Vertical Distribution of Container-Inhabiting *Aedes* in a La Crosse Endemic Area: A Comparative Approach

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Undergraduate Program
La Crosse Encephalitis

- Most common human arboviral disease in NC
- Infections greatly under-recognized (1:150-300)
- Disease most prevalent in children (<15 yrs)
- Western NC counties have the largest burden of LACE
- Primary research focus of the WCU Vector-borne Infectious Disease Lab

La Crosse Encephalitis Cases (NC: 1988-2011)

2005
35 CASES – 5200 INFECTIONS/EXPOSURES?

*Fatal Cases
Aedes triseriatus

LACV Primary “natural” vector: Eastern Tree-hole Mosquito
Sister species: Aedes hendersoni
Background

- *Aedes triseriatus* is the **primary vector** of LACv
- *Aedes hendersoni* does **not vector** LACv
- Accurate identification is required for proper surveillance and public health efforts
- Identification of *Aedes triseriatus* and *Aedes hendersoni* is difficult
- These two species are known to hybridize!
INVASIVES: Secondary/Suspect Vectors

**Aedes albopictus:**
Competent in lab
Found infected in nature


**Aedes japonicus:**
Competent in lab
Found infected in nature???

Identifying Larvae: Primary Characters

*Aedes triseriatus*

- Acus attached
- Anal papillae not bulbous, dorsal pair longer than ventral

*Aedes hendersoni*

- Acus detached
- Anal papillae bulbous, both pairs of anal papillae about same length
Secondary Characters

A: 1-S Setae
Aedes triseriatus: 1-2 setae
Aedes hendersoni: 3+ setae

B: 1-X Saddle Ratio
Aedes triseriatus: Saddle Ratio <1
Aedes hendersoni: Saddle Ratio ≥1

C: 1-X Branch Numbers
Aedes triseriatus: 5+ setae
Aedes hendersoni: 2-3 setae

D: Ventral Brush
Aedes triseriatus: 5 pair/4-3 Br.
Aedes hendersoni: 4 pair/2-3 Br.
Results Summary (2011 Study)

- Pilot Study conducted in 2011 on WCU Campus
- ~6,500 *Aedes* Eggs Collected
- 2,686 reared larvae identified to species
- 63% of identified larvae were invasive species
- 41% hatch rate
Aedes triseriatus vs Aedes hendersoni

Aedes triseriatus: 75% of the total eggs identified were oviposited at 3 or 6 meters

Aedes hendersoni: 67% of the total eggs identified were oviposited at 6 or 9 meters
Invasive Species Oviposition

74% of the total eggs were oviposited at 3 meters or below (ground level)

**Aedes albopictus**

- 74%
- N=1,155

**Aedes japonicus**

- 61%
- N=537

61% of the total eggs were oviposited at 3 meters or below (ground level)
Research Goals

Repeat 2011 vertical distribution study:

*Aedes triseriatus*  (Paradigm: Oviposits at ground level)

*Aedes hendersoni*  (Paradigm: Oviposits well above ground level)

*Aedes albopictus*  
*Aedes japonicus*  

Little known of oviposition in North Carolina

Compare relative abundance and species diversity across three distinct landscapes

Collect *Aedes hendersoni* for future molecular and morphological studies
Wayehutta
Elev. 2674 feet
100+ years

Moore
Elev. 2227 feet
80-100 years

Body Farm
Elev. 2322 feet
30-60+ years
Methods (Field)

- Ovitraps placed in 12 mature Oak trees distributed evenly across 3 focal field sites
- Ovistrips placed in each trap at heights of 0 and 9 meters
- Created standard white oak leaf litter infusion as attractant for field traps: (10.6 grams litter/1 liter DI H$_2$O)
- Traps gathered after 10 days
Methods (Lab)

- Eggs counted/cataloged to calculate hatch rates
- Ovistrips incubated at $28^\circ \text{C} \pm 2$ for 5 days
- Ovistrips flooded with liver powder slurry in DI H$_2$O
- Samples reared to 4$^{th}$ instar larvae, identified to species, cataloged and stored for future study

*Aedes* eggs

Fourth Instar Larvae
Egg Numbers Overall (2012)

Egg Counts by Month:

<table>
<thead>
<tr>
<th>Month</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>7605</td>
</tr>
<tr>
<td>July</td>
<td>1504</td>
</tr>
<tr>
<td>August</td>
<td>1242</td>
</tr>
<tr>
<td>September</td>
<td>1043</td>
</tr>
<tr>
<td>Total</td>
<td>11,394</td>
</tr>
</tbody>
</table>

Body Farm: High average of abundance 57.8% of all eggs

<table>
<thead>
<tr>
<th>Site</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>Overall Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore</td>
<td>1409</td>
<td>240</td>
<td>245</td>
<td>282</td>
<td>19.0</td>
</tr>
<tr>
<td>Body Farm</td>
<td>4984</td>
<td>649</td>
<td>362</td>
<td>593</td>
<td>57.8</td>
</tr>
<tr>
<td>Wayehutta</td>
<td>1212</td>
<td>620</td>
<td>635</td>
<td>168</td>
<td>23.1</td>
</tr>
</tbody>
</table>
Overall Ground Level Oviposition (2012)

* 49% of all *Ae. triseriatus* identified found above 0 meters!
** 35% of all *Ae. hendersoni* identified found at 0 meters!!
Site Specific Ground Level Oviposition (2012)

Moore: More Ae. albopictus (70%) and Ae. japonicus (75%) at ground level at ground level.

Body Farm: Ae. albopictus (85%) and Ae triseriatus (73%) at ground level with a higher level of Ae. hendersoni at ground level.

Wayehutta: Ae. albopictus 100% oviposition, Ae. japonicus over 60% with Ae. hendersoni and Ae. triseriatus below 50%.
Relative Abundance (Site Specific, June 2012)

WCU-Moore (n=1,044)
Egg Count: 1409

WCU-Body Farm (n=540)
Egg Count: 4984

Wayehutta (n=386)
Egg Count: 1212

81%
41%
32%
23%

Ae. triseriatus
Ae. hendersoni
Ae. japonicus
Ae. albopictus
Same Data: Natives vs Invasives

WCU-Moore
Native: n= 665
Invasive: n= 379

WCU-Body Farm
Native: n= 482
Invasive: n= 58

Wayehutta
Native: n= 349
Invasive: n= 37

36% Native 64% Invasive
11% Native 89% Invasive
10% Native 90% Invasive
Where are *Ae. hendersoni*?

Overall, 35% of *Aedes hendersoni* were collected at ground level in 2012 across three different sites:

<table>
<thead>
<tr>
<th>Site</th>
<th>Ground Level</th>
<th>Percent (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore</td>
<td>26.5</td>
<td>(21.2-32.4)</td>
</tr>
<tr>
<td>Body Farm</td>
<td>61.2</td>
<td>(47.2-73.5)</td>
</tr>
<tr>
<td>Wayehutta</td>
<td>36.4</td>
<td>(26.5-47.5)</td>
</tr>
</tbody>
</table>

There appears to be site specific differences (Moore vs Body Farm).

11% of *Aedes hendersoni* collected at ground level in 2011 study.
## Hatch Rates

### Overall Hatch Rate by Site

<table>
<thead>
<tr>
<th>Percent</th>
<th>Moore</th>
<th>Body Farm</th>
<th>Wayehutta</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>10</td>
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<tr>
<td>100</td>
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<td>100</td>
</tr>
</tbody>
</table>

### Hatch Rates by site/month

<table>
<thead>
<tr>
<th>Month</th>
<th>Moore</th>
<th>Body Farm</th>
<th>Wayehutta</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>74%</td>
<td>11%</td>
<td>38%</td>
</tr>
<tr>
<td>July</td>
<td>18%</td>
<td>24%</td>
<td>36%</td>
</tr>
<tr>
<td>August</td>
<td>21%</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>September</td>
<td>0%</td>
<td>0.03%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Overall Hatch rate 2011: 41.0%
Overall hatch rate 2012: 21.8%*

*2482 larvae identified
Conclusions

• Habitat specific (forest age and structure) influences remain unclear
• Poor hatch rates limit conclusions
  – Better hatch methodology
  – Summer 2012 Infrastructure changes
• Weather extremely hot summer
• Future studies must address:
  – Increasing hatch rate and cohort survival
  – Possible substitution of nutrient broth and/or yeast slurry
  – Diapause behaviors
Conclusions

• Ground-level oviposition surveillance efforts do not collect only *Aedes triseriatus*:
  – *Aedes albopictus*
  – *Aedes japonicus*
  – *Aedes hendersoni*

• There is a clear need to delineate additional morphological characters to identify the sister species (*Ae. hendersoni*) and potential hybrids
Next Steps

• New and improved insectary facilities with controlled environmental chamber
• To identify morphological differences and similarities between the two sister species (In progress)
• Using these identifications to find links to possible hybridization between the two sister species (confirm with molecular tools)
References


Questions?
LACV “Life” Cycle

Adapted from Beaty and Marquardt (1996)
Results: Total Eggs Collected

- June: 2791
- July: 2755
- August: 974
- September: 33

Total: N = 6553

US Drought Monitor of NORTH CAROLINA

August 30, 2011
Valid 8 a.m. EDT

Drought Classifications:
- D0 - Abnormally Dry
- D1 - Moderate Drought
- D2 - Severe Drought
- D3 - Extreme Drought
- D4 - Exceptional Drought

County Boundaries

Major River Basins (View Map)

S = Short-Term, typically <6 months (e.g. agriculture, grasslands)
L = Long-Term, typically >6 months (e.g. hydrology, ecology)

The U.S. Drought Monitor focuses on broad-scale conditions. Information provided for North Carolina is relative to the information provided from all other states and the North Carolina Drought Management Advisory Council. Local conditions may vary.

Rainfall (inches)
Toxorhynchites rutilus

July 2011
N=20