

Lab Experiment - Materials List

- 12 HSC
- 2 EGC
- 2 shoeboxes (one for each temp tank)
- 8 resazurin containers
- Well plate
- Acrylic wall (to create narrow path)
- Oyster bag mesh (separate EGC and HSC)
- Video recording device
- Scale
- Red and Blue bead tags for tagging HSC
- Tray for righting time

Experimental Procedure:

Setup:

- Obtain 12 Hairy Shore Crab (which are a 50/50 split of male, female, unparasitized) (which are all one sex)
- Pat dry and weigh individuals for standardization later on
- Tag all 12 Crabs for identification later on - 6 Red tags for elevated group, 6 Green for ambient group, record numbers
- Separate into 2 groups to be placed into temperature treatments later
- Elevated Temperature (24C) Group: 3 Males 3 Females Nonparasitized, 2 of each selected for resazurin test
- Ambient Temperature (14C) Group: 3 Males 3 Females Nonparasitized, 2 of each selected for resazurin test

Respiration Test:

Following Given Resazurin Guidelines -

Working resazurin solution:

Prepared by TA before lab

Protocol

1. Load 35mL of resazurin working solution into crab chambers with transfer pipettor

- Ensure gloves worn
- Make sure to add 3 blank cups with resazurin to create a mean to subtract from your totals
- Ensure resazurin is held at specific temperatures per our crab treatment groups

2. Gently pat each crab dry with paper towels and weigh them to the nearest hundredth of a gram. This will be used to normalize your results across all crabs you test

3. Carefully place a crab within each chamber and quickly start a timer/stopwatch

- Remember to record time when added, T0

4. Every 30 minutes, withdraw 200ul from each chamber and place in the wells of the 96 well plate a. Note which wells contain samples from an individual and at what timestep (ex. Well A2: Crab 1, 60min)

- Carefully label 96 well plate (note, use 96 well plate template, annotate)
- Use template excel sheet to track each well and sample

5. At the end of your trials, withdraw crabs from their chambers using gloved hands and rinse them off with 33-35ppt saltwater

6. Place crabs back in their tanks, ideally within a partitioned off area to identify them as crabs that have already undergone resazurin analysis (can identify using tags)

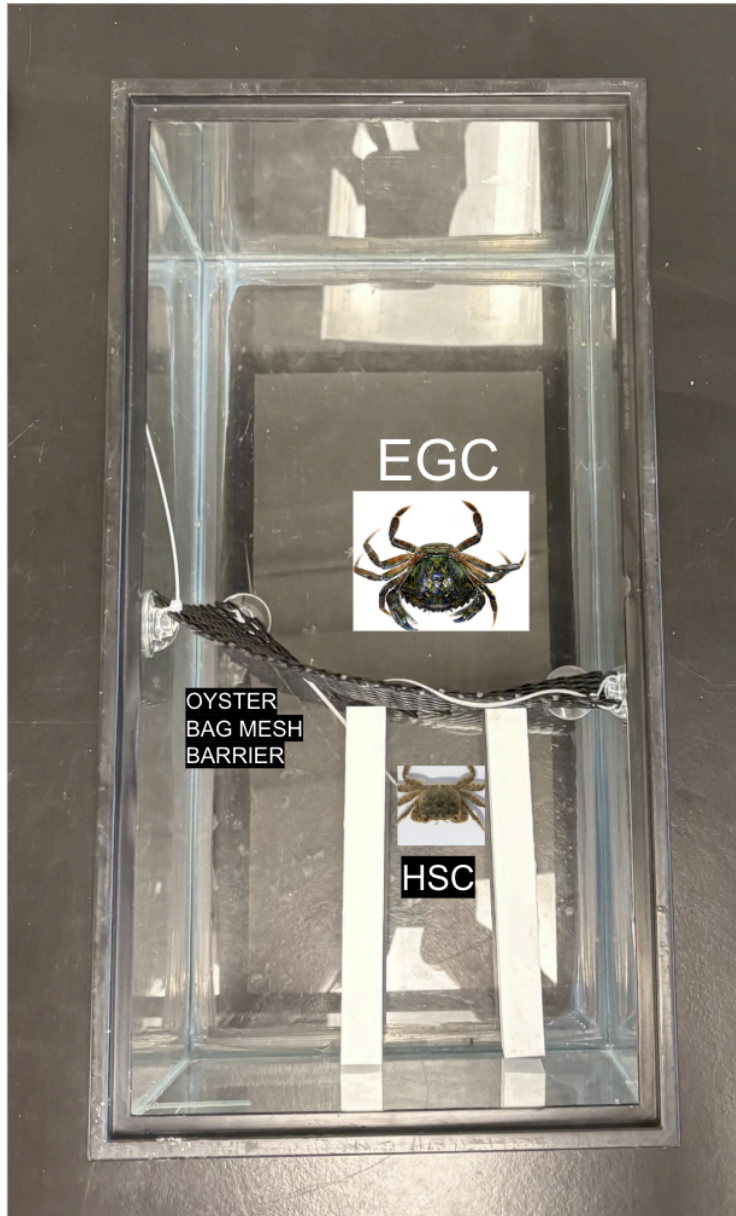
7. Run plate in the plate reader at Excitation 530; Emission 590 to obtain fluorescence values

8. Divide all fluorescence values by crab weight to normalize for differences in crab size

9. Dispose of all resazurin waste in the appropriate chemical waste bottle and thoroughly rinse plates with freshwater You TA will run your samples on the plate reader, but if you're interested in how this is done, reference the plate reader SOP.

Behavioral Test:

Distance Travelled



1. Create two of the behavioral experiment tanks (above). Fill each with ambient and elevated temperature water, respectively.
2. Place the European Green Crab in the tank. The EGC should be placed behind the mesh, opposite of the acrylic walls.

3. Set up a recording device from overhead of the tank.
4. Place one HSC in between the acrylic walls next to the mesh.
5. Record every time the HSC stops and starts, up until final, tucked stop. Calculate total distance moved from EGC.

Righting Time

1. Remove HSC from the tank after the final tucked stop.
2. Place the HSC upside down on a tray. Thumb and Pointer on top and bottom, flip on head, release and start timer.
3. Record time it takes for each HSC to recover after its release.