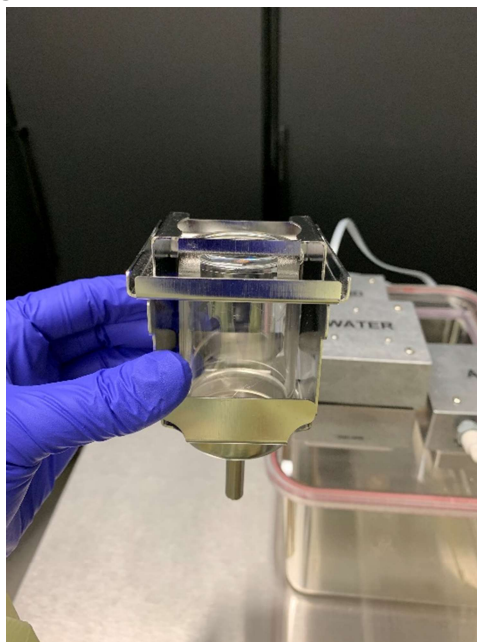


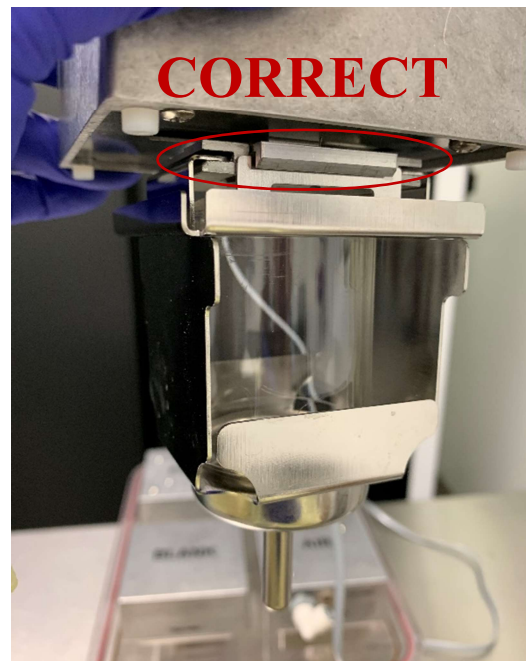
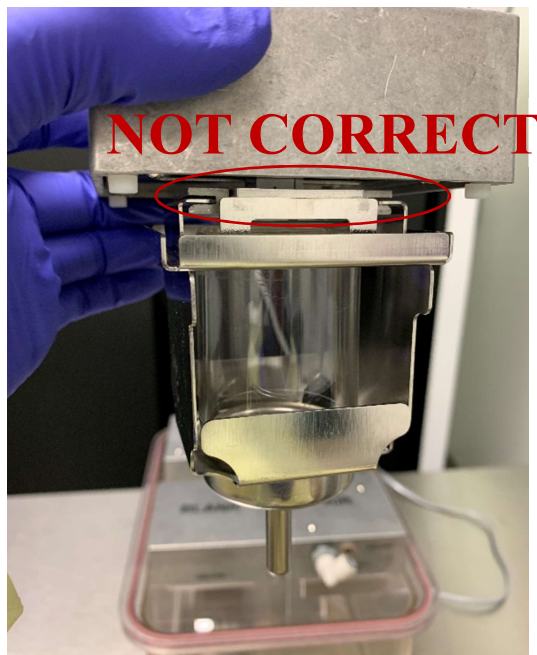
I. Cage Set up

1. Retrieve mice for experiment before CLAMS set up
2. Add bedding to each CLAMS cage that will be used (can use up to sixteen)
 - a. Bedding can be obtained from clean cages in hallway/adjoining rooms or from the CLAMS room if available.
3. Make sure the cages do not have the wheel attachment if you do not need it. If needed, the parts are in lower cabinet and attach magnetically under the air module. Be sure to use the air modules stored with the wheel components.
4. Add clean water to each water bottle and place in carriage shown
 - a. Water can be obtained from racks of autoclaved water bottles from adjoining rooms or from the CLAMS room if available.



For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.

5. Add food to the food containers
 - a. Food can be obtained from adjoining rooms or in the CLAMS room if available
6. Attach food and water modules to appropriate scale block. Use Fingers to ensure it is locked in place. May take a few tries.



For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.

7. Ensure all hookups are attached according to the label attached to the wire. Attach green gas hose to hookup on air module.
 - a. Because all measurements are taken by mass, if you switch food and water, the data will still be correct. Just switch the food and water data readouts.
 - b. Try to thread all wires along the middle between the blocks and make sure none are blocking the black laser arrays that track movement.



For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.

8. Ensure the CI box is fully connected to the computer tower (grey) and to the CLAMS system (blue)



For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.

II. Gas Calibration

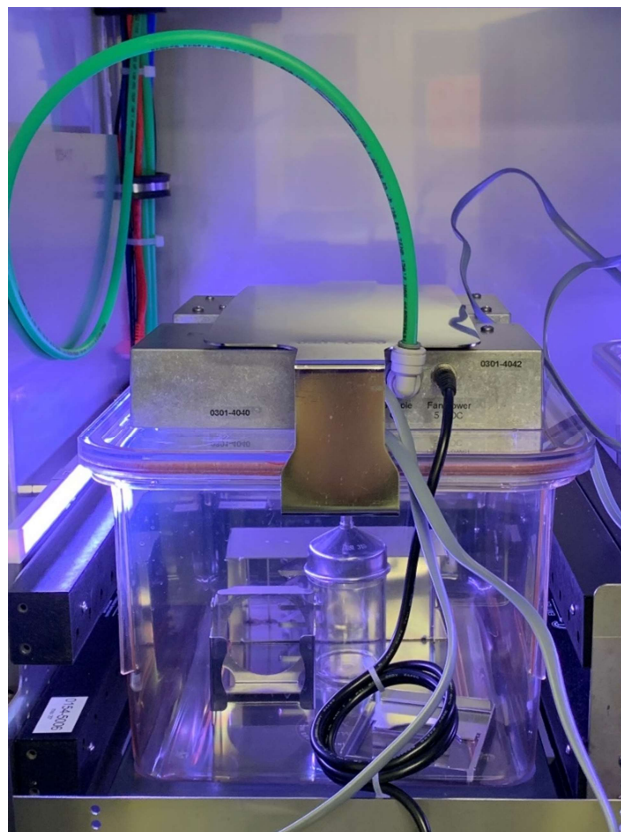
1. Turn on computer.

User: ASB2-COA-3971/CLAMS Password: Cs072219

2. Dialogue box will open. Run “hardware config NO WHEEL” if not using wheel. It should be the default but if not click the second option “open hardware configuration”.
3. Go to file>view hardware config to ensure all hardware is connected and operational.
 - a. If some are failing, ensure all connections on the cages are attached securely and in the correct port. Also make sure the CI Box on the counter is fully connected to the computer tower and the electronics on top of the CLAMS system.
4. To perform gas calibration, go to experiment>calibration and dialogue box will open. Before clicking start calibration, make sure the yellow gas connector is securely connected to the gas tank by the door and that the PSI is around 10psi. A little more or a little less is okay.
5. Start gas calibration and let it run (will take several minutes)
6. Ensure the calculated concentrations of CO₂ and O₂ are close to the actual concentration shown on the papers attached to the tank. Should be around 20.50 for %O₂ and around .5 for %CO₂

III. Experimental Setup

1. In the experiment tab, go to set up.
2. Name each mouse/cage (one mouse per cage) and uncheck cages that will not be used.
3. Weigh each mouse and record it in the appropriate column before placing them in the cage through the blank module space. Be sure to save cage cards so you can put mice back following the experiment, if needed
4. Attach the clamp that secures all modules to the cage by snapping it above each cage. You should hear a snap. Make sure no wires are being compressed by the clamp.



For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.

5. If your mice weigh less than 75 grams, the flow can stay at the default .90
6. A dialogue box will ask you where to save the data.
7. Under experiment tab, go to properties to change environmental conditions in the CLAMS system.
8. If you are running the system when people will not be in the CLAMS room, you can change the reference to be checked every 32 samples.
 - a. You can also change it to 16 if you want the reference to be taken more often. For example, when you know the room will be trafficked through during the experimental period for some reason. (See appendix)
9. Go to the environment tab and ensure temperature is set to about 23 degrees. Also ensure that the light dark cycle is set switch every 720 minutes (12 hours) and make sure the starting time is set to any date at 7:00 am. This will match the light/dark cycle of the other animal rooms. You can change the color for light or dark by clicking the color box. Be sure to either choose black (no light) or red for the dark cycle to ensure the animals aren't disturbed by the light during their dark cycle. **ALWAYS CLICK "SAVE TO ANY CONTROLLERS" AFTER ALL CHANGES**
10. The other tabs do not need to be changed. They just make sure that the food and water measurements do not record when the mouse

isn't actually eating or drinking. For example, when the mouse just brushes against the food/water and a very small amount falls through. (see appendix for default settings in case they get changed). Feeding and drinking should have the same setting.

11. Ensure all doors on the CLAMS system are closed and click run on the bottom right hand of the dialogue box.
12. Experiment will continue running until you click stop. Feel free to post signs on the door to ensure no one will be walking in during the experiment.
13. Be sure to check on the mice EVERY DAY while the experiment is running. When food and water are being refilled during experiment (usually won't be necessary), see page 62 of the software manual.
14. For help with analysis, see software manual on aging server.

IV.Ending and Clean Up

1. To end the experiment, click end run on the lower right side on the dialogue box.
2. Export data going to file>export
3. Put mice back in clean cages with original cage mates and return to appropriate room. Or if you are done with the animals, sac and process according to your workflow.
4. Dump food and bedding into a few clean cages and place them on the dirty cage rack
 - a. If available, dump food and bedding into appropriate bin or chute system to be processed by CCM
5. Dump water down the drain
6. Remove food holder and water bottle apparatuses and wash with soap and water and dry them. Also wash and dry cages using soap. Store food container, water bottle, and water bottle carriage in cage for next use.

APPENDIX (DEFAULT SETTINGS)

The screenshot shows the 'Experiment Setup' window. At the top, there are two text fields: 'Data Filename: C:\Users\rvasquez\Desktop\' and 'Output Directory: C:\Oxymax for CLAMS'. Below these is a table with 11 rows, each representing a subject. The table has columns for 'Subject ID', 'Mass (g)', and 'Flow (LPM)'. A mouse cursor is pointing at the 'Flow (LPM)' column header. The 'Flow (LPM)' column has a value of 0.90 for subjects 101-108 and 109-111. The 'Mass (g)' column has a value of 25.00 for all subjects. The 'Subject ID' column is empty for all subjects. To the right of the table is a large grey rectangular area, likely a placeholder for a graph or image.

Subject ID	Mass (g)	Flow (LPM)
101	25.00	0.90
102	25.00	0.90
103	25.00	0.90
104	25.00	0.90
105	25.00	0.90
106	25.00	0.90
107	25.00	0.90
108	25.00	0.90
109	25.00	0.90
110	25.00	0.90
111	25.00	0.90

For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.

Experiment Properties

Metabolic | Feeding | Drinking | Activity | Environment | Misc | Alert Email Setup

Measurement Settings

Intervals: 0

Cage Settle (s): 17

Cage Measure (s): 3

Reference Settle (s): 17

Reference Measure (s): 3

Reference Method: 16 Samples

Cage	Settle	Measure
Ref.	00:00	00:17
1	00:20	00:37
2	00:40	00:57
3	01:00	01:17
4	01:20	01:37
5	01:40	01:57
6	02:00	02:17
7	02:20	02:37
8	02:40	02:57
9	03:00	03:17
10	03:20	03:37
11	03:40	03:57
12	04:00	04:17
13	04:20	04:37
14	04:40	04:57
15	05:00	05:17
16	05:20	05:37

Heat Calculation

Standard: $(3.815 + 1.232 \times \text{RER}) \times \text{VO}_2$

K1: K2: K3:

☒ kcal ☐ kjoule

☐ Normalize Heat

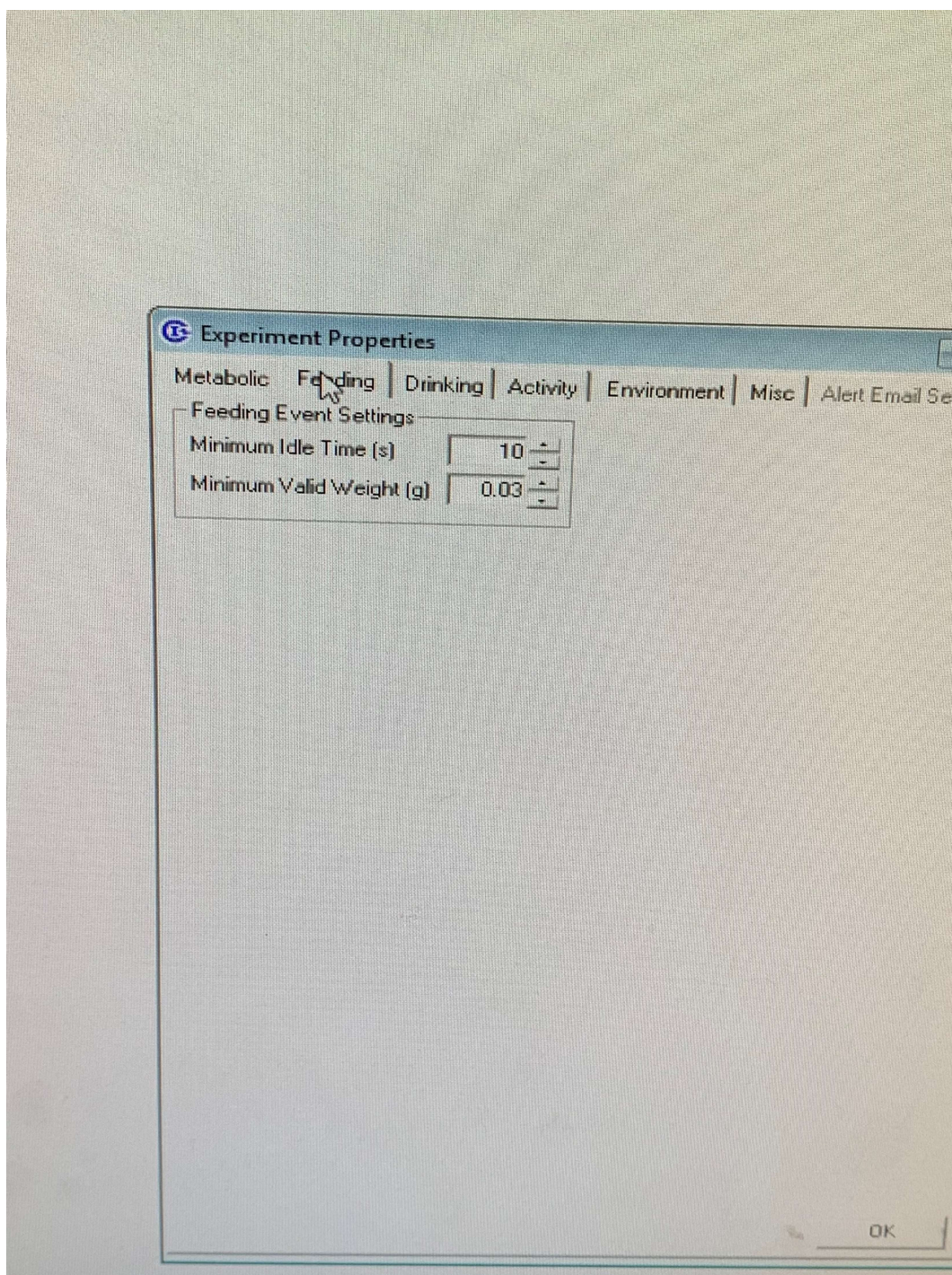
Calculation Parameters

Volume Rate Units: ml / kg / hr

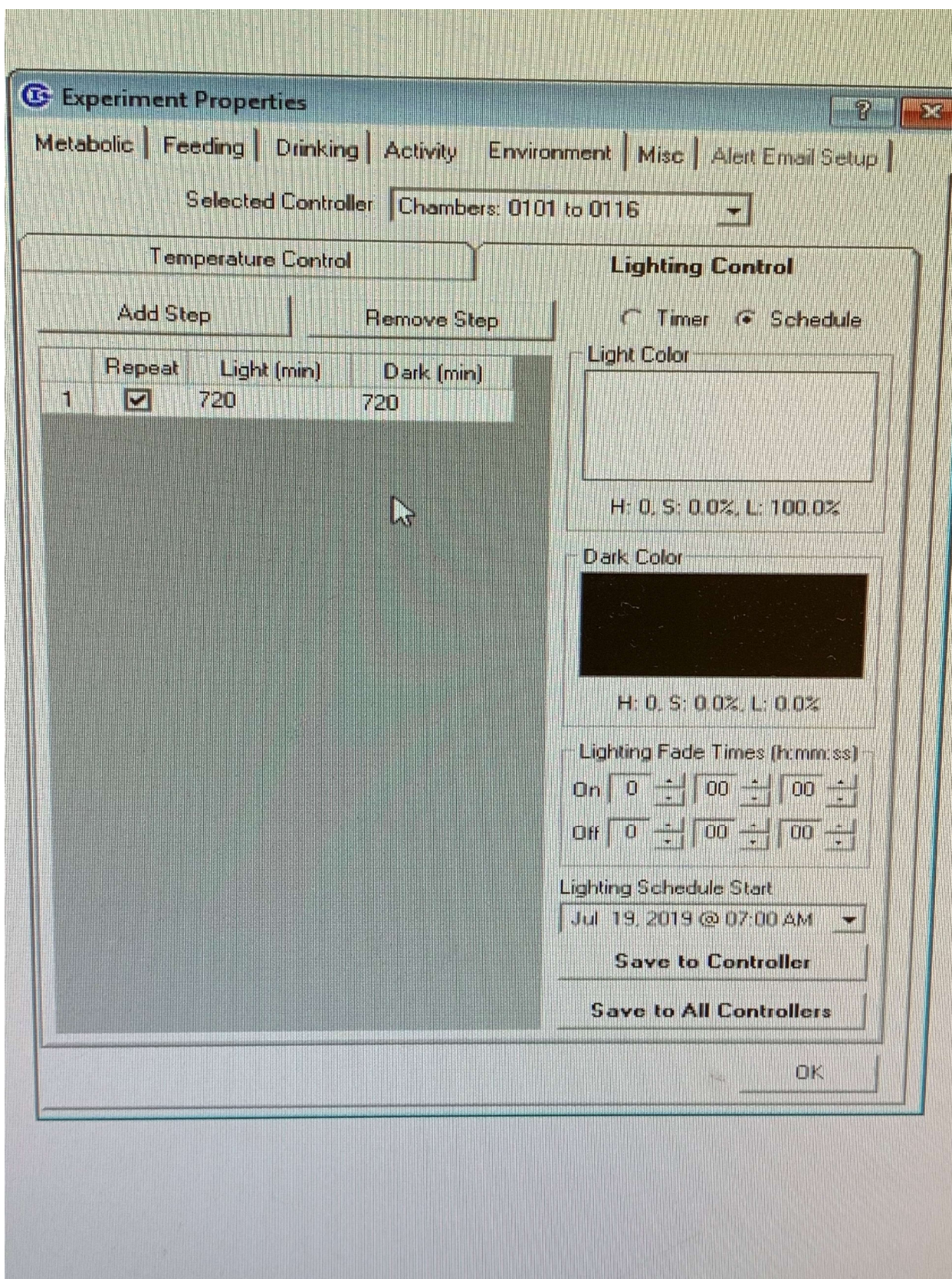
Accumulated Gas Units: l

OK

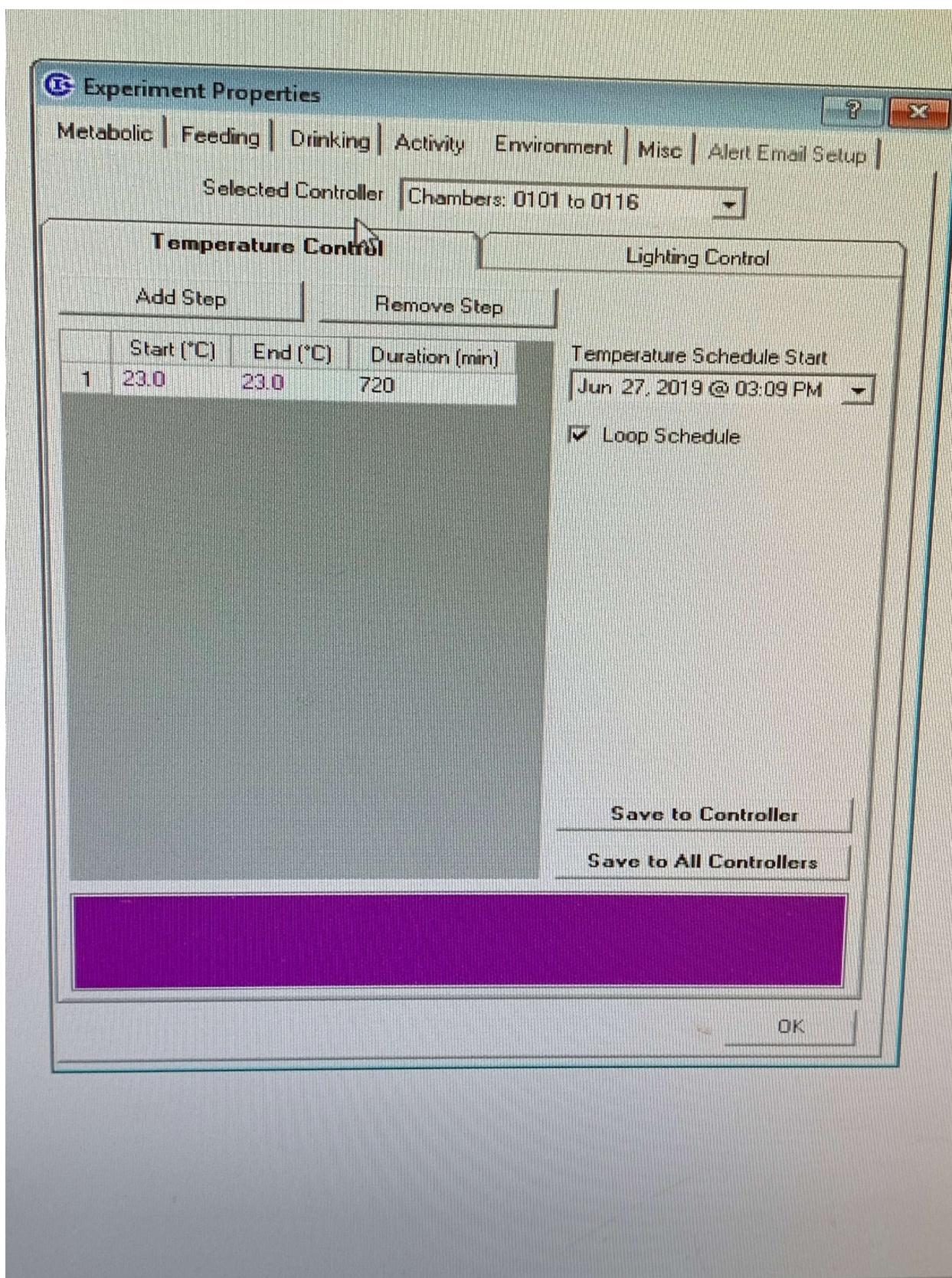
For troubleshooting, refer to hardware or software manual. Found in CLAMS room and also on the aging server.



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