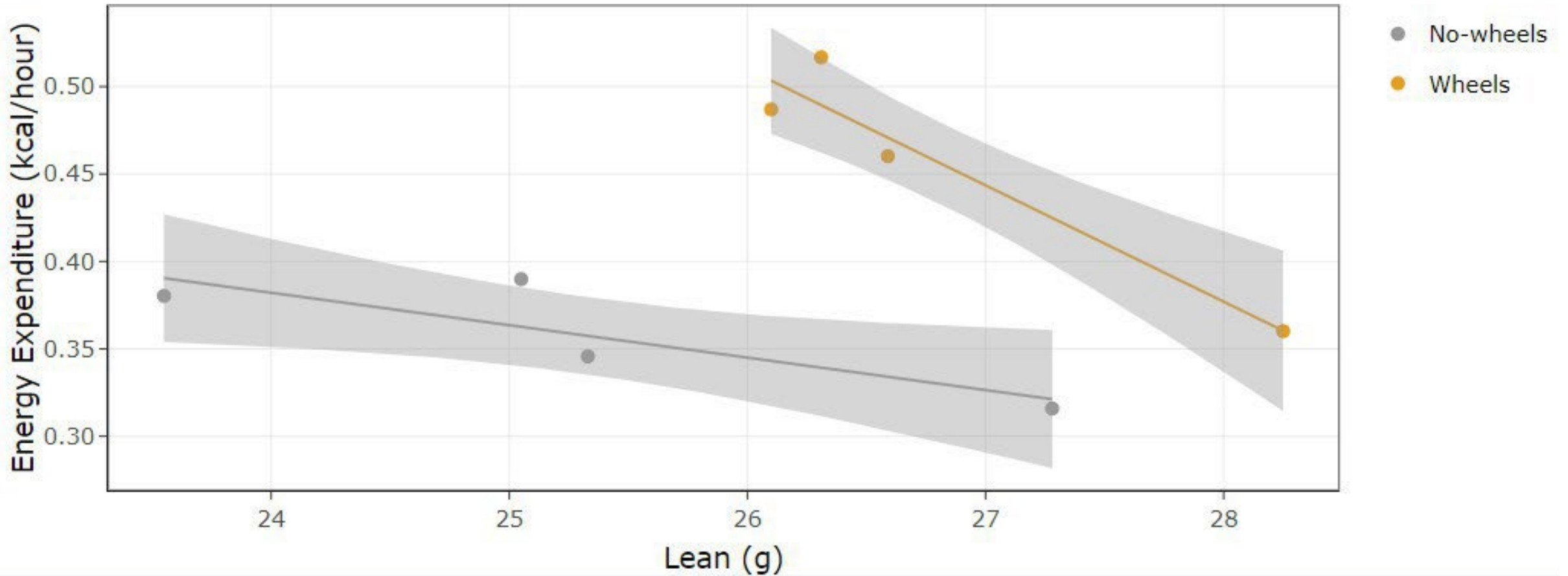
	Mass effect	Group effect	Interaction effect
p-value	0.0851	0.0173 *	0.0245 *

And rotatable 3-D plots
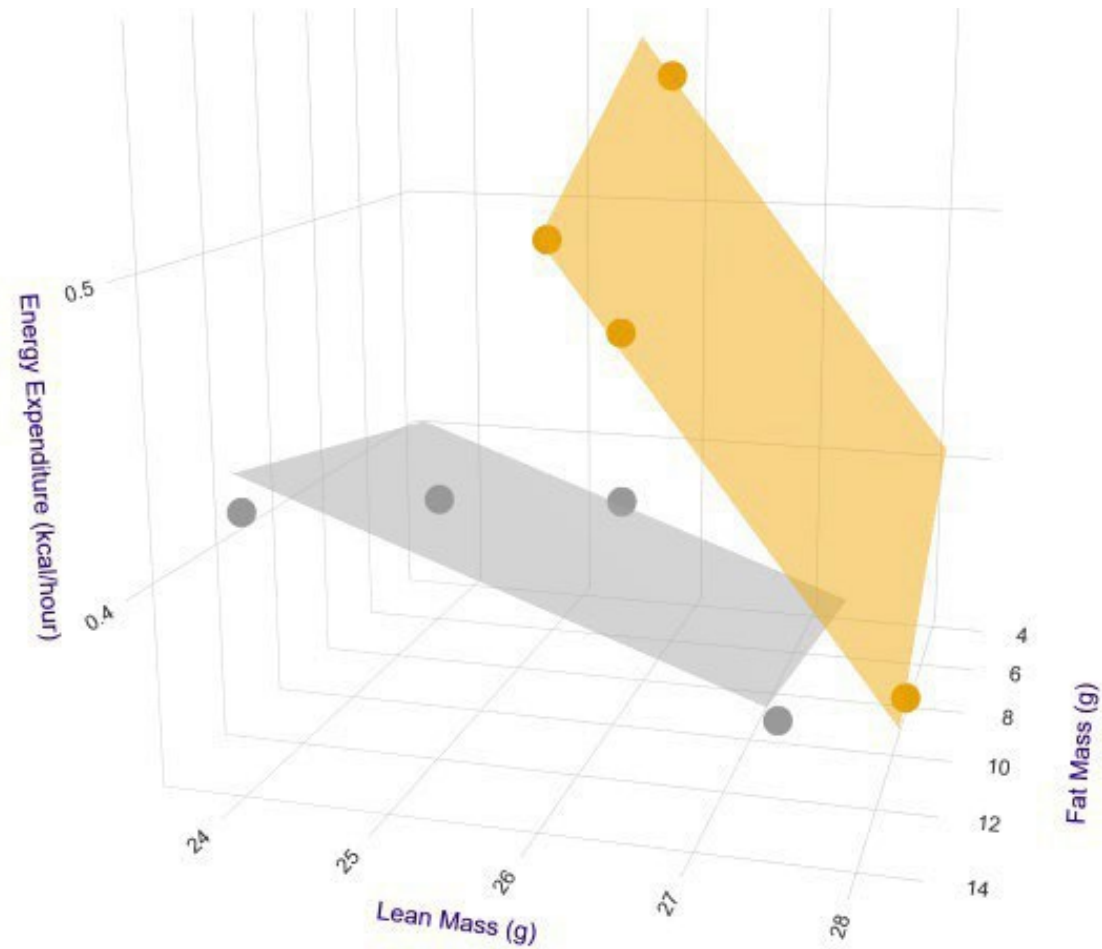
	Mass effect	Group effect	Interaction effect
p-value	0.0796	0.0165 *	0.0235 *

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
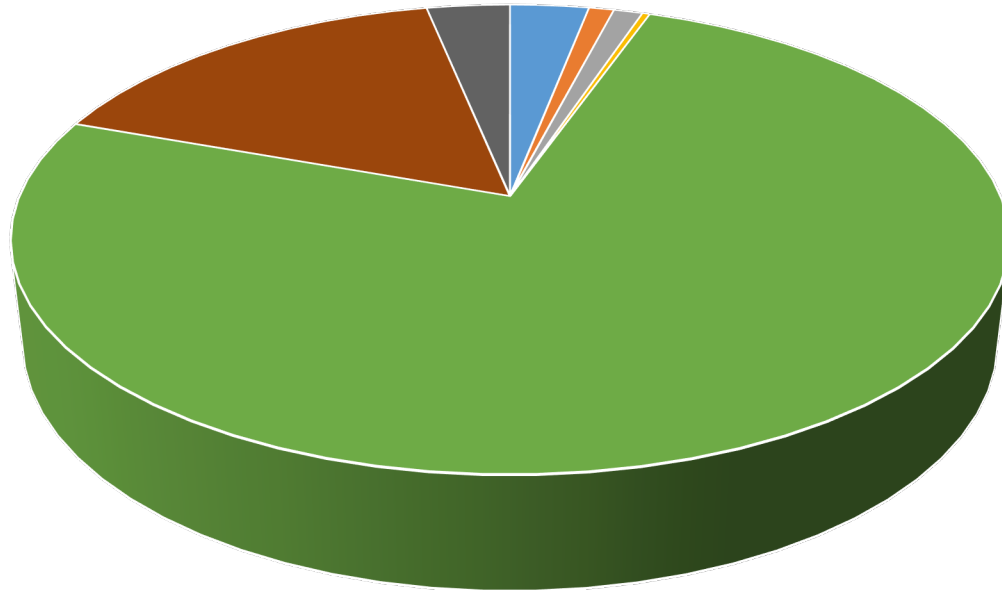
No Wheels   Wheels
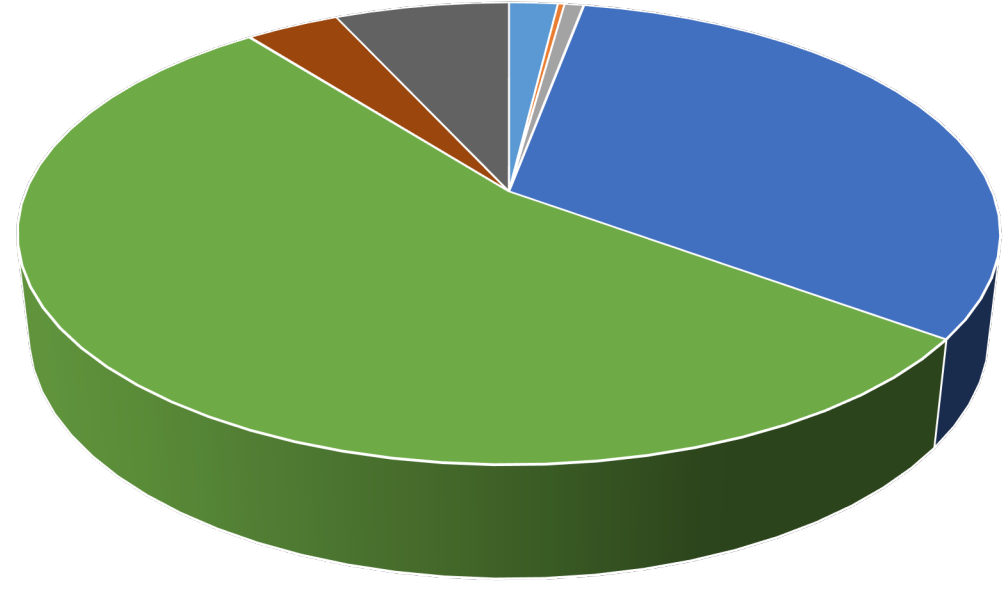
EthoScan: additional tables

- **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
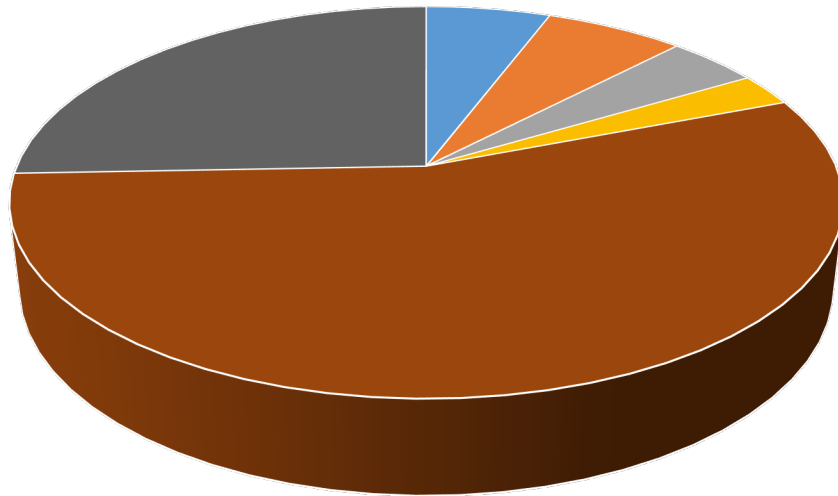
Locomotion % Budget: Wheels

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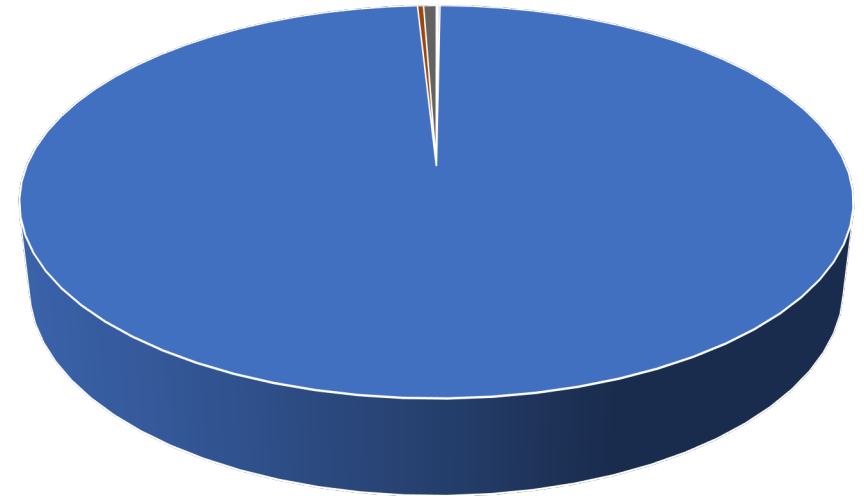
EfodA TfodA DWatr TWatr Wheel IHome THome LLnge SLnge

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 VO2,   Avg VCO2
  - Avg EE,   Total EE
  - Avg RQ
  - Avg VH2O

  - 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:

Marsha Ensor

ml.ensor@uky.edu