Status of Year 1 and Year 2 Refuge Research

Weston Nowlin
Status of Year 1

- Proposed two experimental studies
- Factors that affect pupation and adult eclosion in CSRB
- Experiment 1
  - Biofilm origin and OM types
    - Field vs SMARC
    - Wood and leaves (WL) vs wood, leaves, cloth (WLC)
- Experiment 2
  - Conditioning of material prior to feeding
  - Conditioned with adult CSRB vs conditioned without
Status of Year 1

- Experiment 1 – Origin and OM type
  - Experiments run from July 2019 to Dec 2019
- Experiment 2 - Conditioning prior to feeding
  - Experiments run from December 2019 to April 2020
Experiment 1

- Treatments replicated 5 times
  - 3 late-stage larvae per tube

- Assessed
  - Larval and pupal mortality
  - Pupation rate
  - Adult eclosion
  - Composition of biofilms (microbial)
  - Nutritional composition of biofilms
    - Carbohydrates, lipids, proteins, C:N
Experiment 1

• Summary
  • Larval mortality ~15% on average across the entire experiment
  • Similar to previous work
  • Pupation occurred in all treatments
    • 53 pupae
    • ~200 larvae
  • Very limited adult eclosion
    • 3 adults produced
    • From Comal biofilms
    • 0.05 adults/pupae
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Experiment 1

- **Microbial biofilm composition**
  - Sequenced biofilms on different substrates grown at different locations
  - >5200 microbial genera detected
  - Dominated by Proteobacteria (26% of sequences) and Bacteriodetes (8.2%)
  - Substrate type and location contribute to microbial composition (PERMANOVA, Jacard distances)

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Experiment 1

- SMARC biofilms
- Higher dominance score
- Lower Shannon diversity
- Typical of captive food sources
Experiment 1

- *Chrysochromulina*
  - Most differential microbial genus between sites
- Eukaryotic haptophyte
- Wood biofilms from SMARC lack genes involved in denitrification
Experiment 1

- Carbohydrates, lipids, proteins, C:N
  - Analyses ongoing
- Expectation to complete all work by Feb 2021
  - Final report
Research in Year 2

- Pupal survival rates low
- Understand reasons for this

- Experiment 1
  - Effects of access to air – water interface and facilitation of pupal survival

- Experiment 2
  - Effects of frequent handling/checking on pupal survival
Access to Air – Water Interface

- Pupae likely hydrophobic
  - Buoyant
  - Coated in hydrophobic setae
  - Not well documented

- Other Elmidae pupate above water, but preliminary data (Huston et al.) suggests that CSRB need at least partial submergence

- Current practice – keep in flow through chambers
Access to Air – Water Interface

- Experimental design
  - House late-instar larvae in two chamber types
    - Standard flow though chambers
    - Flow through chambers with air – water interface
**Access to Air – Water Interface**

- Experimental design
  - House late-instar larvae in two chamber types
    - Standard flow though chambers
    - Flow through chambers with air – water interface
  - Larval and pupal survival, adult eclosion
- Photo-document and describe pupal setae and potential hydrophobicity
Access to Air – Water Interface

• Status
  • Experiments conducted from July – October 2020

• Mortality, pupae produced, adults produced
Frequency of Handling

• Current round of experiments check larvae and pupae weekly
  • Coated in setae
  • Fragile?
  • Preliminary observations suggest rough or frequent handling reduces hydrophobicity
• How often should we check pupae?
  • Does handling damage setae?
Frequency of Handling

• Used “air – water interface” chambers
• Check larvae on either (a) weekly or (b) monthly basis
  • Track survival of larvae and pupae
• Sub-set of pupae and photo-document/describe pupae external morphology at the two handling regimes
Frequency of Handling

• Experiment conducted July – October 2020

• Data analysis phase

• Pupal photography is ongoing
  • Slowly proceeding

• Hopeful to have things completed by February 2021
Timeline

• **Year 1**
  • Finish final lab analyses by Feb 2021
  • Final Report by end of Feb

• **Year 2**
  • Finish data analysis
  • Final Report by end of Feb