

# Refugia Research:

## Development of Husbandry and Captive Propagation Techniques for Invertebrates Covered Under the Edwards Aquifer Habitat Conservation Plan

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# Background

- Five invertebrate species are covered under the HCP
  - Comal Springs riffle beetle
  - Comal Springs dryopid beetle
  - Peck's Cave amphipod
  - Edwards Aquifer diving beetle
  - Texas troglobitic water slater
- SMARC maintains individuals of these species, but information on maintaining captive populations, husbandry, and ecology unknown
  - Riffle beetles and amphipod reproduction and long-term captive breeding are not good

# Project Questions and Goals

- Conduct four preliminary studies that will aid in the husbandry and maintenance of populations in a refuge context
  - Anesthesia of individuals
    - Immobilize individuals to monitor growth and development, keep alive, unharmed
  - Light responses and requirements
    - Preferences or avoidance of light? Effects of light on growth and survival?
  - Mating behavior and requirements
    - Peck's cave amphipod mating is not well documented and individuals eat each other (cannibalism); how to mate and keep alive?
  - Holding methods in a refuge
    - Peck's cave amphipods will eat each other; survival is better in what kind of set-up?

# Anesthesia Study

- Focus on MS-222, clove oil, and EtOH
  - CO<sub>2</sub>, ether, cold not good options
- Literature is not good with setting initial thresholds
  - Use of surrogates initially to get rough idea of thresholds
  - *S. flagellatus* and/or *C. pseudogracilis* for *S. pecki*
  - *H. glabra* for *H. comalensis*
  - *Lirceolus*



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# Anesthesia Study

- Trials performed individually
  - Duration after exposure to LOR
  - Duration to recovery after removing from chamber
  - Longer-term survival (72 h after exposure)
  - Individual wet weights post experiment
- 5-10 individuals per species per anesthetic
  - ANOVA

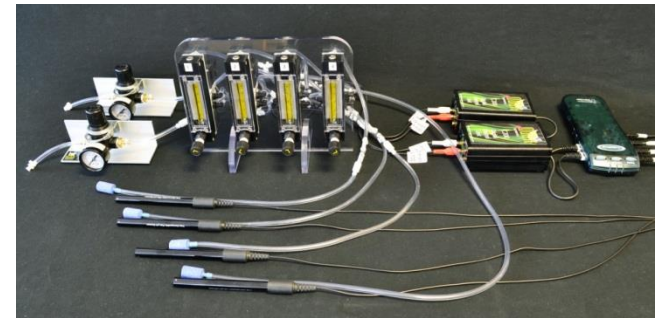
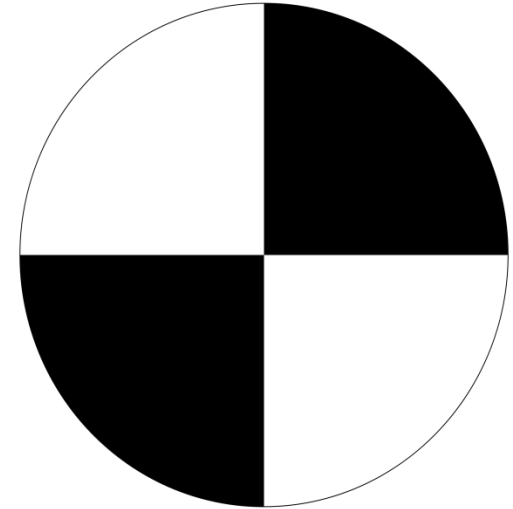
# Light Responses and Requirements



- Light conditions are critical
  - Epigeal vs hypogean
- Hypogean/stygobitic organisms negatively respond to light
  - Little idea for many of the Edwards organisms
  - *S. flagellatus* shows no preference, *S. pecki* hides from light
  - Observations on mating, housing, growth
  - *Lirceolus* and other spp.

# Light Responses and Requirements

- Trials performed individually
- Examine light responses
  - Red light, full-spectrum light
  - Two types of responses
    - Motility responses
    - Metabolic responses ( $O_2$  consumption)
- Motility responses
  - Filmed for 1 hour on black/white disc
  - Proportion of time in contrasting areas
- Metabolic responses
  - Individual closed chambers
    - No light, red light, FS
    - Measure respiration for 1-h interval
    - Qubit system (4 channel)
    - Standardized to body size



# Peck's Cave Amphipod Mating



- A lot known about amphipod mating, in general
- Strong size-selection component
- Mate guarding (amplexus)
- Molt cycle stage important
- Little is known about *S. pecki* mating
  - Preliminary observations
- Look like?
- Length of time?
- Preferences/success and size differences?
- Cannibalism?



# Peck's Cave Amphipod Mating

- Male – female pairs in plastic containers
  - Video camera set up, continuous filming
- Three size treatments: Male larger than female, male and female equal, male smaller
  - Gravid females
- Incidence of amplexus, length of amplexus prior to mating, incidence of cannibalism
- Note any other characteristics of mating

# Housing of Peck's Cave Amphipod

- Current set up is a “group holding system”
  - Flow through plastic containers
  - Cannot track development of individuals
  - Cannibalism
- Contrast the survival of individuals in two alternate set-ups
  - Flow through systems
  - Static arrays



# Housing of Peck's Cave Amphipod



- Three to four set ups of each type
- Stock with 10-20 individuals
- Monitor survival weekly for at least two months
- Compare % survival in each set up

# Study Time Line

- Start the Housing Experiment immediately
- Anesthesia within the next few weeks
- Light by July
- Mating study by July