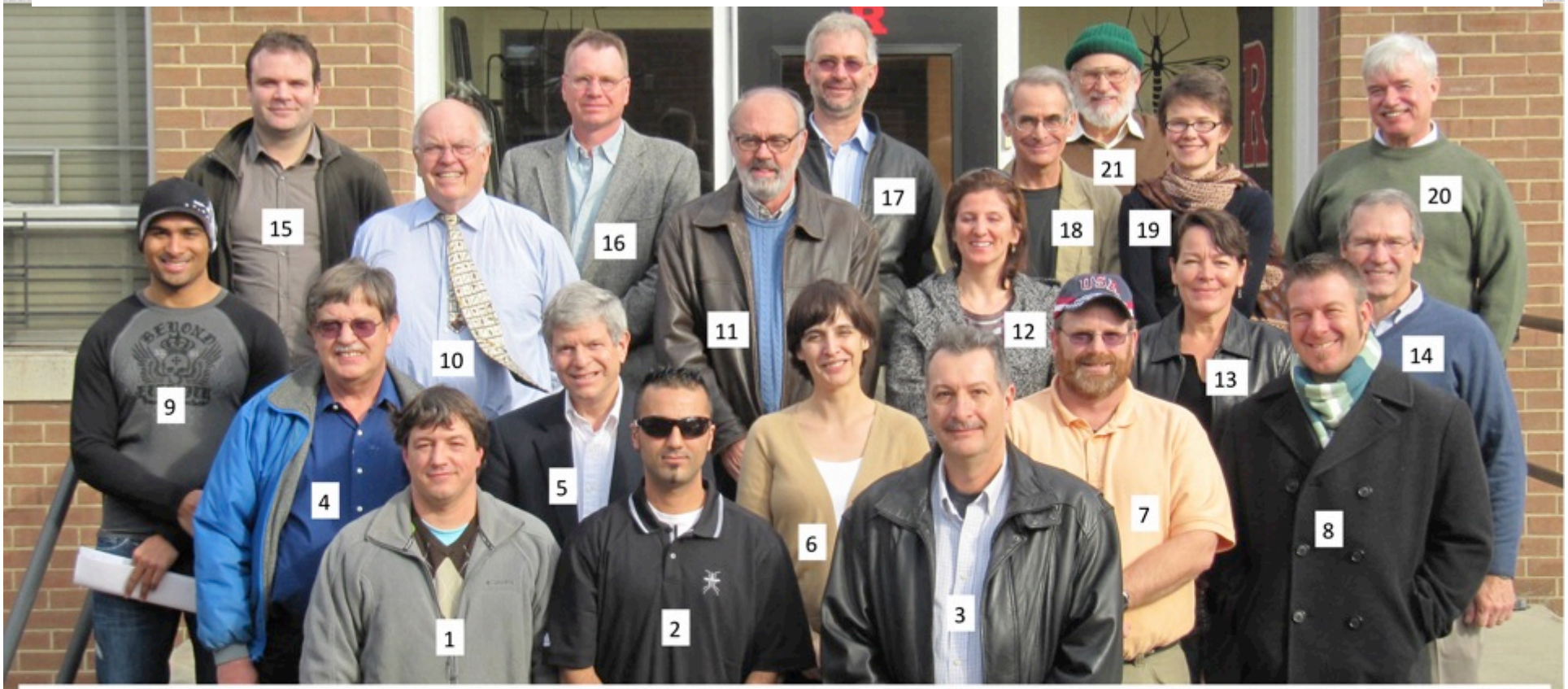
THE PROS AND CONS OF EGG COUNTS

Dina Fonseca, Rafael Valentin,
Taryn Crepeau, Sean Healy,
Ary Farajollahi, Isik Unlu

78th Annual Meeting of the American Mosquito Control Association
March 1, 2012
Austin, Texas

The ATM team

Study integrated in the Area-wide ATM Management Project



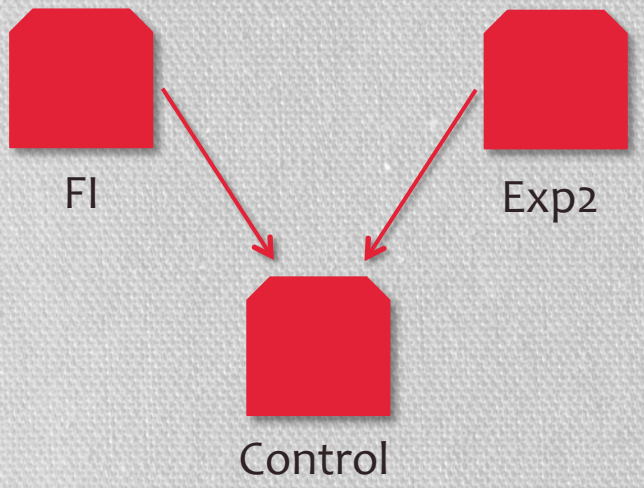
January 6th 2012 ATM Review Board Meeting Participants:

1 Sean Healy , **2** Ary Farajollahi, **3** Dominick V. Ninivaggi, **4** Dan Kline, **5** Don Shepard, **6** Dina Fonseca, **7** Scott C. Crans, **8** Greg Williams, **9** Rafael Valentin, **10** Graham White, **11** Gary Clark, **12** Isik Unlu, **13** Dawn Wesson, **14** Roger S. Nasci, **15** Sebastien Marcombe, **16** Douglas Burkett, **17** Karl Malamud-Roam, **18** Daniel Strickman, **19** Emily Zielinski-Gutierrez, **20** Randy Gaugler, **21** John Petersen

Not shown: Kristen Bartlett-Healy, George Hamilton, Taryn Crepeau, Yara Halasa, Eve Wittenberg, Mike Hutchison

Area-wide ATM Management Project (USDA-ARS)

- Surveillance
- Source reduction
  - Door-to-door control
  - Education
- Larvicides, Adulticides
- Economic Analysis



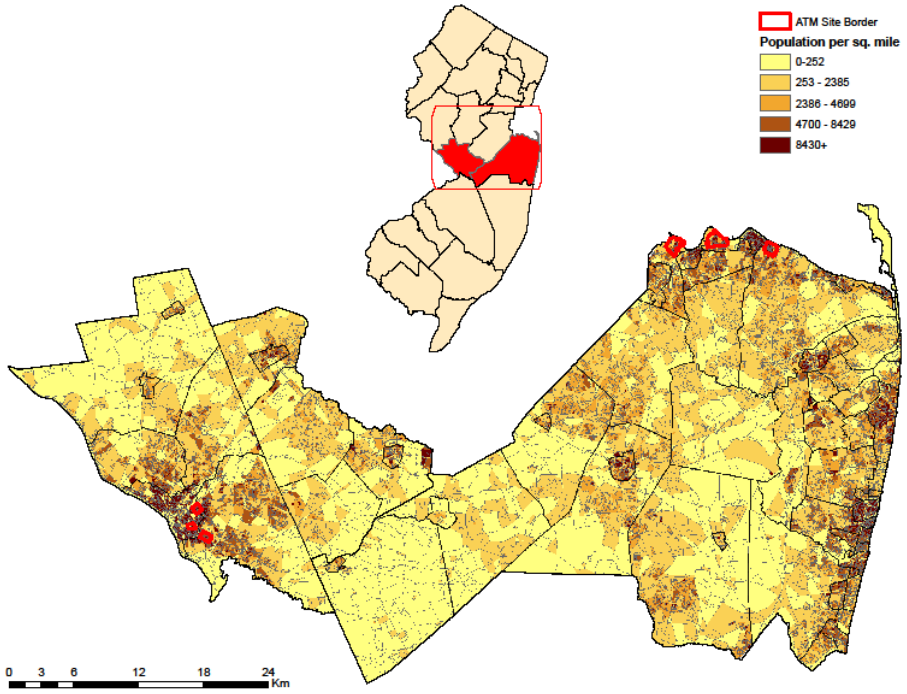
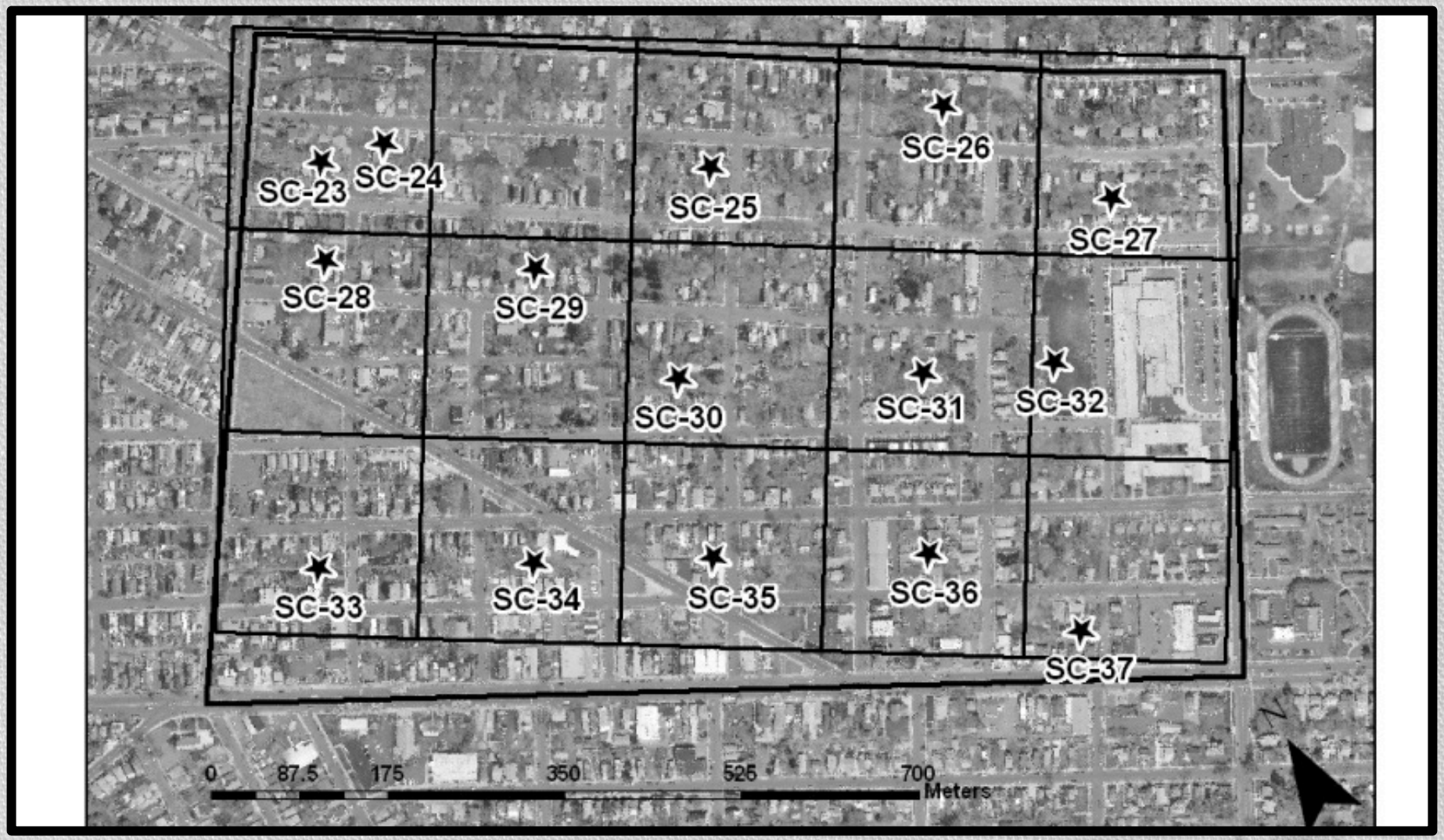
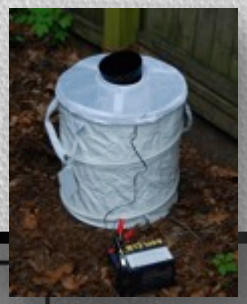


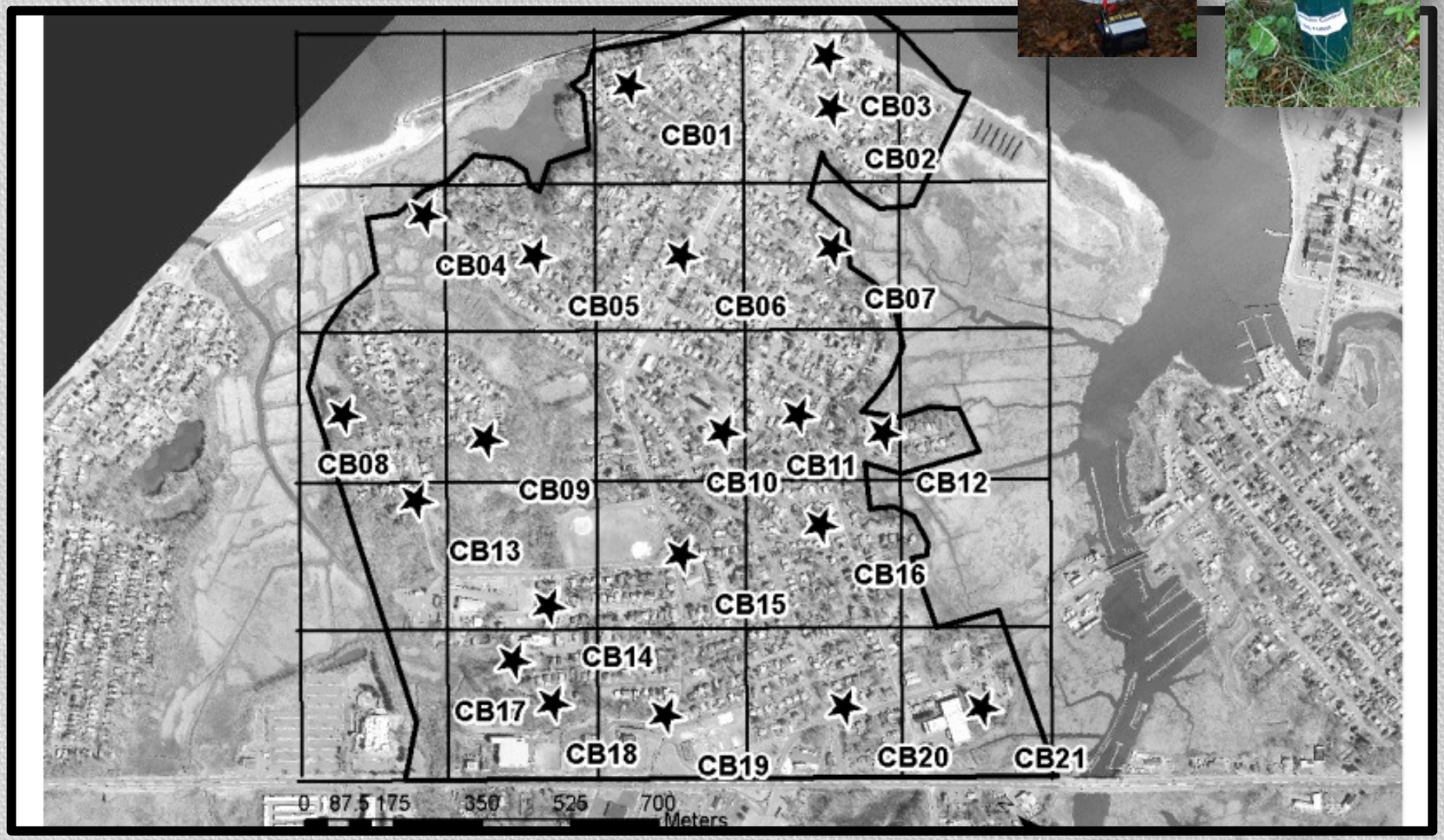
Figure 1: Relative locations of the two counties and sites within each county. Only the 3 experimental sites in each county examined in 2009 (Year 2) are depicted.

Mercer sites



Monmouth sites





Ovitraps

- 400 ml dark green plastic cemetery vases (Eaton Brothers Corp., Hamburg NY)
- Germination paper (brown, textured)
- 300 ml of oak leaf infusion (Trexler 1998)
  - 5 g of dry oak leaves per 8 L of water
    - White oak, *Quercus alba*
    - Trash cans with tap water
  - 1-2 week infusion was used
  - New batches were prepared every 2 weeks

1. Ovitraps were left out for about 1 week

(all egg counts are corrected to 7 days)

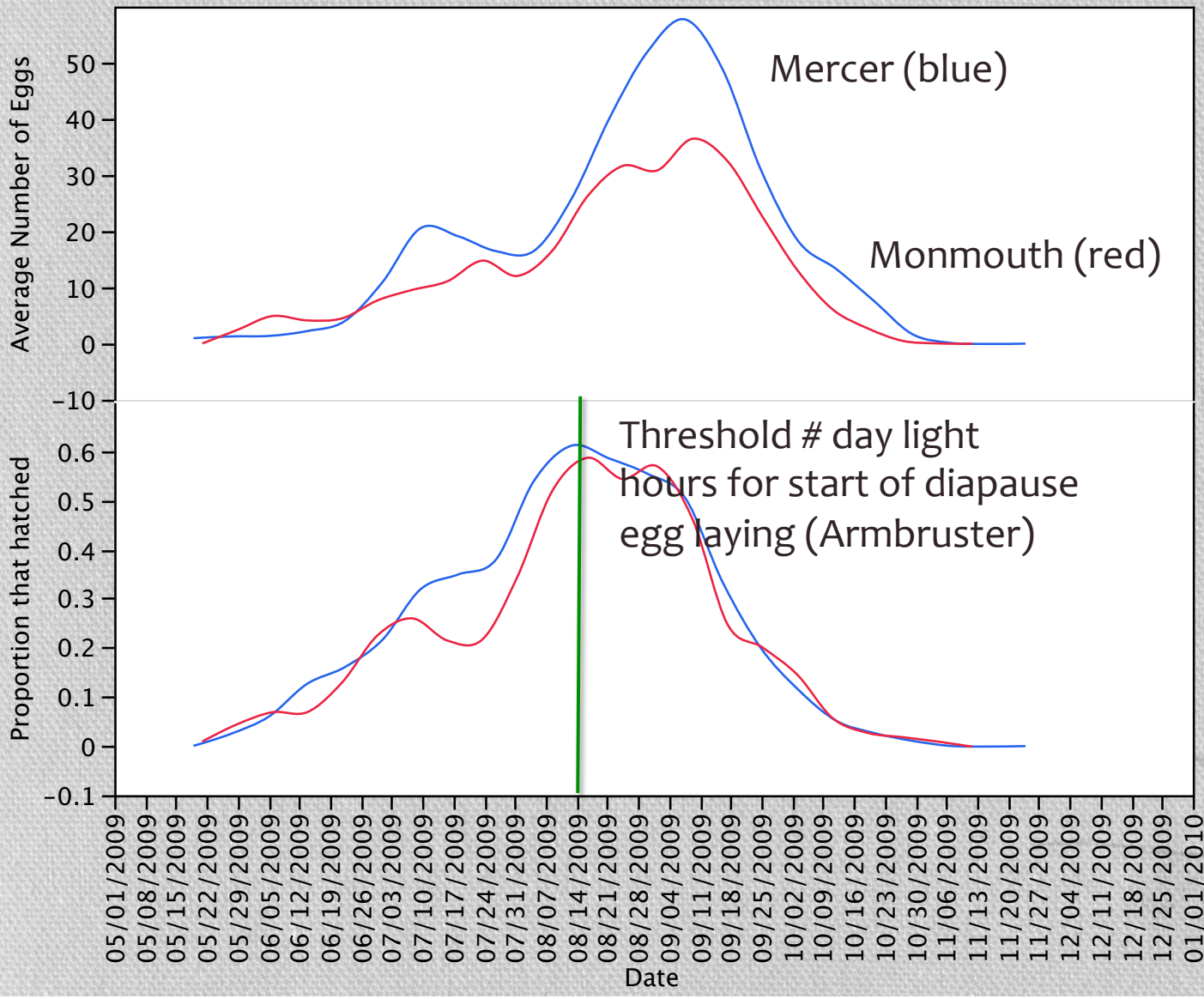
2. Upon collection egg papers were placed in zip-lock bags and taken to lab for processing

3. New germination paper and fresh infusion added to cups

Egg processing

- Eggs were counted under a dissecting microscope
  - >90% of the times all eggs were counted
  - If more than 500 eggs present we subsampled (average of 3 replicates)
- In 2009 egg papers were placed in plastic 500 ml containers, flooded with tap water and kept at 27°C for up to 1 week.
  - Hatching stimulus: 5 mg of ground rat chow/500 ml of tap water
  - 4^{th} instar larvae were identified to species (Farajollahi and Price pictorial key)
- In 2010 we noticed very low hatch rates, especially in Mercer sites
- Developed a TaqMan (qPCR) rapid assay to ID eggs.
- In 2011 after eggs were counted we collected 3 groups of 10 eggs randomly from each egg paper.
  - Eggs were immersed in DNA buffer solution (T.E) and frozen

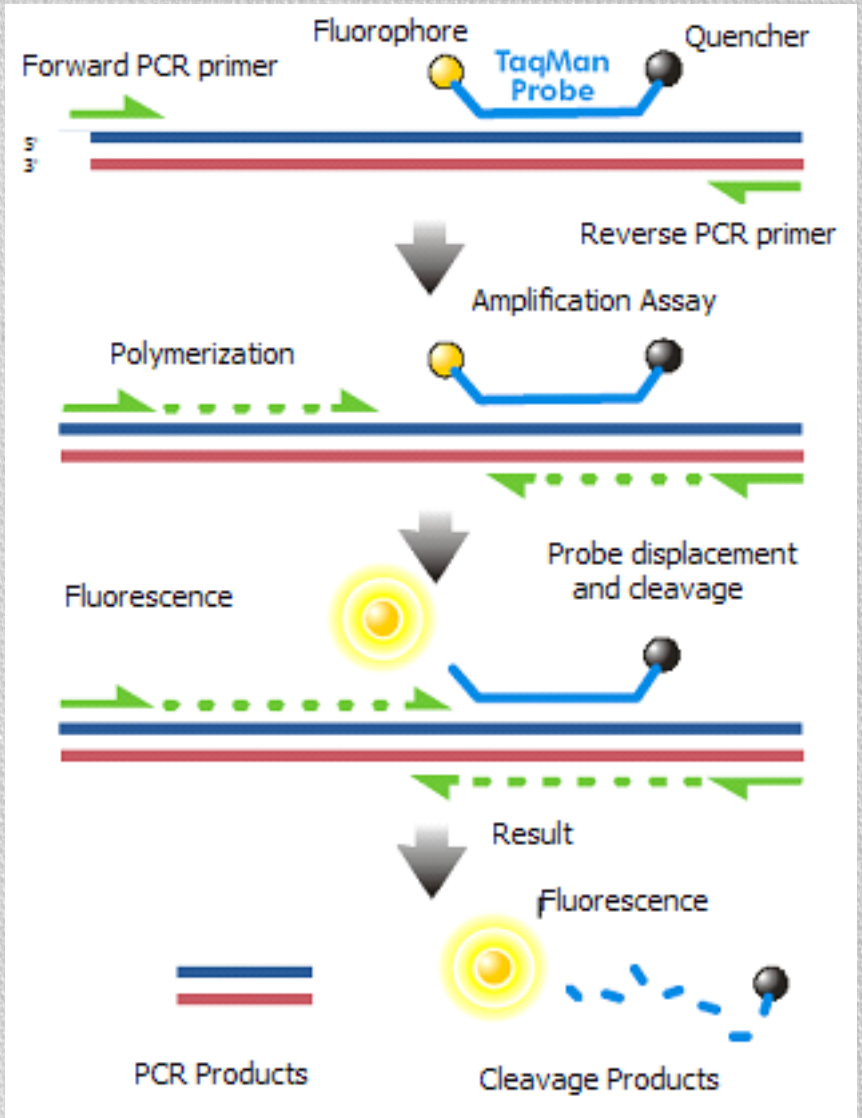
Low hatch rates – CON and PRO
INFO on onset of diapause - PRO



AVERAGE #EGGS/TRAP /7DAYS

HATCH RATE

TaqMan Rapid assay



- Very high specificity
- Can be multiplexed
- Because eggs are discrete units this assay is being optimized semi-quantitatively
  - i.e. how many eggs of *Aedes triseriatus* vs. *Ae. albopictus*
  - All eggs are allowed to embryonate before collecting, to decrease variance

TaqMan Rapid assay

- Has been developed to identify all species of *Aedes* that commonly lay eggs in containers similar to the ovitraps.
  - ✓*Aedes albopictus* (used primers and probes from Hill et al AJTMH 2008)
  - ✓*Aedes japonicus*
  - ✓*Aedes triseriatus*
  - ✓*Aedes atropalpus*
  - ✓*Aedes aegypti* (used primers and probes from Hill et al AJTMH 2008)

- *Aedes hendersoni*
- *Aedes epactius*

- ✓*Aedes koreicus*

TaqMan Rapid assay

To cut costs:

1. **Rapid DNA extractions (boil+freeze+spin)**
2. **High sensitivity:** detects a single egg of *Ae. japonicus* in 1,000 eggs of *Aedes albopictus*
3. **Multiplexed** (up to 3 primer/probe combinations)
   - *Ae. albopictus* + *Ae. japonicus*
   - *Ae. albopictus* + *Ae. triseriatus*
   - *Ae. aegypti* + *Ae. atropalpus*
     - These can be juxtaposed with *Ae. koreicus*
4. **1 replicate (instead of 3)**
5. **Maximum likelihood approach** (similar to virus testing – MLE)

Egg Diversity in ATM project sites (2009)

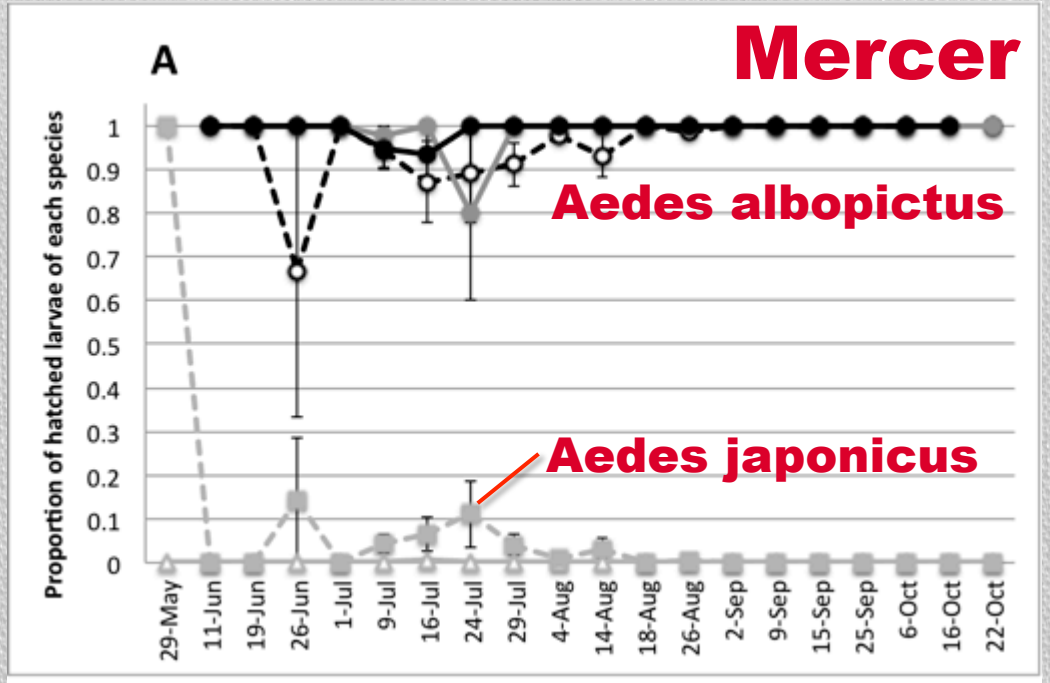
Aedes japonicus
Aedes triseriatus

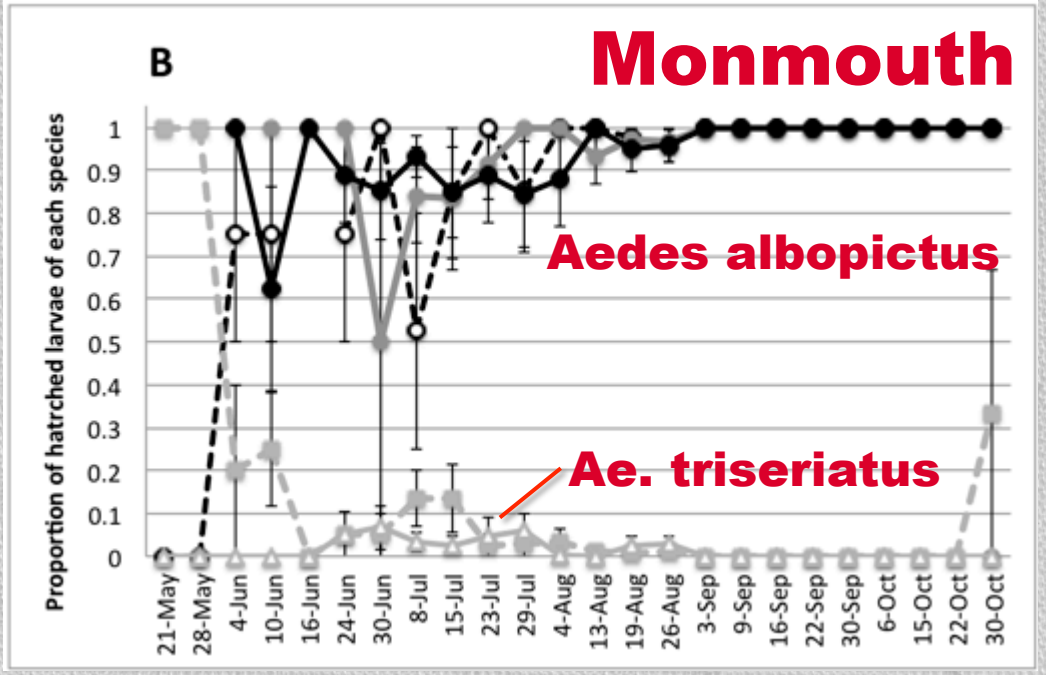
No *Ae. atropalpus*

Highest species richness occurs before early August

Highest species richness in Monmouth (suburban)

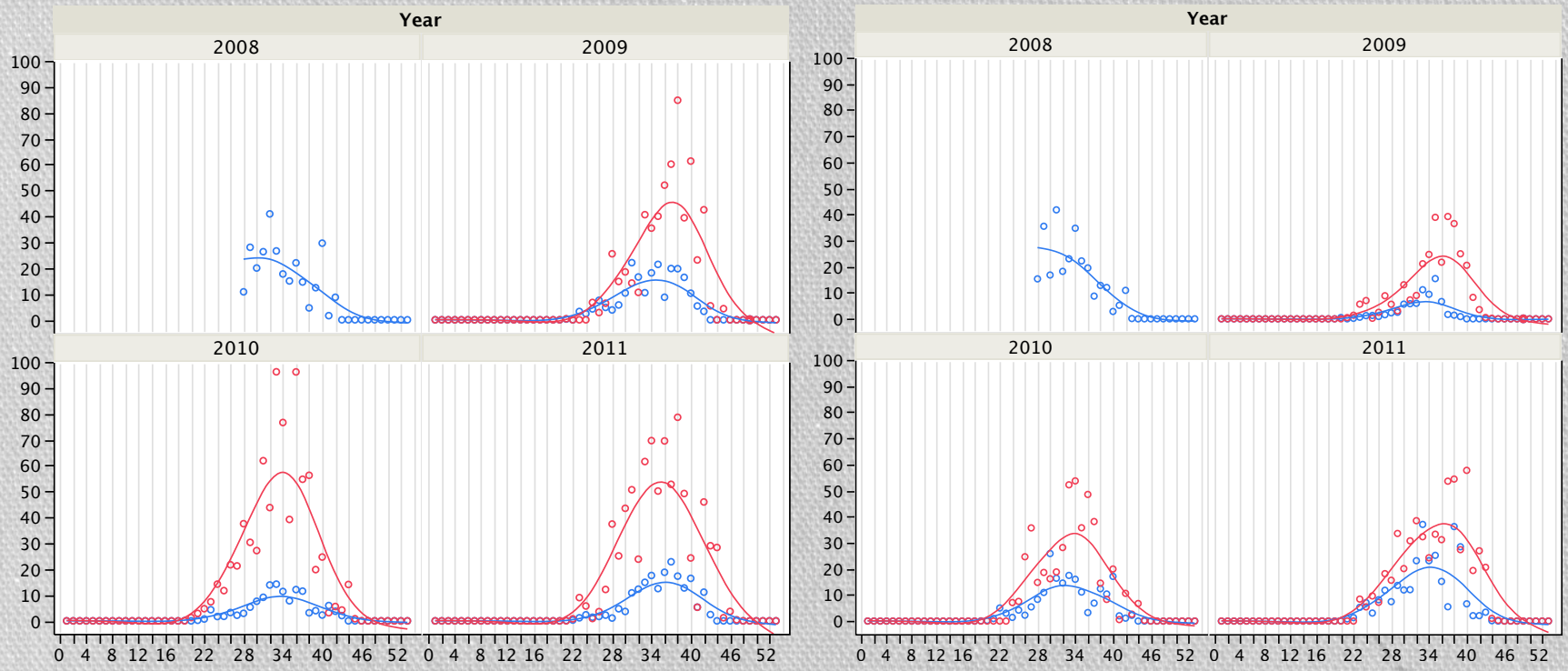
Mercer egg catches are overwhelmingly composed of *Aedes albopictus*





How do egg catches predict adult populations?

Mercer – Untreated site only - Monmouth



Julian week

Mean # adult ATM/trap/day

Mean # eggs/trap/7 days

How do egg catches predict Adult ATM populations?

Summary of Fit for Mercer Untreated site 2009-2011

R^2 **0.72**

Observations (or Sum Wgts)155

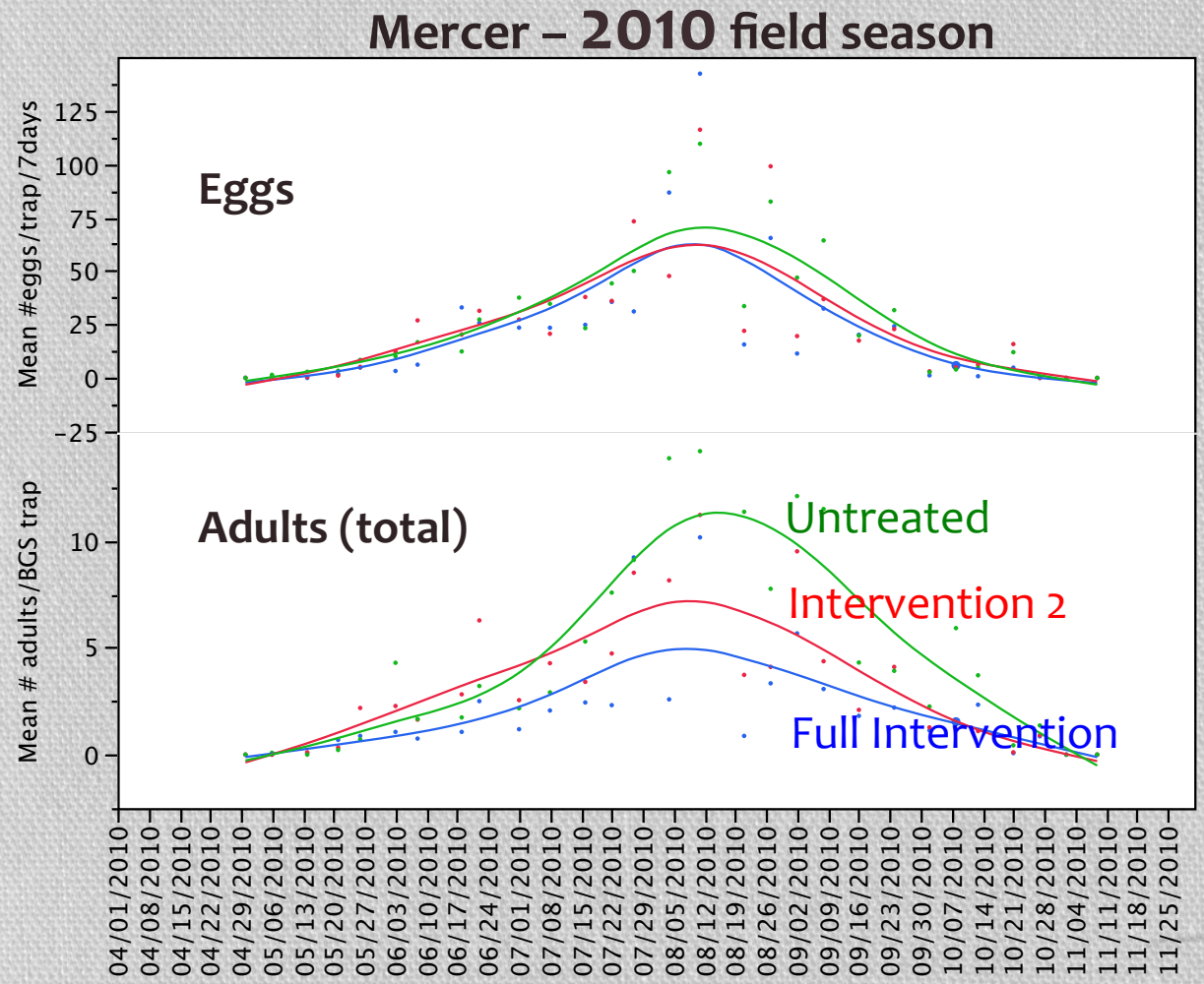
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	
Model	6	3792.04	632.01	64.34	
Error	148	1453.8	9.8		**Prob > F**
C. Total	154	5245.8			<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Eggs	1	1	576.3	58.7	<.0001*
Mean T Trent	1	1	113.8	11.6	0.0009*
Winter Temp	1	1	85.9	8.7	0.0036*
Mean H Trent	1	1	72.5	7.4	0.0074*
CumDD	1	1	53.4	5.4	0.0210*
Mean T Trent*CumDD	1	1	85.7	8.7	0.0037*

Do egg catches reflect accurately the results of interventions?



No.

Not always

Why?

Skip oviposition (most likely)

Less of a problem in areas with lower rates of oviposition

Conclusions

Cons

1. **Egg ID is cumbersome**
   - **Low hatch rates**
   - **Time and space intensive**

     Taq Man assay can help but facilities and funds need to be available
2. **Egg catches may not reflect Intervention efficiency**

Pros

1. **Cheap and easy – 95.9% of hatched eggs in NJ were ATM**
2. **Higher catches = higher statistical power**
3. **Information on field egg mortality rates**
4. **Information on onset of diapause**
5. **Egg catches** corrected by Cumulative DD, Weekly Temperature, Winter Temperature, and Weekly Humidity **explain 72%** of the variance in ATM adult catches in **Untreated sites.**

Expansion to other counties and states

- Established collaborations with mosquito control programs in
  - Bergen County, NJ (Warren Staudinger) — 3 sites
  - Pennsylvania (Mike Hutchinson)
    - York (Andrew Kyle) — 1 site
    - Harrisburg (Matt Helwig) — 1 site
    - Philadelphia (Andrew Kyle) — 2 sites
  - Virginia
    - Portsmouth (George Wojcik) — 4 sites
  - Louisiana
    - New Orleans (Dawn Wesson) — 4 sites
    - St. Tammany (Chuck Palmisano) — 2 sites
  - Florida
    - St. Augustine, Florida (Rudy Xue) — 2 sites

Total = 19 sites

>400 egg papers/week