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Collections

- We will collect 1080 total snails of three different species coming from two different locations (MA and NJ)
 - 60 snails / treatment per population * 2 populations * 3 treatments
 - 360 per species (180 per population) in each treatment
- **Each snail we be labelled tagged with a four-digit number that will correspond to a longer, more informative label on the container (see below) in which the snail will be held.**
 - Treatment_Population_GenusSpecies_Block_SampleIndicator
 - E.g. HS_MA_LS_03_0120
- After labelling, each snail will be measured for the following after collection and retrieval to the MSC:
 - shell height and width
 - wet weight
 - buoyant weight of shell

Acclimation Period

- Snails will be held in individual urinalysis containers filled with aquarium gravel and Fucoid algae. The containers will be covered with Nitex mesh to allow for water drainage.
 - I think we should maybe drill holes at the bottom of the container to allow for reliable water drainage.
- **Each container will be placed in the sea tables for 60 days** with the following:
 - 12 hr light regime
 - Ambient temperature of sea water system
 - Table drained to simulate tidal events

Treatments and Controls

- Each treatment and control will be carried out over a **4 hour period for 3 consecutive days**.
- There will **12 replicates** of each treatment and control
 - By doing 12 replicates, the experiment will be broken down to 30 snails per treatment at one time (90 snails in total)
- Each replicate of treatments and control will be repeated over 3 days with **living snails**
 - Snails that die will be recorded along with the day of treatment (or control) in which it dies recorded.
 - Snails that live will be carried through to the next day of treatment (or control)

Cold Shock Treatment

- Cooler filled with ice
 - If space is limited, snails may be stacked with ice in between layers
- Need thermometer in cooler to measure temperature

Heat Shock Treatment

- Environmental Chamber (Helmuth Lab)
 - 40 C
- The chamber will easily hold 30 urinalysis containers
 - Could be more if we would like to decrease replicates

Control

- Held in acclimation conditions

Post-Treatment

Measure Respiration

- Respiration will be measured by placing snails in scintillation vials equipped with PreSens sensor dots.
- We will measure post-treatment respiration of **36 randomly selected of individuals** from each replication of a treatment.
 - The individuals cannot be selected prior to treatment given they the individual may die.
- **We have 9 sensor spots right now.** We need more than that and the amount of individuals we are able to do respiration measurements on will be limited by how much we are willing to spend.
 - **I would like to do 1 individual from 1 of the species / population / treatment over half of the replicates.**
 - $1 * 1 * 2 * 3 * 6 = 36$
 - Each sensor costs \$25. $36 - 9$ (sensors we already have) = \$625

Post-Treatment

Tissue Sampling

- All snails will be measured for shell height and width, wet weight, buoyant weight of shell prior to being sampled destructively
- Tissues we be sampled from **all surviving individuals** after the three day replication of blocks.
- The **foot and remaining tissue** will be collected, placed separately in cryo-vials, and flash frozen.
- The foot tissue will be used for RNA extractions for gene expression analysis.

Death during treatment

- Snails that die during treatments will be measured for post-experiment shell height and width, wet weight, buoyant weight of shell.
- **The day of treatment that the snail died will be recorded in data**

Data table for data entry

```
> print(head(data))
  block sample_n sample_indicator      genus_species population collection_location treatment shell_height shell_width wet_weight buoyant_weight respiration_measured respiration treatmentDay1_survived treatmentDay2_survived treatmentDay3_survived tissue_collected
1    07     0001 HS_MA_LS_07_0001 littorina_saxatalis        MA                NA      HS            NA            NA            NA            NA            FALSE            NA              TRUE              TRUE              TRUE              FALSE
2    11     0002 CS_NJ_LS_11_0002 littorina_saxatalis        NJ                NA      CS            NA            NA            NA            NA            FALSE            NA              TRUE              TRUE              TRUE              FALSE
3    06     0003 CS_NJ_LL_06_0003 littorina_littorea        NJ                NA      CS            NA            NA            NA            NA            FALSE            NA              TRUE              TRUE              TRUE              FALSE
4    12     0004 CS_NJ_LL_12_0004 littorina_littorea        NJ                NA      CS            NA            NA            NA            NA            FALSE            NA              TRUE              TRUE              TRUE              FALSE
5    03     0005 CS_NJ_LL_03_0005 littorina_littorea        NJ                NA      CS            NA            NA            NA            NA            FALSE            NA              TRUE              TRUE              TRUE              FALSE
6    08     0006 NT_MA_LO_08_0006 littorina_obtusata        MA                NA      NT            NA            NA            NA            NA            FALSE            NA              TRUE              TRUE              TRUE              FALSE
>
```