*** Each link directs to information behind the each step of the experiment

Collections and pre-experiment measurements

Acclimation Period

Treatments and Control

- Cold Shock Treatment
- Control
- Heat Shock Treatment

Death during treatment

Post-Treatment

- Tissue Sampling
- Measure Respiration

Collections

- We will collect 1080 total snails of three different species coming from two different locations (MA and RI)
  - 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 (maybe only 30 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 accilimation 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

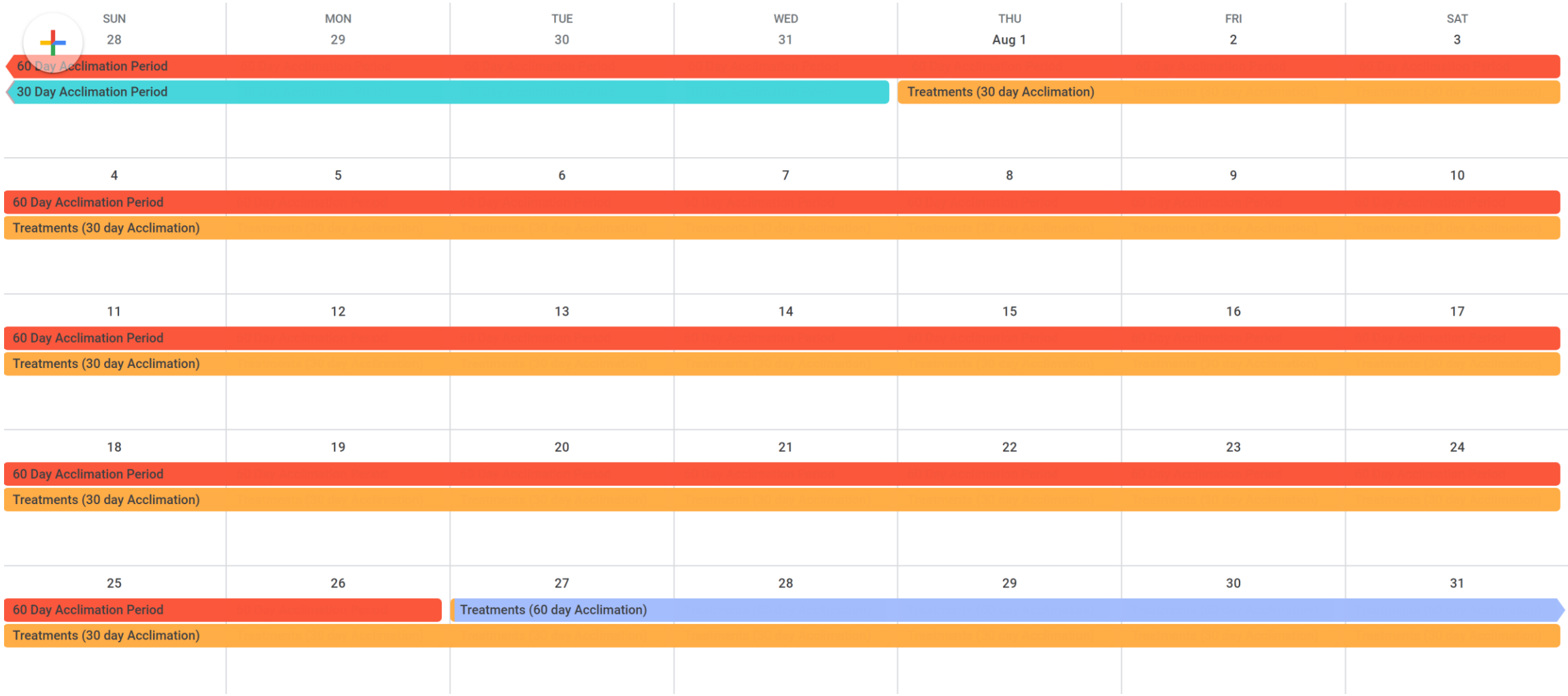
- Snails that die during treatments will be measured for post-experiment shell height and width, wet weight, buoyant weight of shell.
- **The day of treatment that the snail died will be recorded in data**

Data table for data entry

```
> data[1:25, ]
   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_RI_LS_11_0002 littorina_saxatalis         RI                 NA        CS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
3     06     0003 CS_RI_LL_06_0003  littorina_littorea         RI                 NA        CS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
4     12     0004 CS_RI_LL_12_0004  littorina_littorea         RI                 NA        CS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
5     03     0005 CS_RI_LL_03_0005  littorina_littorea         RI                 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
7     03     0007 NT_MA_LO_03_0007  littorina_obtusata         MA                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
8     05     0008 CS_RI_LS_05_0008 littorina_saxatalis         RI                 NA        CS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
9     10     0009 HS_RI_LL_10_0009  littorina_littorea         RI                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
10    06     0010 HS_MA_LO_06_0010  littorina_obtusata         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
11    08     0011 NT_RI_LO_08_0011  littorina_obtusata         RI                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
12    01     0012 HS_RI_LL_01_0012  littorina_littorea         RI                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
13    10     0013 NT_RI_LO_10_0013  littorina_obtusata         RI                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
14    07     0014 HS_MA_LO_07_0014  littorina_obtusata         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
15    10     0015 HS_MA_LO_10_0015  littorina_obtusata         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
16    04     0016 HS_MA_LO_04_0016  littorina_obtusata         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
17    04     0017 HS_MA_LS_04_0017 littorina_saxatalis         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
18    06     0018 NT_MA_LO_06_0018  littorina_obtusata         MA                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
19    12     0019 HS_MA_LO_12_0019  littorina_obtusata         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
20    04     0020 HS_MA_LL_04_0020  littorina_littorea         MA                 NA        HS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
21    03     0021 NT_MA_LS_03_0021 littorina_saxatalis         MA                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
22    11     0022 CS_MA_LS_11_0022 littorina_saxatalis         MA                 NA        CS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
23    06     0023 NT_MA_LS_06_0023 littorina_saxatalis         MA                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
24    04     0024 CS_MA_LL_04_0024  littorina_littorea         MA                 NA        CS           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
25    10     0025 NT_MA_LS_10_0025 littorina_saxatalis         MA                 NA        NT           NA          NA         NA             NA               FALSE          NA                  TRUE                  TRUE                  TRUE             FALSE
>
```

SUN	MON	TUE	WED	THU	FRI	SAT
26	27	28	29	30	31	Jun 1
				Ordering Supplies		
2	3	4	5	6	7	8
9	10	11	12	13	14	15
	Preparing Urinalysis Containers					
16	17	18	19	20	21	22
	Preparing Acclimation Conditions				Evolution	
23	24	25	26	27	28	29
Evolution				Labelling and Initial Measurements		60 Day Acclimation Period
			Sampling in Rhode Island	Sampling in Nahant		30 Day Acclimation Period
30	Jul 1	2	3	4	5	6
60 Day Acclimation Period						
30 Day Acclimation Period						

SUN	MON	TUE	WED	THU	FRI	SAT
30	Jul 1	2	3	4	5	6
60 Day Acclimation Period 30 Day Acclimation Period						
7	8	9	10	11	12	13
60 Day Acclimation Period 30 Day Acclimation Period						
14	15	16	17	18	19	20
60 Day Acclimation Period 30 Day Acclimation Period						
21	22	23	24	25	26	27
60 Day Acclimation Period 30 Day Acclimation Period						
28	29	30	31	Aug 1	2	3
60 Day Acclimation Period 30 Day Acclimation Period				Treatments (30 day Acclimation)		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60 Day Acclimation Period
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Treatments (30 day Acclimation)
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60 Day Acclimation Period
Treatments (30 day Acclimation)
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Treatments (30 day Acclimation)
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60 Day Acclimation Period
Treatments (60 day Acclimation)
Treatments (30 day Acclimation)