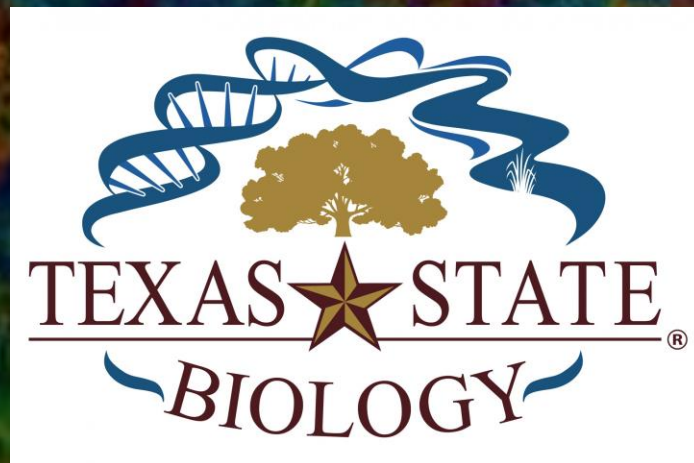


Status of
Year 1
and
Year 2
Refuge Research

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

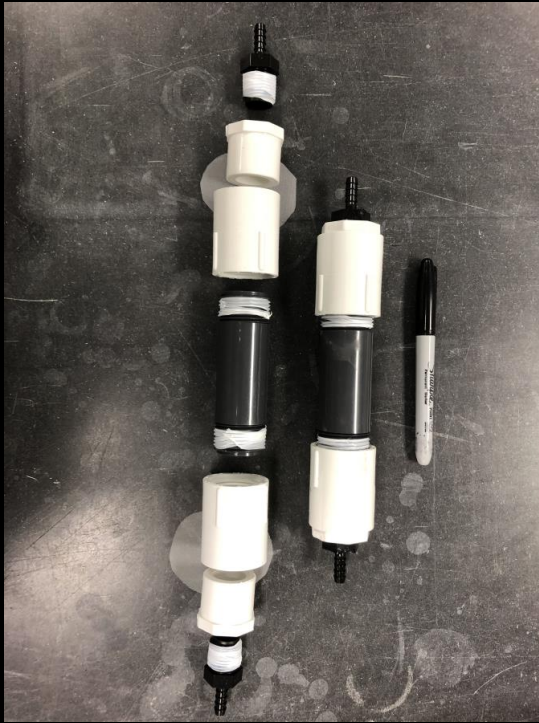
Status of Year 1



- 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



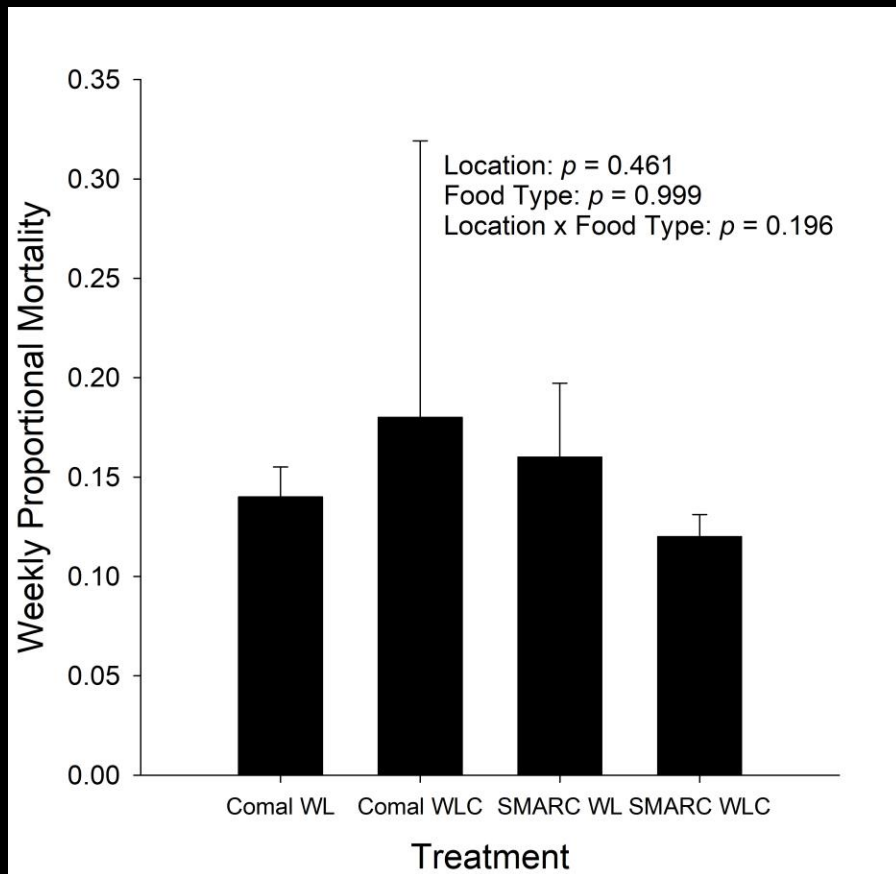
- Treatments replicated 5 times
 - 3 late-stage larvae per tube
- Assessed
 - Larval and pupal mortality
 - Pupation rate
 - Adult eclosion
 - Composition of biofilms (microbial)
 - Nutritional composition of biofilms
 - Carbohydrates, lipids, proteins, C:N



Experiment 1

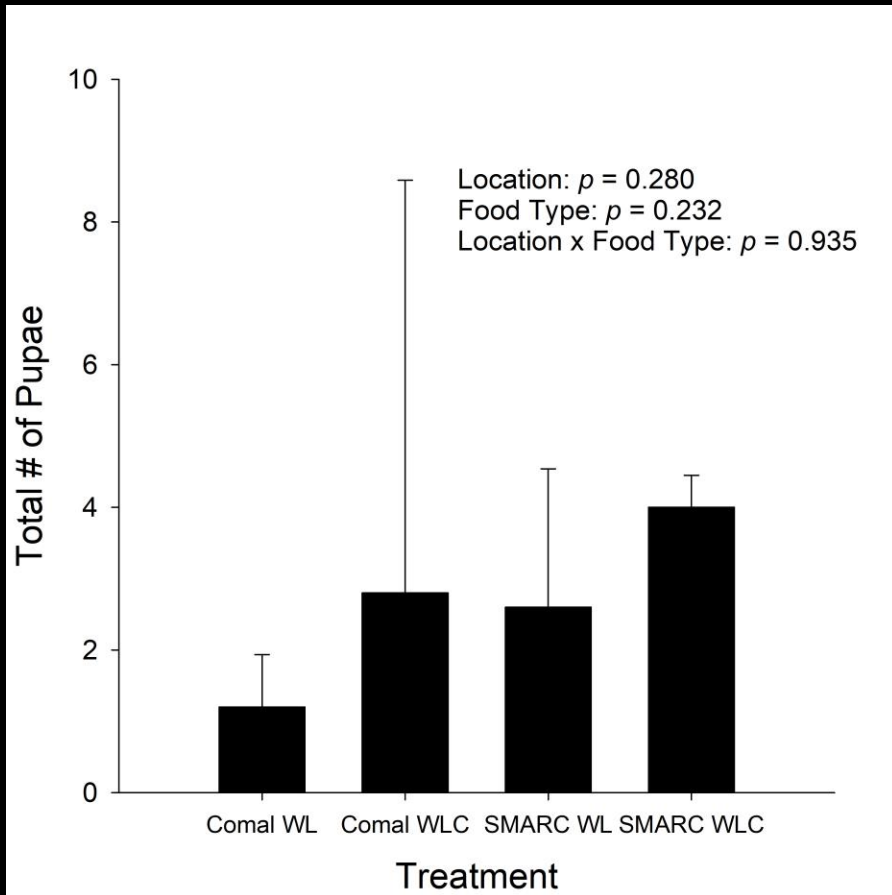
- Summary

- 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



Experiment 1

- Summary



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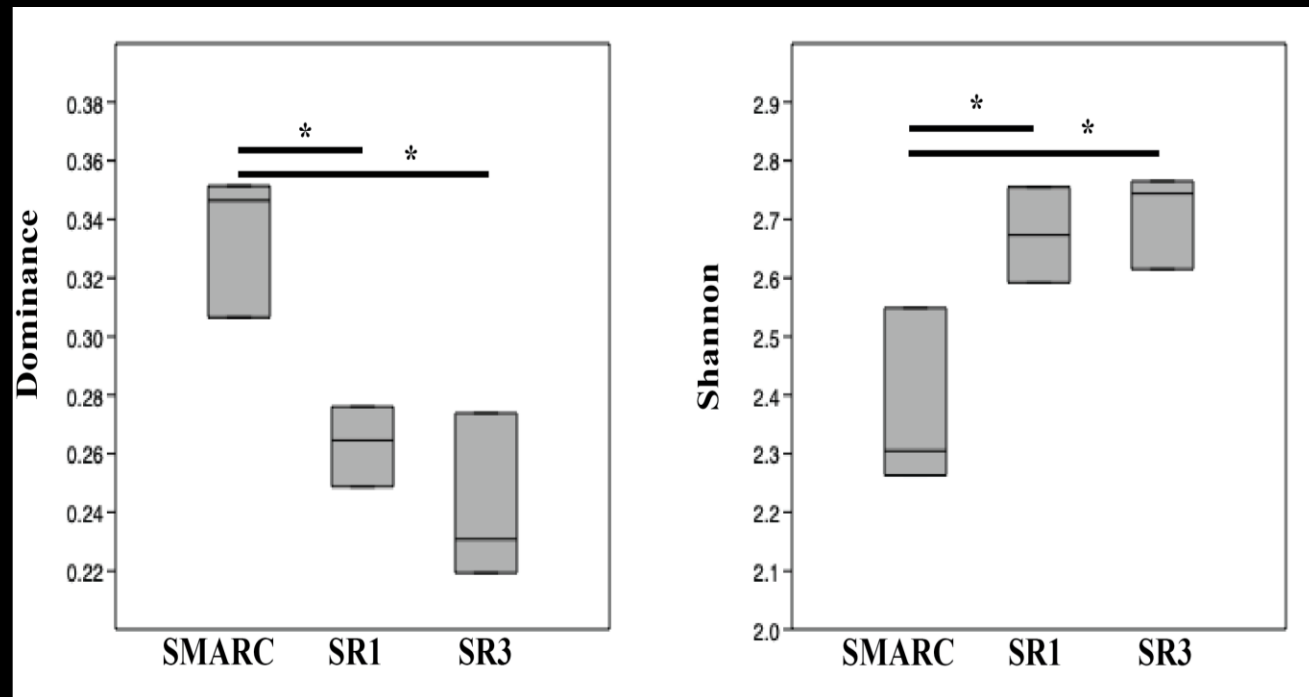
Experiment 1

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

Source	Sum of sqrs	df	Mean square	F	p
Substrate	0.012435	2	0.0062174	1.149	0.0013
Location	0.013812	2	0.0069062	1.2763	0.0001
Interaction	0.002153	4	0.00053825	0.099469	0.1133
Residual	0.070346	13	0.0054112		
Total	0.098746	21			

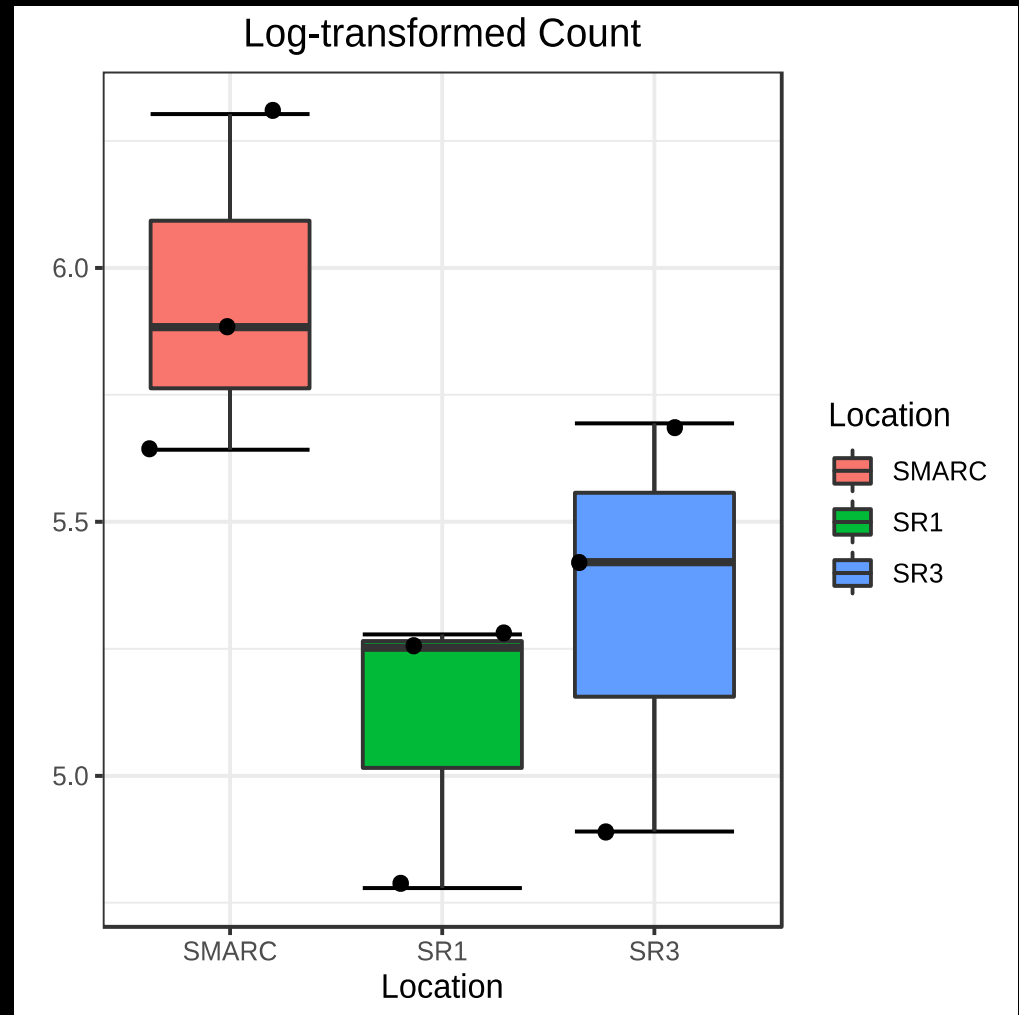
Experiment 1

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

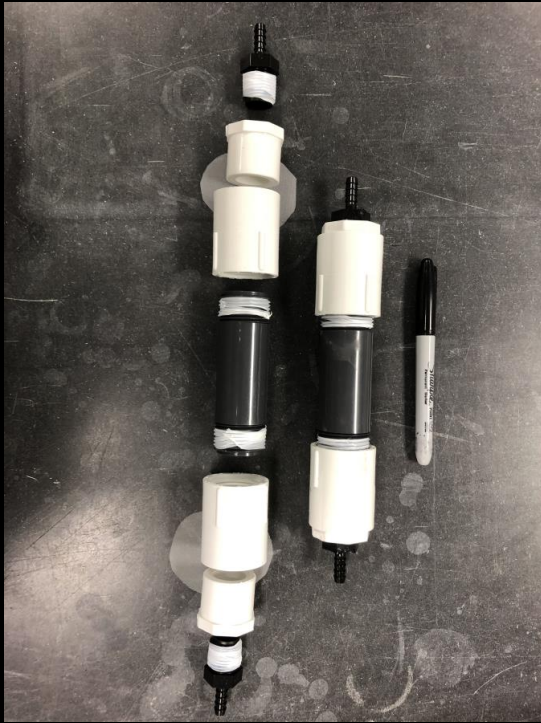


Experiment 1

- *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



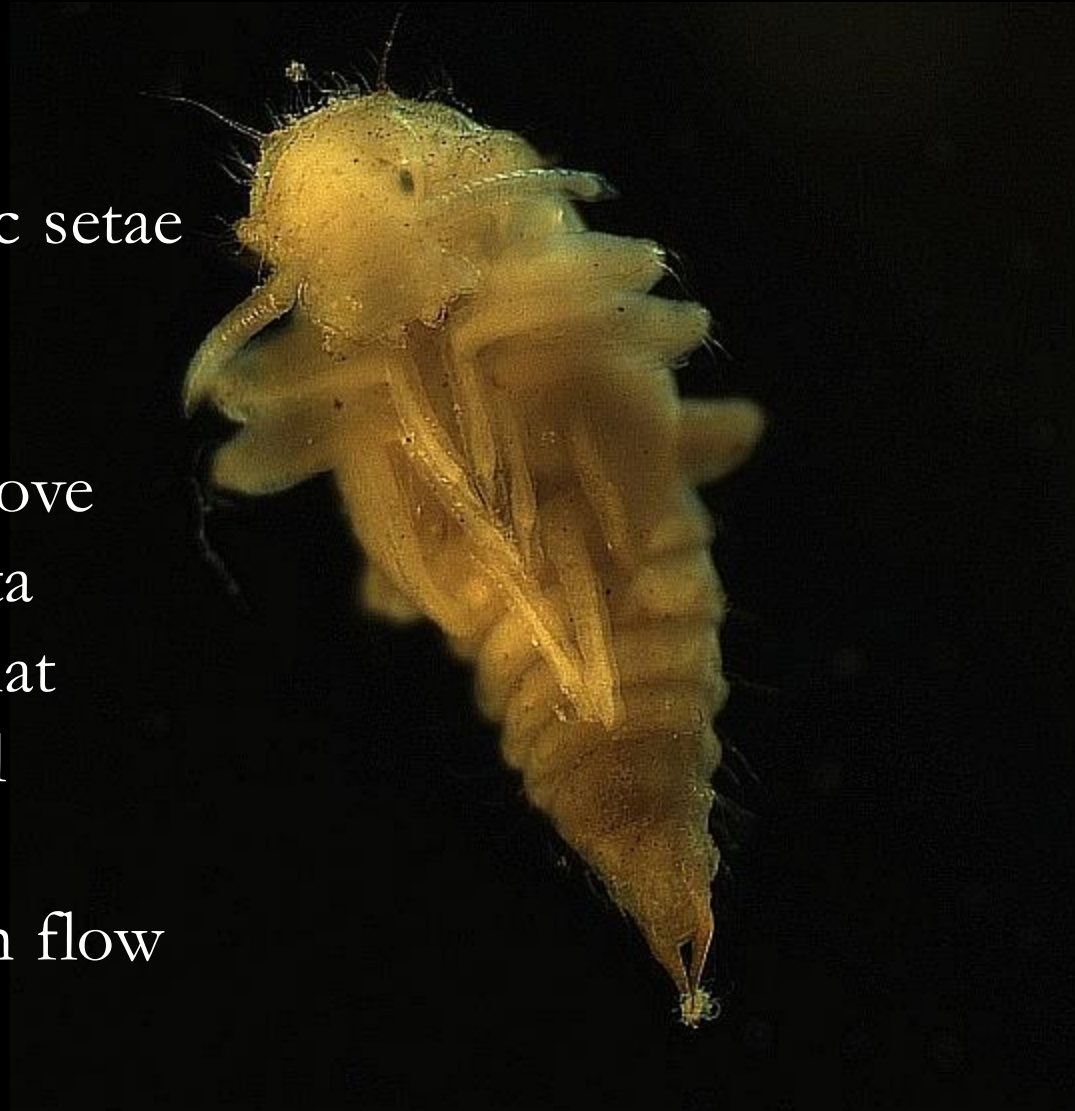
Research in Year 2

- 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



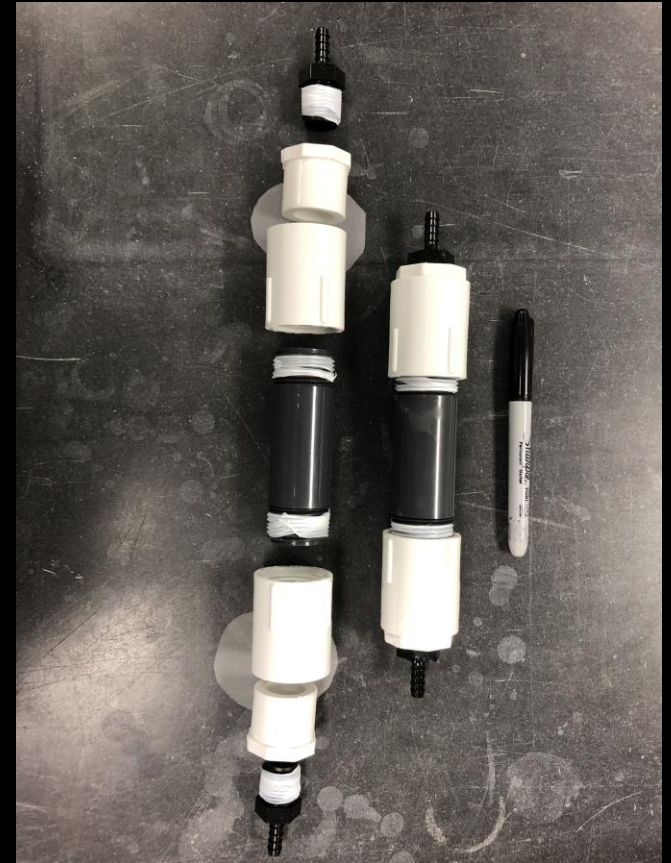
Access to Air – Water Interface

- 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



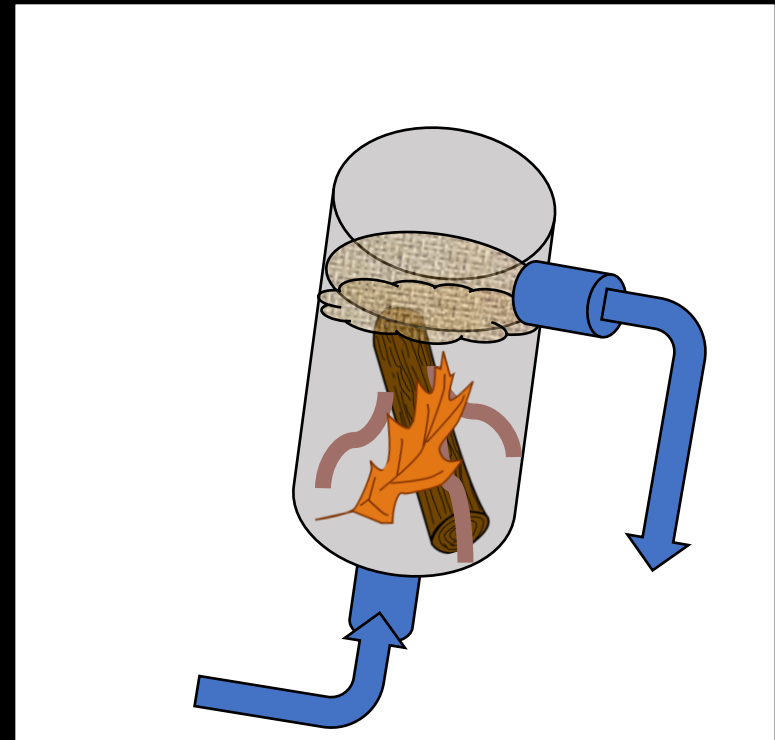
Access to Air – Water Interface

- Experimental design
 - House late-instar larvae in two chamber types
 - Standard flow through 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 through 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





