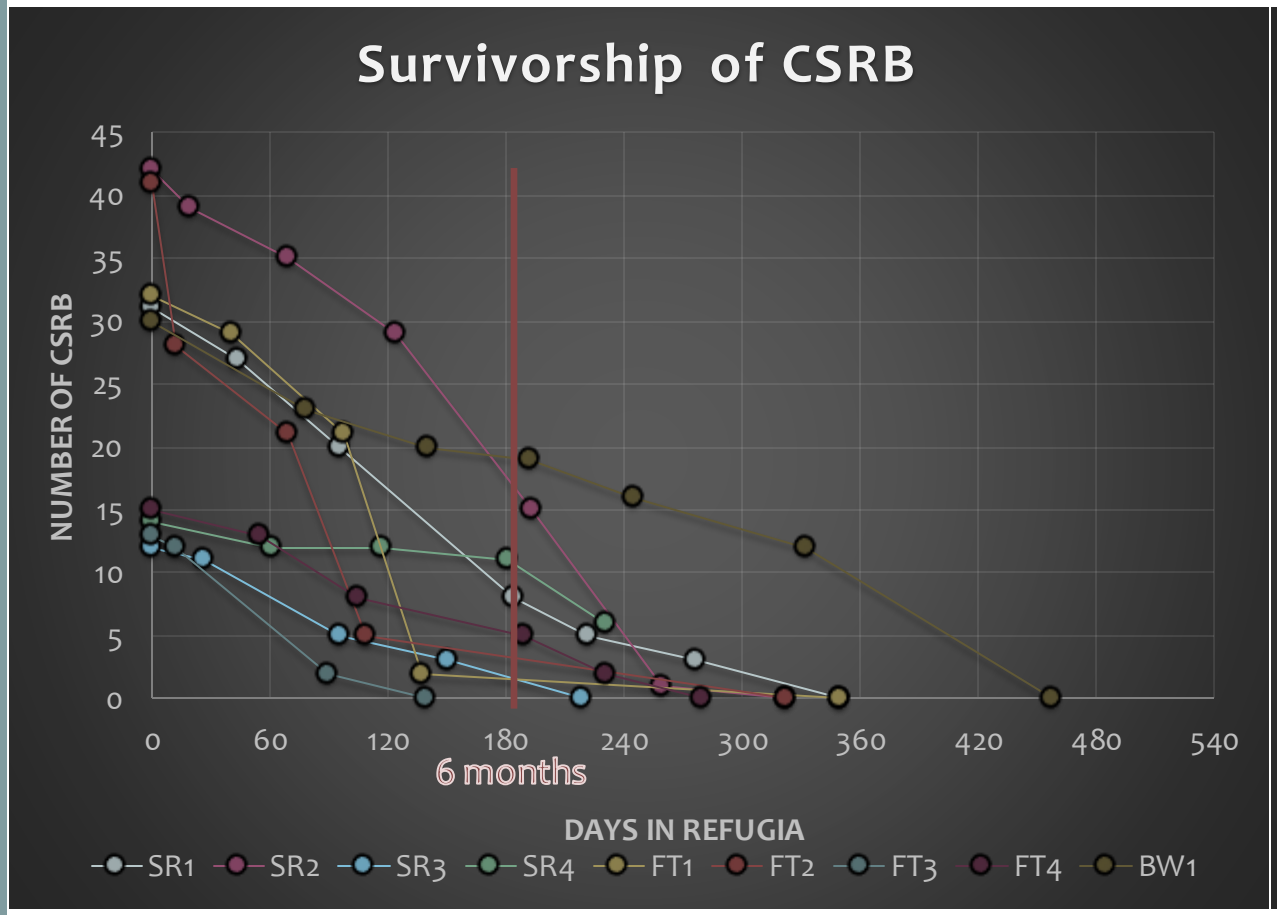
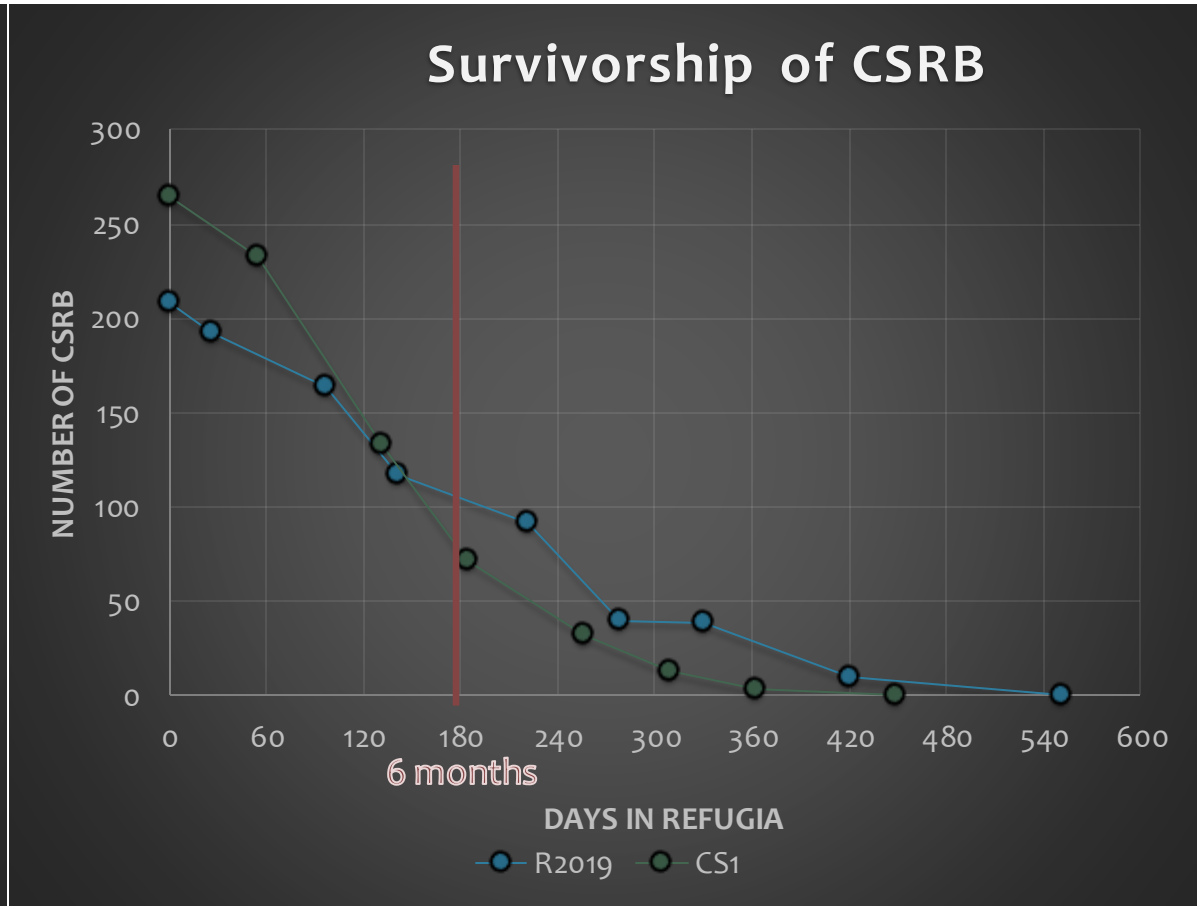
Increasing Comal Springs riffle beetle F1 adult production at the Refugia level

Dr. Lindsay Campbell

Review of Research - Survivorship

- Survivorship decreases rapidly after 5-7 months

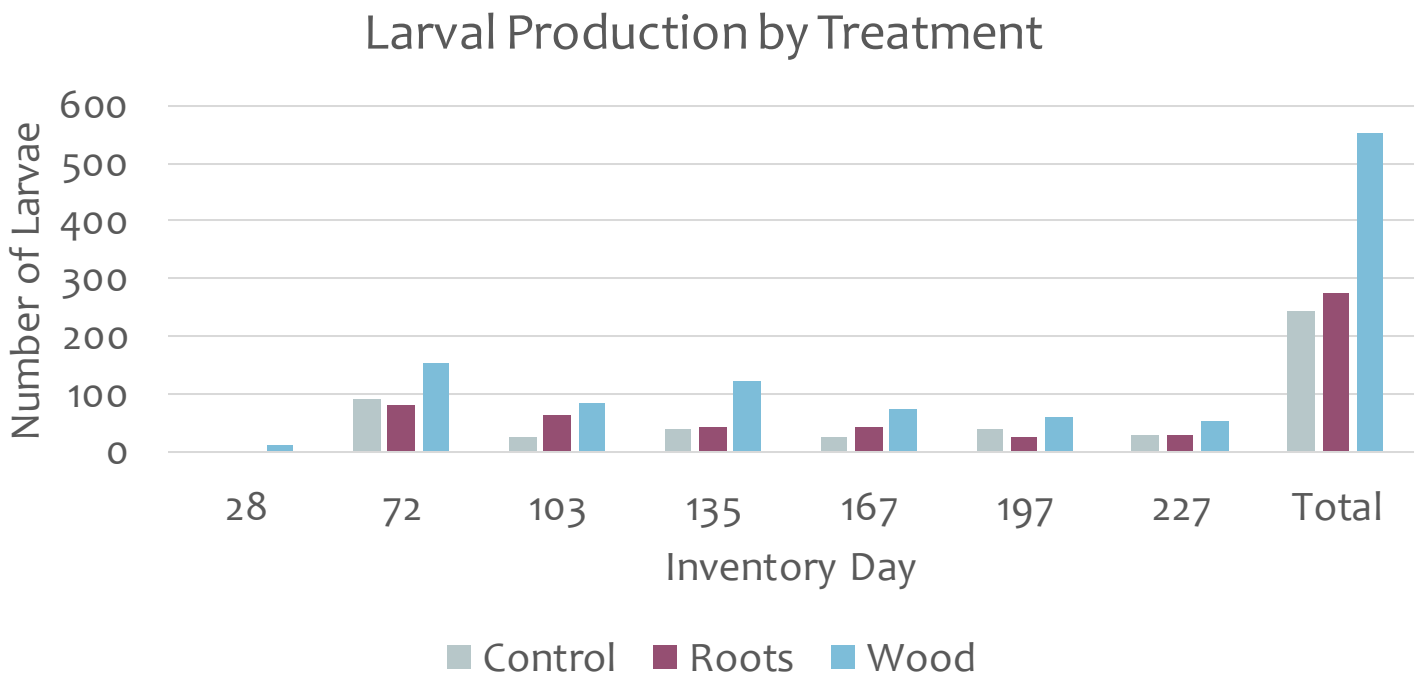




Review of Research

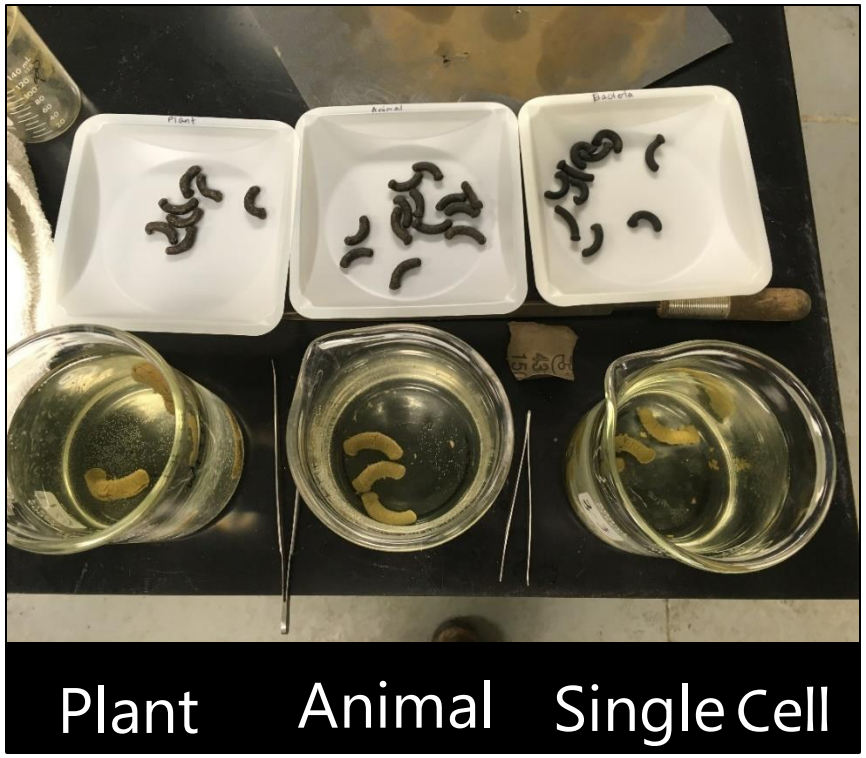
- Tested adding wood and roots to tubes
- Slightly higher survivability for Wood and Roots, but not statistically significant
- Significantly higher larval production for the Wood treatment

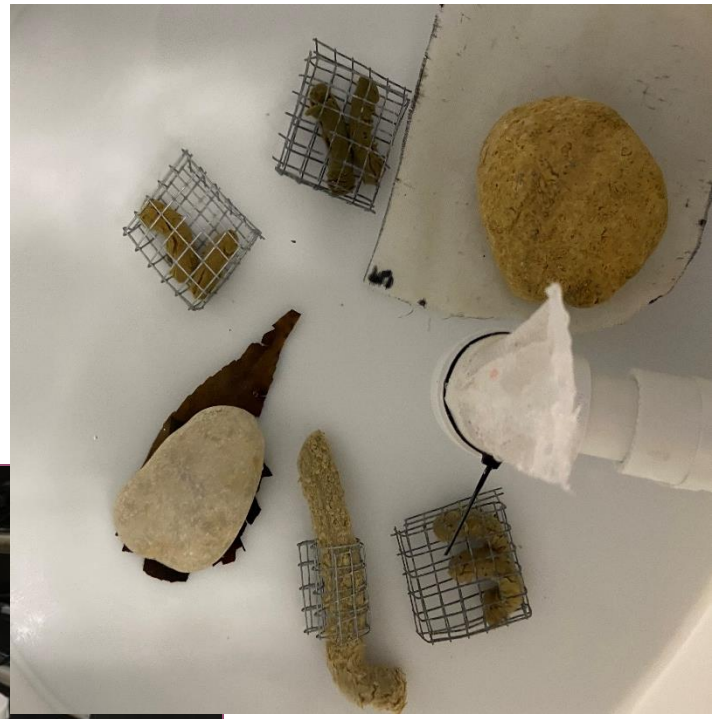
Control Roots Wood



Review of Research – Manufactured Feeds

- Four types
  - Plant protein, Animal protein, Single Cell protein, Artificial Log shape
- Offered with leaved and biofilm cloth
- Stable isotopes analyzed

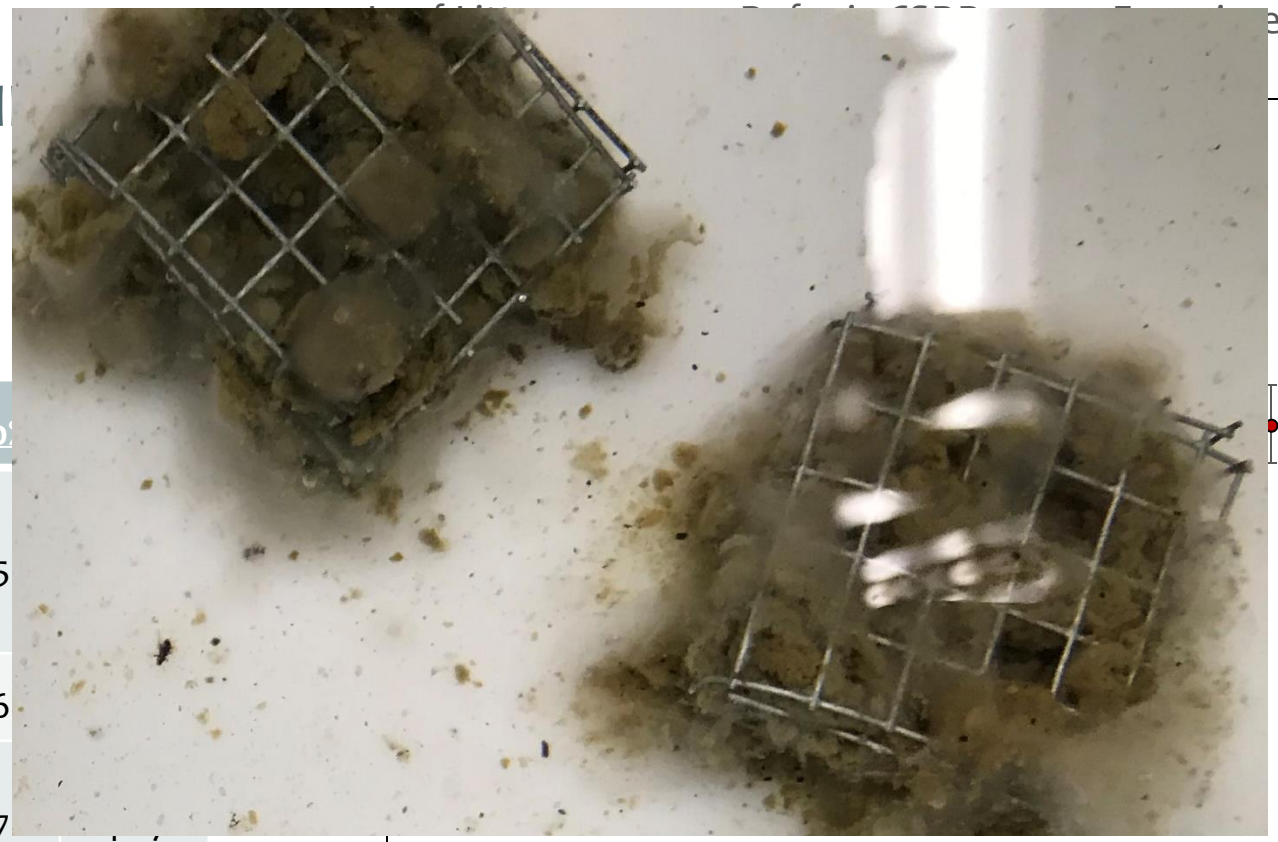




Diet Analysis

- Did eat some of the pel
  - Plant Pellet the most
- Mostly ate leaves

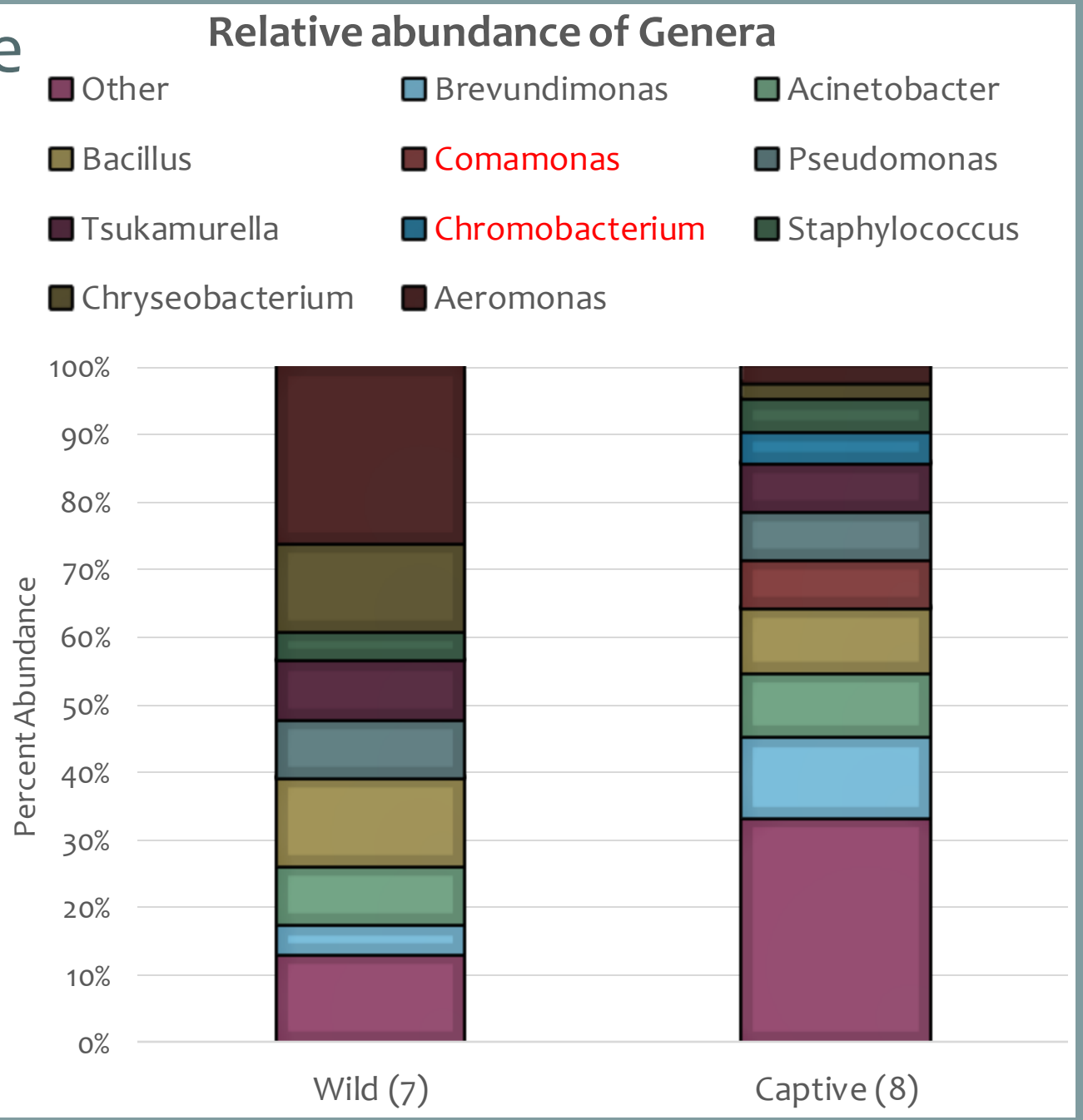
Diet Item	Mean	SD	2.50	
Leaves	22.6	10.9	0.5	
Plant Pellet	19.3	14.4	0.6	
Single Cell Pellet	16.0	13.0	0.7	
Artificial Log	13.3	15.2		
Biofilm Cloth	13.3	11.6		
Animal Pellet	10.7	10.1	0.3	37.1



But pellets fungus and degrade water quality, so not usable in current form

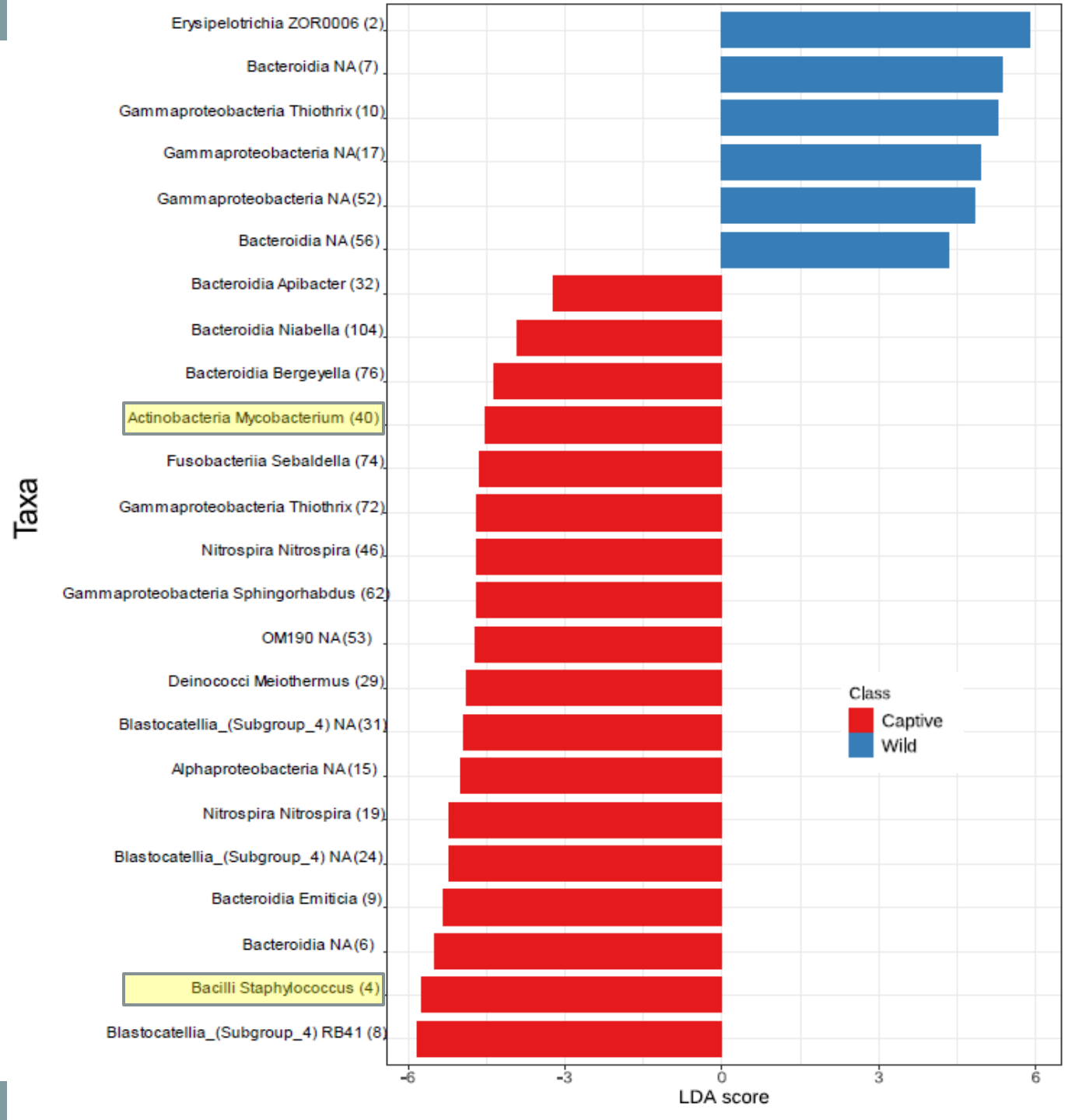
Review of Research- Microbiome (on-going)

- Significant differences between wild CSRB and captive CSRB
- Did find *Brevundimonas* genus, associated with wood-feeding insects in both
- Greatest difference was *Acidobacteria*
  - 17% captive CSRB
  - 0.6% wild CSRB
- Also found higher levels of *Staphylococcus* in captive beetles
- Only found *Comamonas*, Chromobacterium, and *Mycobacterium* genera in captive CSRB



Microbiome (on-going)

- Linear Discriminant Analysis found 24 sequences that were significantly different between the two groups
  - 6 (blue) greater in wild CSRB
  - 18 (red) greater in captive CSRB
- Captive CSRB missing *Acidovorax*, *Enterococcus*, and *Roseateles* genera
- Water at SMARC missing 10 different species of bacteria that is found in Comal Springs water



Review of Research – F1 Pupation & Eclosion

- Dr. Ely Kosnicki from BIO-WEST, Inc had a 60% pupation/eclosion rate (interim report)
  - Horizontal tube orientation
  - Found air-water interface important
  - Increasing replicates in 2020
- Dr. Weston Nowlin's group had initially higher success rates with wild cultured biofilm
  - Also found air space important to pupation
  - Grant delayed, so second year work on-going

Refugia Research 2021-2022 on CSRB
Scaling-Up findings to Refugia Level Production

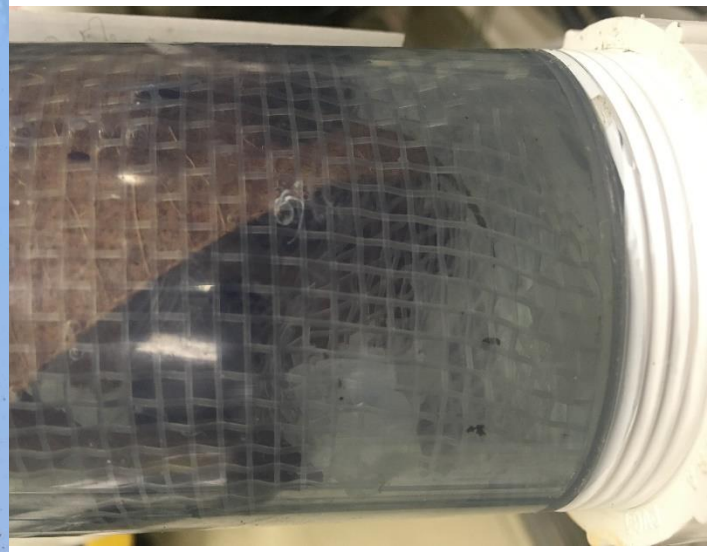
- **EFFICACY** – how well a treatment/design works in controlled research environment
- **EFFECTIVENESS** – how well a treatment/design works on larger groups, "real world"
- **Why the difference is important**
  - While a particular design might work well in low numbers or with much time commitment, this might not be feasible to large production or time that would be limited to each container.
- Based on a 60% success rate with 20 larvae in a tube:
  - In order to produce **1,000 F1** beetles to re-populate **83 flow-through tubes** needed, all at once
  - **1,660** late-stage larvae → at least **3,320** larvae would need to be produced
  - **195** pairs of adult beetles would be needed to produce that many larvae

Objectives

- Determine if higher larvae densities in a flow-through tube design can maintain or improve upon measured pupation/eclosion rates?
- Determine if a tube design modified as a small, rectangular flow-through box and maintain or improve upon measured pupation/eclosion rates?
- Based on optimization of above factors, determine if adding wild, cultivated biofilm (on leaves, wood and cloth for larvae) will improve pupation/eclosion rates?
- Try supplementing biofilm/microbiome with cultured bacteria
- Test exposure of *Staphylococcus* at high levels to larvae

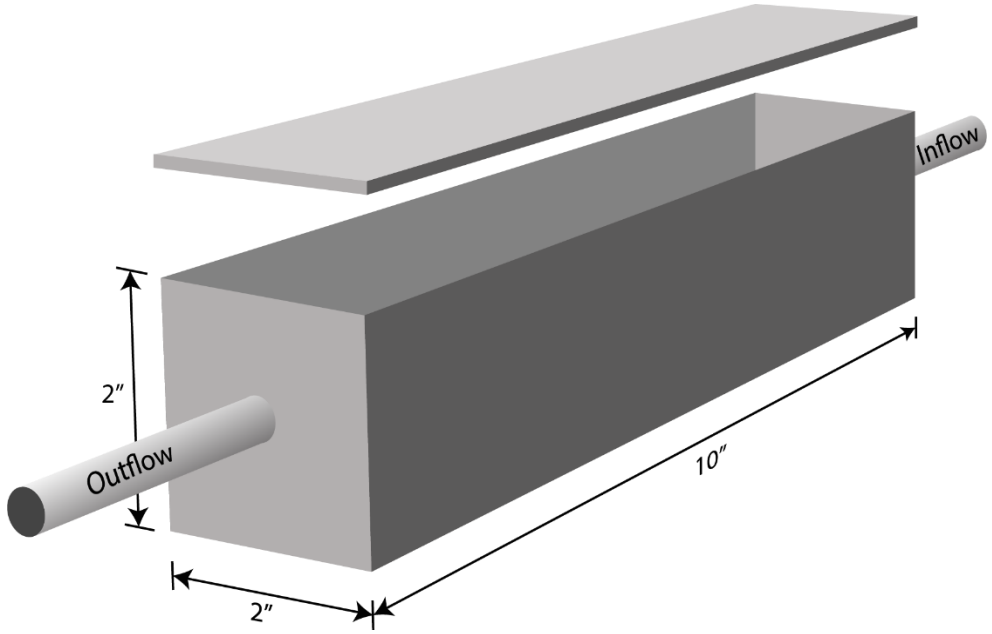
Density

- Use the most effective tube design from BIO-WEST, Inc.'s research
- Test 20 larvae (control) against 30 and 40 larvae per tube
- Three to five replicates each
- Check after 80 days (recommended in their interim report)



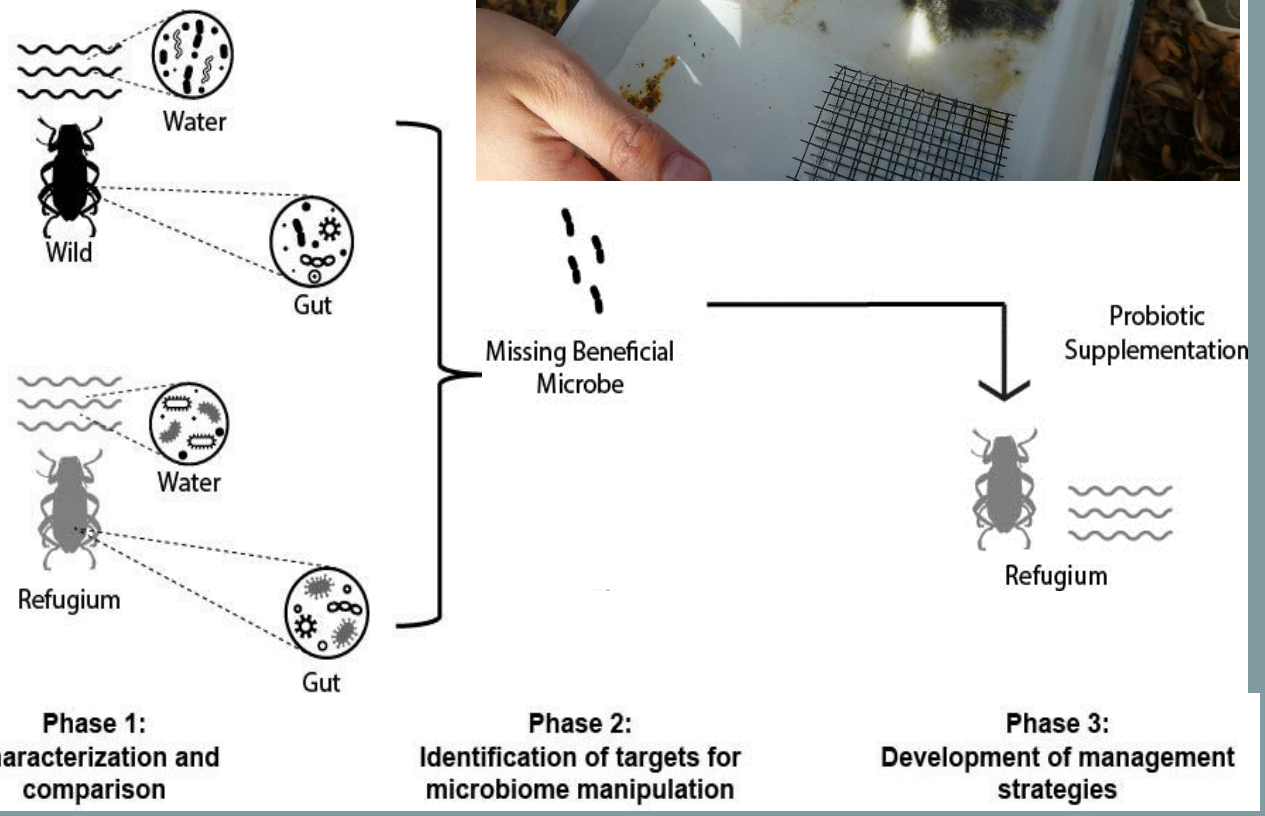
Modified tube design

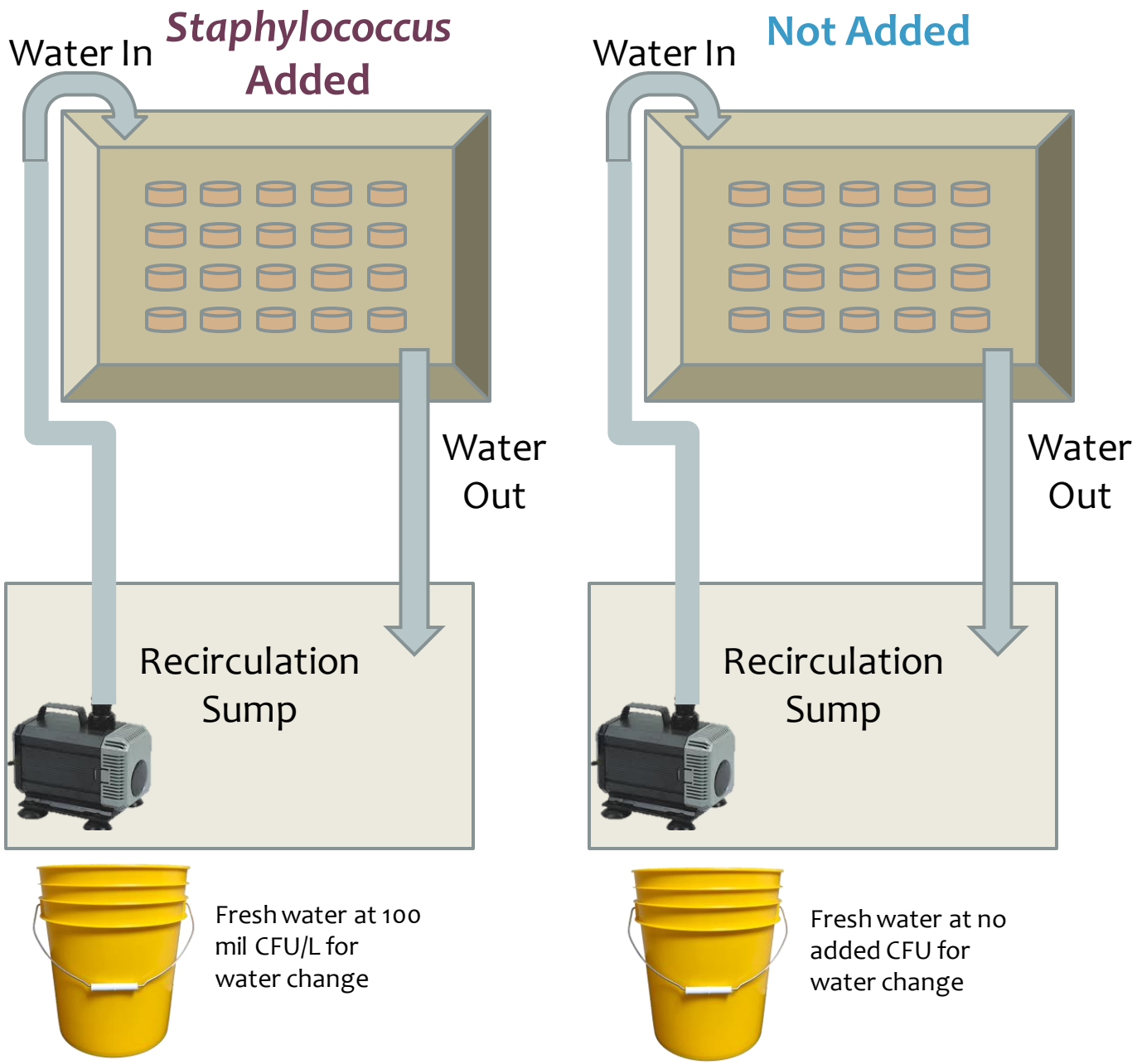
- We have found flow tube tubes easily clog
- Risk of damage to enclosed organisms when moved and opened to clear
- Cannot easily see if food items are compromised or need replenishing
- Have been testing a modified rectangular boxes for adult beetles
  - Easier to clear any obstructions
  - Can see food items
  - Easy open lid
  - Air space and water level can be adjusted
  - Same general dimensions as tubes, 70% water fill
- Three replicates of 20 larvae each



Biofilm - SMARC, Wild, Microbiome Supplement

- Use the best tube design and density from previous experiments
- Compare biofilm cultured at SMARC as usual (control)
- To that of biofilm cultured in Comal Springs (like Nowlin's group)
- And that of biofilm cultured with bacteria from microbiome study
  - Own recirculating tank using sterilized water
  - Bacterial spikes given twice a month
  - Let grow for three months
- Three replicates of each





Exposure to Detrimental Bacteria

- Identify deleterious bacteria step to healthier Refugia population
- Work not able to complete in 2020
- Based on sequencing first test *Staphylococcus*
- Expose late stage larvae to high levels and not added bacteria
  - Test Refugia larvae for base line levels
  - Test larvae after high exposure
- Check survival rate after 30 days
- Allow remaining to grow out and document survival and pupation rates

QUESTIONS?