# Mosquitoes, CSOs and Stable Isotopes

# Where's the link?

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Outline
CSO Introduction
Tanyard Creek *Cx. quinquefasciatus*Stable Isotopes
Ongoing Research
Conclusion



# What is a CSO?

- Combined Sewage
  Overflow
- Collect
  - Rainwater runoff
  - Domestic sewage
  - Industrial wastewater
- Combined Sewer
   System (CSS)

# Atlanta has:

- •7 CSO facilities
  - •Clear Creek
  - Custer Avenue
  - •Greensferry
  - Intrenchment Creek
  - McDaniel Street
  - North Avenue
  - •Tanyard Creek
- •2 CSO Regulators •Boulevard
  - Confederate Avenue

#### Combined Sewage =

#### Wastewater + Stormwater

# What causes overflows?

#### Dry weather 🏹

- All wastewater goes to WRC & is fully treated
- Returned to Atlanta water supply
- Wet weather
  - Stormwater exceeds WRC<sup>0</sup><sup>0</sup><sup>0</sup> capacity
  - Combined sewage goes to CSO facilities for disinfection & screening

Heavy rain

 Combined sewage
 bypasses CSO facility and flows directly into stream

### Rain, rain....Go Away!!!



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# Tanyard Creek CSO -is unique-

- Loring Heights residential area
- Serves West Atlanta combined sewershed
- 1,955 acres of urban area served
- Smallest volume capacity of <u>all facilities</u>
  - Maximum overflow capacity=3,600 mgd

Paved Conveyance situated at headwater of urban stream. Less than 1/10 in. of rain will cause Tanyard Creek facility to overflow.

- Primarily Treated?
  - Coarse screening
    - Remove large pollutants from street
      - Trash, basketballs, etc.
  - Fine screening
    - Remove solid sewage
  - Disinfection
    - Cl- injected
      - Kills some bacteria & pathogens

# Discharge at Tanyard Creek



## How do overflows affect us?

- Primarily disinfected sewage & rainwater runoff go directly into Tanyard Creek
- Still contains some sewage solids & harmful bacteria

- Rainwater runoff contains organic & inorganic pollutants
  - Inorganics: oils, metals, and grease
- Sewage is a major source of excess Nitrogen
  - High organic content
  - Eutrophication
  - Algae deplete oxygen
  - Natural enemies such as fish cannot survive
  - Mosquito larvae thrive in anaerobic conditions

# The good, the bad & the ugly

- Without overflow
   pipes sewage would
   backup into homes
   & streets
- Reconstruction in progress (reduce events)



- Tanyard Creek is used for many types of recreation
- CSO events can affect human health

 CDC/Emory Study shows correlation with *Culex* population & CSO events
 WNV+ surrounding Tanyard Creek CSO

#### Combined Sewage Overflows (CSO) Are Major Urban Breeding Sites for *Culex* quinquefasciatus in Atlanta, Georgia

Lisa M. Calhoun, Melissa Avery, LeeAnn Jones, Karina Gunarto, Raymond King, Jacquelin Roberts, and Thomas R. Burkot\*

Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia



American Journal of Tropical Medicine and Hygiene, April2007

"The overall goal of this study is to define the role of CSO streams for WNV vector mosquito production in Atlanta, GA."

An inverse relationship was found between the volume of water released in the previous 1–5 days and the prevalence of dips positive for any mosquito stage."

# Culex quinquefasciatus

- Larvae can be found in bodies of water with a high degree of organic pollution
  - Drains, cesspits, polluted streams
    - CSO streams
- Close to human habitation
- Adults readily enter homes to bite humans at dawn and dusk



Southern House Mosquito

- Blood meals:
  - Birds & mammals, especially humans
    - Contradicting data for % human blood meals
- Vector for
  - Lymphatic filariasis
  - MVE
  - RRV
  - Rift Valley Fever
  - SLE
  - Dog heart worm
  - Avian malarias
  - WNV!!!!

# What is an isotope?

Atoms of same element!
 # neutrons in nucleus
 Mass difference-separation
 Example: <sup>1</sup>H and <sup>2</sup>H



1.00797

### Isotope analysis is determination of isotopic signature

 Relative abundance of isotopes of given element in particular sample

<u>Stable</u> isotopes are <u>Non-Radioactive!</u>
 Share chemical characteristics

# Stable Isotopes In Question

# Carbon12C



- 6 protons+6 neutrons
- 98.89% natural abundance
- ► 13C
  - 6 protons +7 neutrons
  - 1.11% natural abundance
- Nutrient rich-13C

- Nitrogen
- ▶ 14N



- 7 protons +7neutrons
- 99.63% natural abundance
- ▶ 15N
  - 7 protons +8 neutrons
  - 0.37% natural abundance
- Nutrient rich-15N

## Stable Isotope Analysis

### Mass Spectrometry

- Delta 15N vs. Air
- Delta 13C vs. PDB

### Preliminary Data 06/05/2008

	Avg. Delta 15N vs Air	Avg. Delta 13C vs PDB
CSO	8.5	-21.7
Control	3.3	-29.2

Statistical Analysis
 SAS-ANOVA test



# Ongoing Research



# Study #1 Isotopic Analysis of Mosquito Larvae

- CSO vs. non-CSO
  - IS THERE A DIFFERENCE???
- Fourth instar larvae & pupae reared to adult
  - 10 females from each site analyzed
    - Culex quinquefasciatus
    - Culex restuans

- Control Site
  - Problem → Created Control Site!
- Spatial variation
- Does signature change down stream?
  - Sample sites along entire stream
    - 0m, 250m, 500m, 750m, 1000m, & ~2000m

# Study #2 Isotopic Analysis of Adult Mosquitoes

### Adult Traps

CO<sub>2</sub>-baited
 Light Traps
 (



Culex dispersal

- 2 Transects
  - 0 km, ½ km, 1 km, 1½ km, & 2 km
     Westward
- Adults definitively from CSO stream?

Gravid Traps



### Stable Isotopes & WNV

### Isotopic Signature Does the signature of captured adults relate to established CSO signature?

WNV

- Are captured mosquitoes WNV+?
- Do WNV+ mosquitoes have same signature as larvae from CSO?
- Definitive?
  - What questions are left?
    - Temporal & Age variation

## Study #3 Temporal Variation

- Does stable isotope signature for larval samples change over time?
- Larvae sampled throughout 2009
   CSO
   Control

- Does stable isotope signature for trapped adults change over time?
- Adult traps throughout WNV peak season
   July-October

## Study #4 Age Variation

Does signature change after blood meal? Does signature change after oviposition?

Larvae reared in breeders
Females are fed blood meal
Mated
Allowed to oviposit *10 FEMALES REMOVED FOR ANALYSIS*Females are fed second blood meal
Allowed to oviposit a second time *10 FEMALES REMOVED FOR ANALYSIS*

# Conclusion

- It has been established that CSO streams yield high populations of immature mosquitoes
- WNV+ mosquito pools in close proximity to CSO
- WNV human cases in close proximity to CSO

- Stable Isotopic tracing
  - Problems with mark/ release/re-capture
  - Can captured adults be definitively tied to CSO stream larval habitat through isotopic tracing?
- CSO input raises <sup>13</sup>C
   & <sup>15</sup>N levels in creek
  - Unpublished data (Downey, et. al., 2008)

# Why is this important?

we can definitively correlate captured mosquitoes in residential areas to CSO streams...

we can provide solid scientific evidence to support the need for mosquito control in these streams... ...starting with Atlanta and then 771 other cities...

**N H E N** 

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