# House of 1,000 Corpses

## Pesticide Resistance Testing in Suffolk, VA

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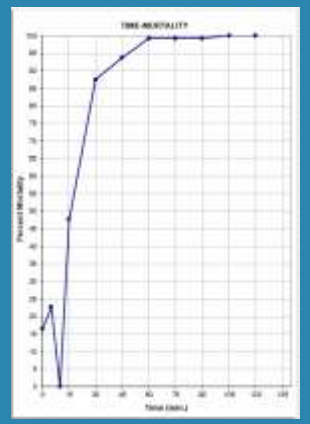


2021 Suffolk In-house Pesticide Resistance Testing Results

(CDC Bottle Bioassay)

Cornell (NEVBD) Pesticide Resistance Testing Results with Inhibitors

(CDC Bottle Bioassay)

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

Exploratory Testing to Determine Optimal Health & Survivability of *Cx. pipiens*

Future Outlook for Pesticide Resistance Testing in Suffolk



# Suffolk In-house Resistance Testing

# *Aedes albopictus*

**In-house Testing Results –**
Chlorpyrifos, Permethrin, Sumithrin

- End of Season 2020 – mortality at diagnostic time

| Chemical Tested | Zone #1 (Downtown 1) | Zone #2 (Downtown 3+4) |
|---|---|---|
| Chlorpyrifos | 100% | 100% |
| Permethrin | n/a | 100% |
| Sumithrin | n/a | 100% |

3 failed tests due to high death in control bottles – depleted stock

- End of Season 2021 – mortality at diagnostic time

| Chemical Tested | Zone #1 (Downtown 1) | Zone #2 (Downtown 3+4) |
|---|---|---|
| Chlorpyrifos | 100% | 100% |
| Permethrin | 96.97%* | 100% |
| Sumithrin | 100% | 100% |

*indicates may be developing resistance

# *Culex pipiens* complex

**In-house Testing Results –**
Chlorpyrifos, Permethrin, Sumethrin

- Mortalities at diagnostic time

| Chemical Tested | Zone #1 (Downtown 1) | Zone #2 (Downtown 3+4) |
|---|---|---|
| Chlorpyrifos | 30 - 74% | Test #1: 38 - 82%<br>Test #2: 54 - 91% |
| Permethrin | Test #1: failed<br>Test #2: 31% | 2 failed tests |
| Sumithrin | 2 failed tests | 2 failed tests |

*Range exists for Chlorpyrifos results where diagnostic times vary for *Cx. pipiens* and *Cx. quinquefasciatus* (unable to morphologically separate in Suffolk)

*7 test failures all due to high death in control bottles – unsure of exact cause(s)

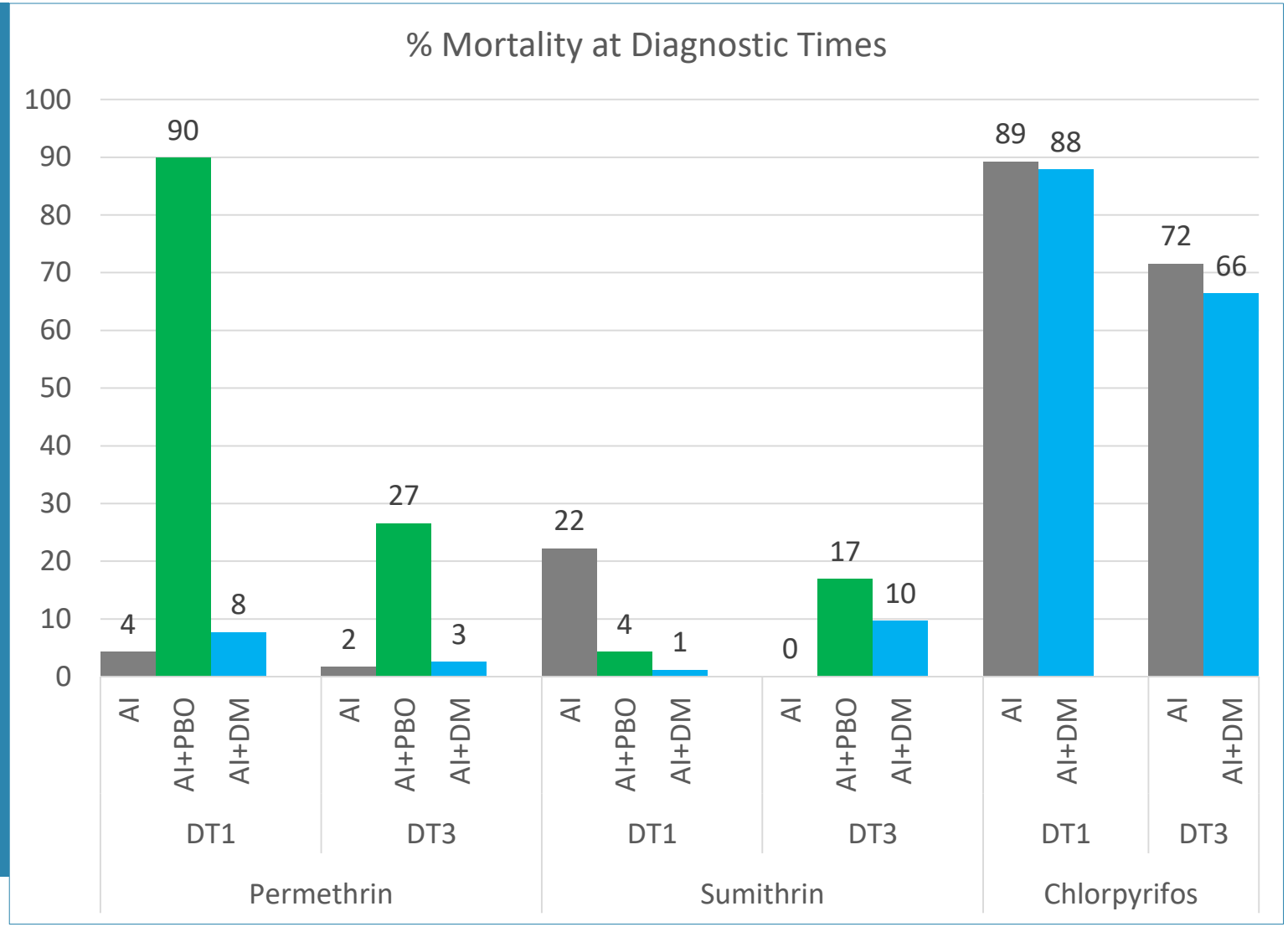
# Submissions to NEVBD-Cornell for Resistance Testing

# *Culex pipiens* complex

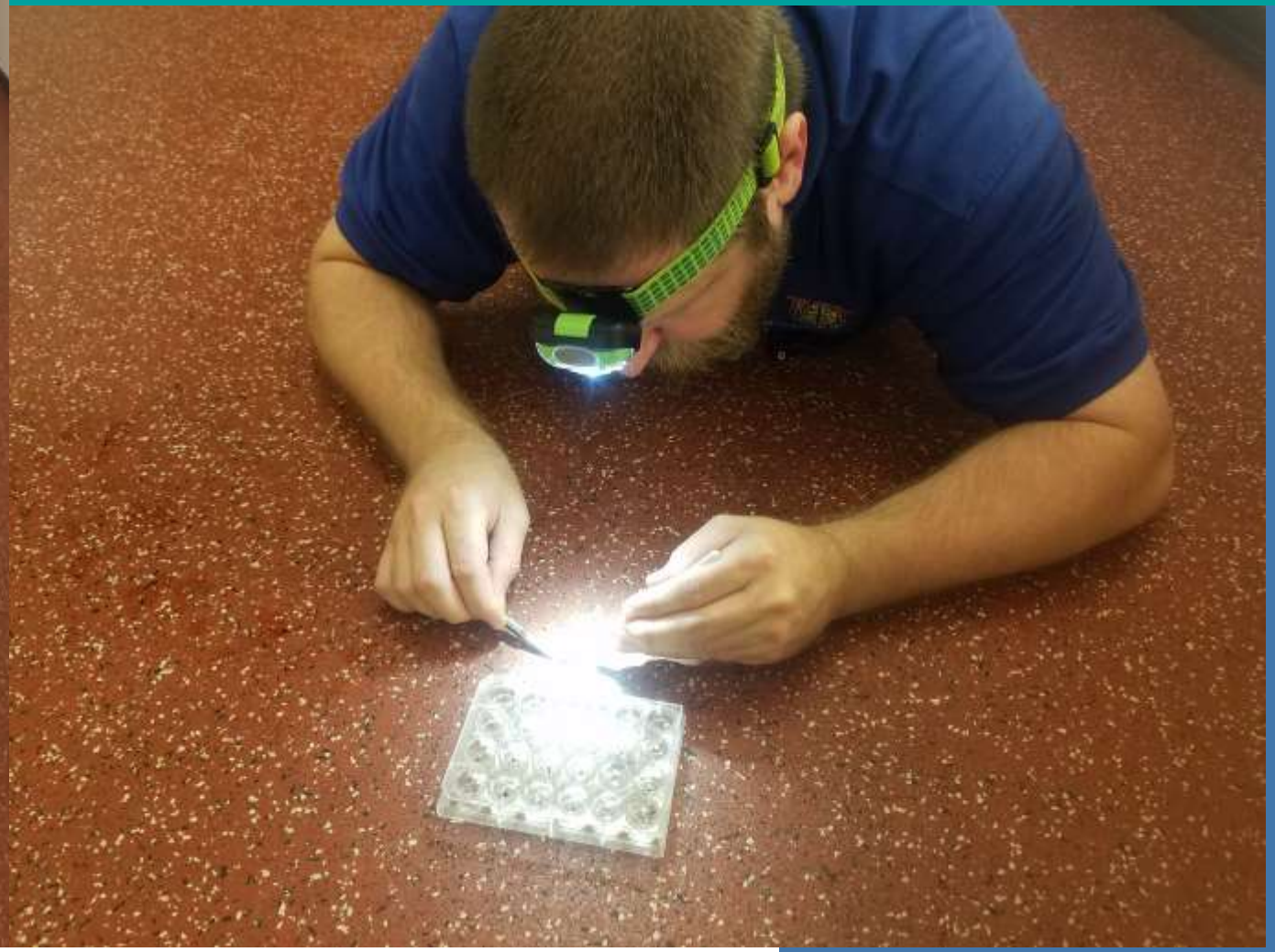
Cornell-NEVBD Results –

Permethrin and Sumithrin with inhibitors Piperonyl butoxide (PBO) and Diethyl maleate (DM) + Chlorpyrifos with DM

- Sent 40 egg rafts (20/zone)
- Performed 16 tests: greater success than Suffolk in-house (11 tests: 4 successful, 7 failures)
- High resistance to pyrethroids
- Low resistance to organophosphate
- <u>Increase in mortality with addition of PBO</u> (except Sumithrin in DT1 zone) indicates resistance to pyrethroids is due to metabolic mechanism related to oxidase activity
- DM did not have an effect on either chemical class



# Exploratory Testing to Determine Optimal Conditions for *Cx. pipiens* Health and Survivability



# Preliminary data from 4 factors potentially affecting survival

## Egg raft/Larvae Crowding

2 replicates

3 : 5 : 7 rafts per larval tray

## Adult Sugar-feeding Options

3 replicates

sucrose-soaked cotton balls

sucrose wicked via paper towel

suspended sugar cubes

## Pupal Separation Techniques

4 replicates

Ice water (Kauffman et al. 2017)

Pipette (turkey baster) removal

## Pupae/Adult Crowding

4 replicates

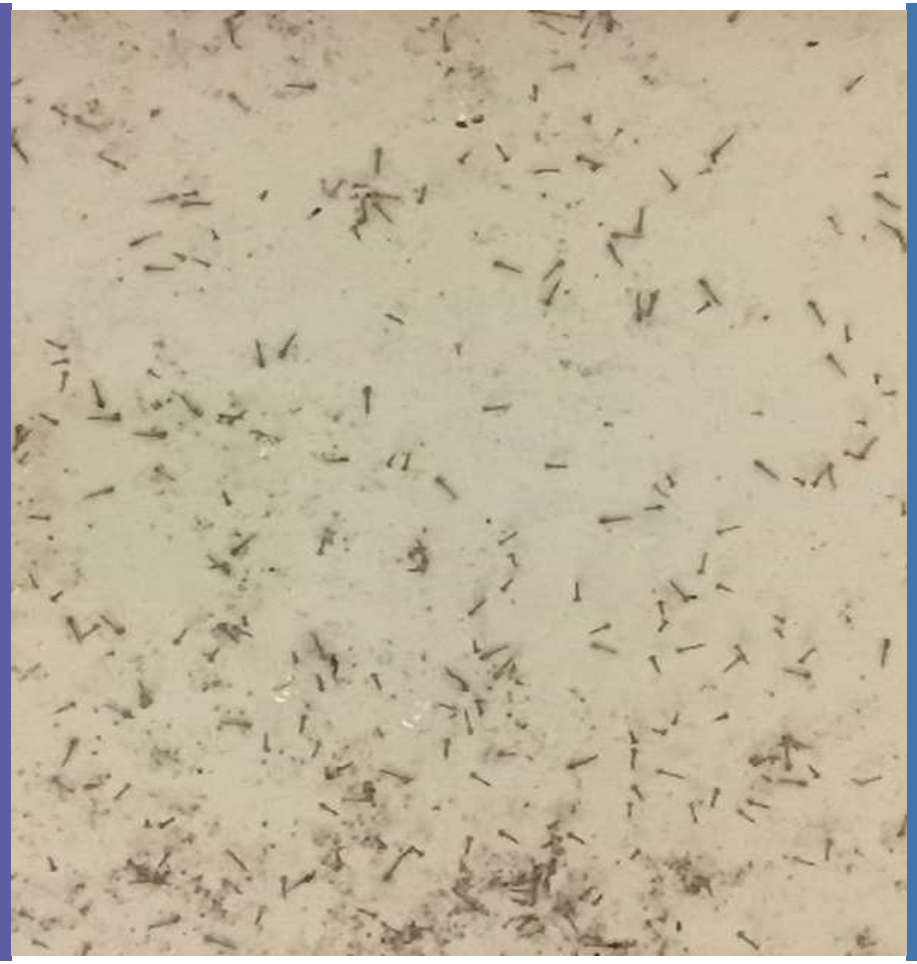
300 : 600 pupae per rearing cup/dorm

# Egg raft/Larval Crowding

**Question: Are we putting too many egg rafts in a tray resulting in overcrowding/malnutrition and subsequent low pupal growth rates?**

## Methods

- Field-collected egg rafts*
- Verified *Cx. pipiens* as 1st instar
- 6 trays** total : 2 trays each with 3, 5, and 7 egg rafts
- 2 L of filtered tap water
- Food: 200 mL of liver powder-water mix and pinch of fish food flakes on Day 1, 3, 6***
- Kept at room temperature (approx. 24°C)****



## Results

Expected number of pupae grew in each tray without significant differences (average of 100-200 pupae per raft)

**Takeaway message:**
We do not need to limit to <7 rafts per tray. They have plenty of space and food to develop into pupae.

*confirm collection containers are not contaminated with pesticide
**18” x 12.5” x 2”
***in the past we may have only fed 2x
****room temp questionable – office building hvac shuts off on weekends sometimes

# Adult sugar-feeding options

**Question: Is our sugar-feeding method suboptimal?**

## Methods

- 9 dorms total : 6 large (24” cube-tent), 3 small (12” cube)
- 300 pupae added to each dorm
- All dorms given water-soaked cotton pads**
- Kept at room temperature, covered with towels (wet daily) for increased humidity
- Recorded # living adults on Day 5 post-emergence
- Tested physiological health of adults by exposing to acetone-coated bottles (mimic PR controls) on Day 5

## Results/Observations

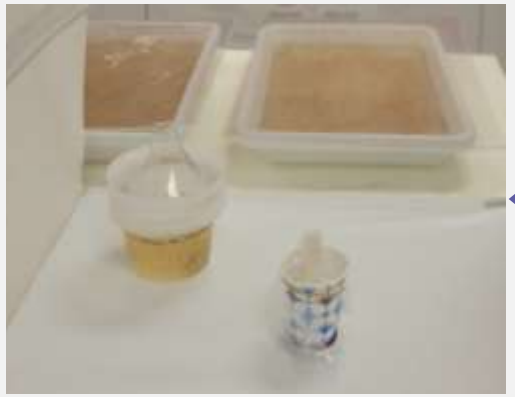
- Observational notes: observed large number feeding on cup with wicking paper
- No significant difference in living adults on Day 5 between sugar methods
- No significant difference in ability to survive control bottles between sugar methods

HOWEVER…

*10% sucrose changed from previous years’ 5% concentration

**additional measure not used in past years

*10% sucrose-soaked cotton balls



*10% sucrose in cup wicked via paper towel

Sugar cubes suspended in mesh bag

# Adult sugar-feeding results

- Significantly more living adults on Day 5 in small cubes ($p = 0.01$)
- Ability to survive in control bottles not significantly different ($p = 0.08$) <u>BUT</u>

- Difference in humidity levels : large tent (70-75%) vs. small cube (81%)
- Smaller area = easier aspiration/observed more feeding

**Significant differences between dorm types: large tent vs. small cube**


Takeaway message:
Need larger sample size, but we feel that the smaller cubes and the cup with wicking paper is best

# Pupal Separation Techniques

**Goal: Assess the most efficient way to separate pupae from larvae in rearing trays without affecting survivability and adult health**

## Pipette (turkey baster) removal

- One by one; for higher pupae:larvae ratio, use the swirl method (above)
- Hypothesized minor mechanical stress to pupae/larvae
- can be **VERY** time-consumptive

**Methods**

8 trays:
4 pipetted, 4 iced
into
8 dorms
300 pupae each
4 pipetted, 4 iced
Water
soaked cotton pads
Sucrose
wicking paper cup
Room temp
Humidity
via wet towels
Recorded
adults on Day 5 & in control bottles

## Ice water method

- Described in Kauffman et al. 2017; pupae float, larvae sink
- Hypothesized more stressful – temporary (but severe?) physiological shock
- Significantly faster than manual removal, especially in situations with low pupae:larvae ratio

# Pupal Separation Techniques Results

- No significant difference in living adults on Day 5
- No significant difference in ability to survive control bottles
- Significant time difference
  - Pipette 1 tray = 30 min-1 hr
  - Ice water 1 tray = 5 min

Takeaway message:
Since neither method seems to affect survival,
ice water is better logistics

# Pupae/Adult Crowding

**Goal: To determine optimal amount of pupae per cup and subsequent hatched adults per dorm to avoid overcrowding**

*Cx. quinquefasciatus* pupa by: Stephanie Hill, UF

**Standard rearing cups in 12” cubes**

**4 dorms with 300, 4 dorms with 600 pupae in a cup**

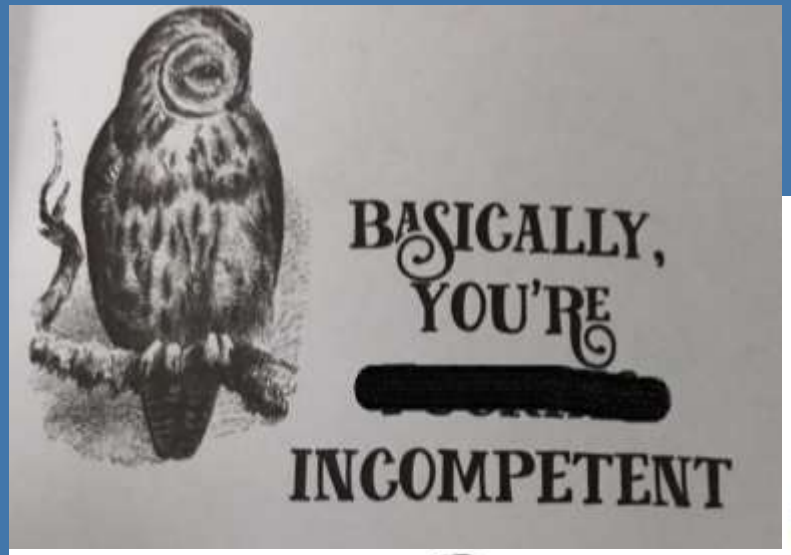
**Water, sucrose, temp, and humidity as previous exp**

**Recorded adults & control bottle survival on Day 5**

# Pupae/Adult Crowding Results

- No significant difference in living adults on Day 5
- No significant difference in ability to survive in control bottles

Takeaway message: Up to 600 pupae in a standard rearing cup/600 adults in a 12” cube does not seem to result in overcrowding that affects hatch/survival rates





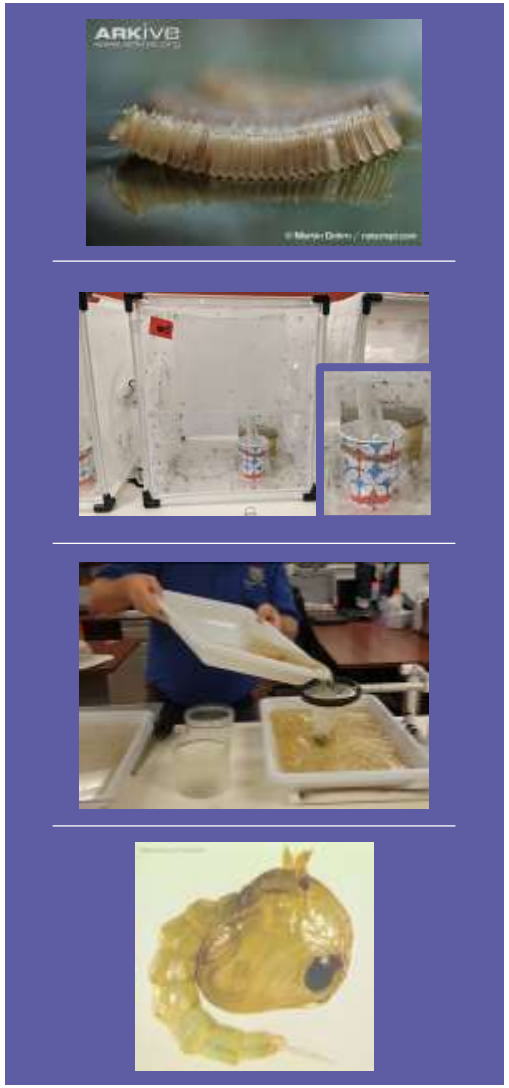



# Conclusions and future directions?


Screenshot sentiments from Reynolds, Aaron (2019). *Effin' birds*. Berkeley, CA: Ten Speed Press. & on IG @EffinBirds

# What (we think) we have learned

Notes:
Small sample sizes,
Temp & Humidity
control is questionable




Our protocol of <u>7 egg rafts per larval tray</u> does not seem to negatively affect pupal development

<u>Any of the 3 sugar-feeding methods</u> is ok; however, wicking paper in cup seems best.

<u>Smaller cubes are better</u> than large tents.

<u>Either method of removing pupae</u> is ok; however, ice water method is much faster.

<u>600 pupae per cup/dorm</u> does not result in overcrowding - low hatch rates or poor adult health

# New equipment and considerations

- Use small cube dorms
- Stick to 7 egg rafts per larval tray and 600 pupae per cup/dorm to avoid overcrowding
- Provide water pads and 10% sucrose solution in wicking cup
- Send more to NEVBD
- Purchase (hopefully) an incubator for better temperature and humidity control
- Use deionized water
- Test on Day 3 or 4 instead of 5
- Try different aspirator(s)

# Thank you!

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