

# **Correlates of urban mosquito fitness: CSO streams and Culex oviposition choices**

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# West Nile Virus in Atlanta: Combined Sewer Overflow (CSO) study

## Collaborators

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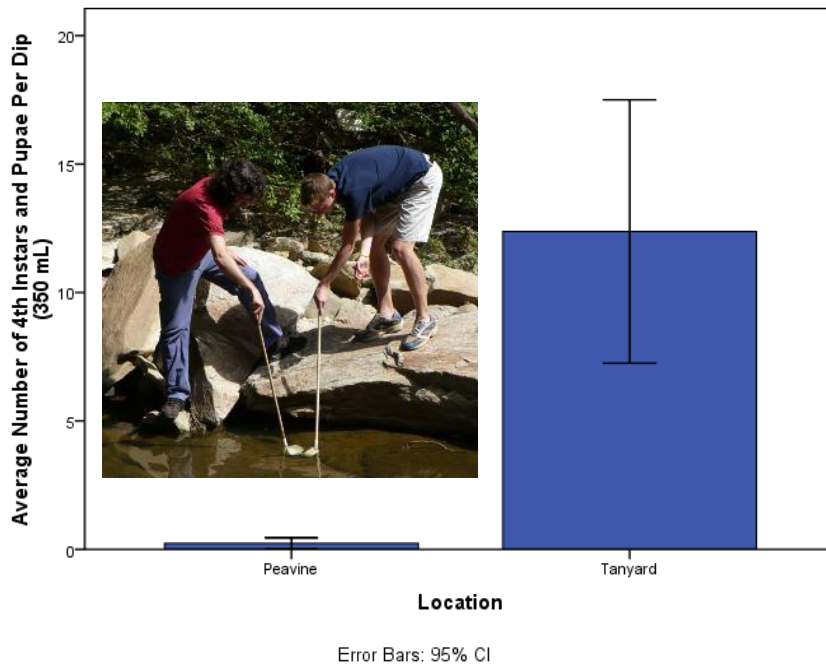
ENVS Undergraduate students (and recent graduates): Will Galvin, Alex VanNostrand, Andy Nguyen, An Nguyen, Greg Decker, Miho Yoshiaka, Kevin Lanza, Aubrey Dennis-King

# Atlanta Combined Sewer Overflow (CSO) study – ongoing (and growing!)

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

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### Key questions

Does the high mosquito productivity in CSO's translate in a higher WNV transmission risk?

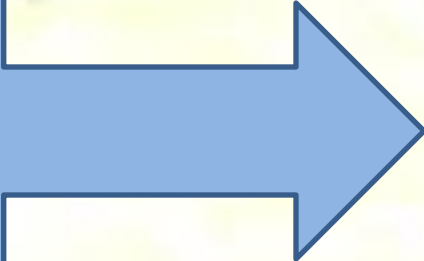
Particularly,

→ How does the CSO larval habitat affect *Culex* spp. fitness, and vector potential?

→ How do the basics of oviposition biology change in CSO streams, and how might this relationship affect fitness?

Descriptions of recent and current projects addressing these questions:  
<http://www.envs.emory.edu/research/WNV/index.htm>

# Determinants of Mosquito Fitness

- Adult survival
  - Adult fecundity
  - Juvenile survival
  - Juvenile fecundity
    - Larval nutrition
    - Body size
- 

Female oviposition  
choices

Affected by

- Nutrient availability
- Presence of microorganisms
- Presence of conspecifics

How does a CSO contribute to  
attractiveness?



# This talk...

- Semi-natural experiments
  - Preferences for CSO versus non-CSO oviposition habitats
  - Egg rafts as an indicator of oviposition choice
  - Effect of oviposition habitat size on oviposition preference
- Further studies on fitness effects (preliminary data), if time permits!

## Experiment 1: Preference for CSO versus an alternative oviposition site

Mosquito oviposition is not uniform across different media, so:

- Is there a preference for CSO water in comparison to non-CSO (tap) water?
- Do protein-rich nutrients enhance attraction?
- Can egg rafts be used as an indicator of oviposition choice, or is a trade-off involved?



# Preference for CSO versus non-CSO oviposition site: methods

Carried out on the bank of Tanyard creek, near one of the field sampling sites, using 10gal Rubbermaid containers (with 6 gallons of water each)

Experimental design: 2 x 2 factorial = 4 treatments

1. CSO water (collected 48 hours after a 9400 kGal overflow)
2. Tap water
3. Presence (+) of additional nutrient (24g of dogfood)
4. Absence of added nutrients

Data collection:

Counted and removed egg rafts after 3 days and after 6 days

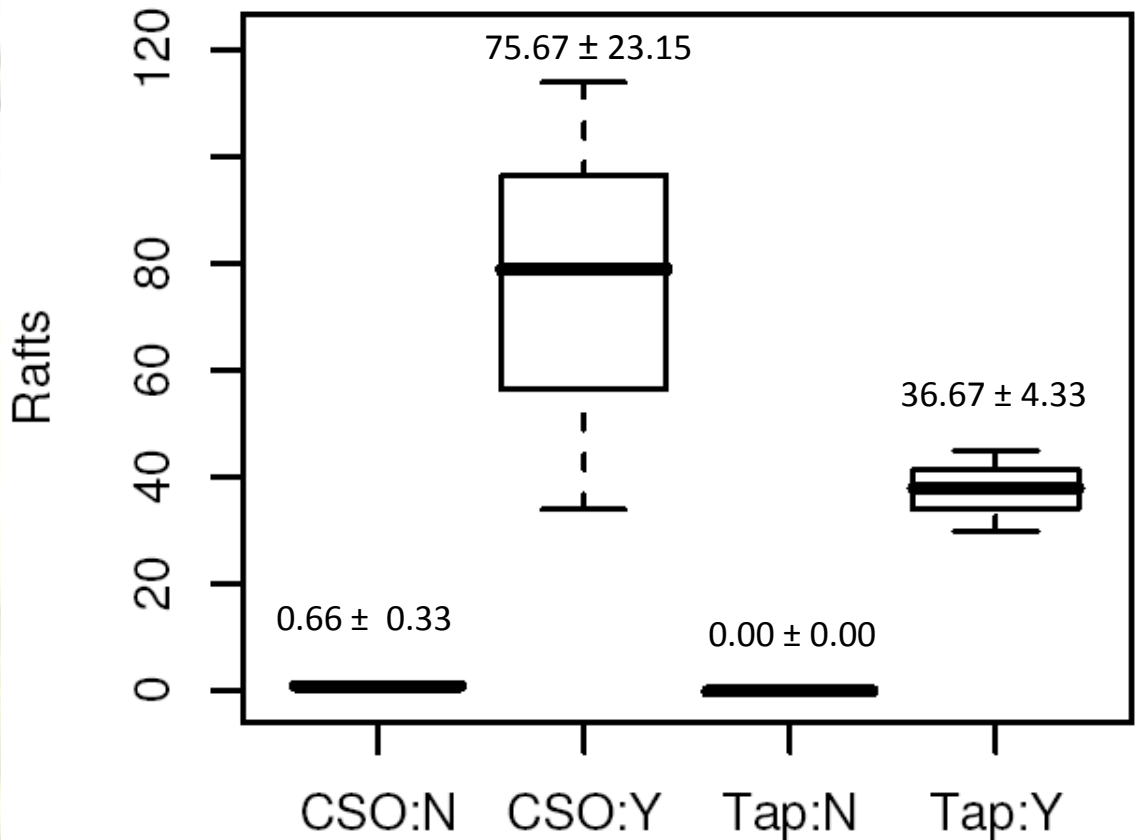
Tap	CSO	Tap+	CSO +
Tap	CSO	Tap+	CSO+
Tap	CSO	Tap +	CSO +

Individuals from 5 randomly chosen rafts per sampling period were allowed to hatch in the lab, identified at 4<sup>th</sup> instar as *Cx. quinquefasciatus*

# Preference for CSO versus non-CSO oviposition site: results

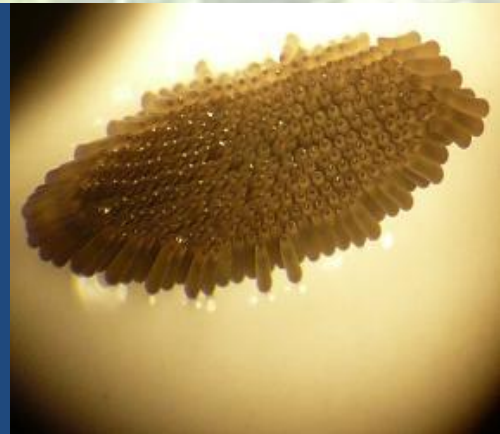
## Results:

1. Females oviposit preferentially in containers with added nutrients
2. CSO containers with added nutrients are significantly more attractive than non-CSO habitats with added nutrients





Is the number of rafts a reliable indicator of number of eggs, or is there a trade-off in the number of eggs oviposited per raft in an “undesirable” habitat?



### Methods

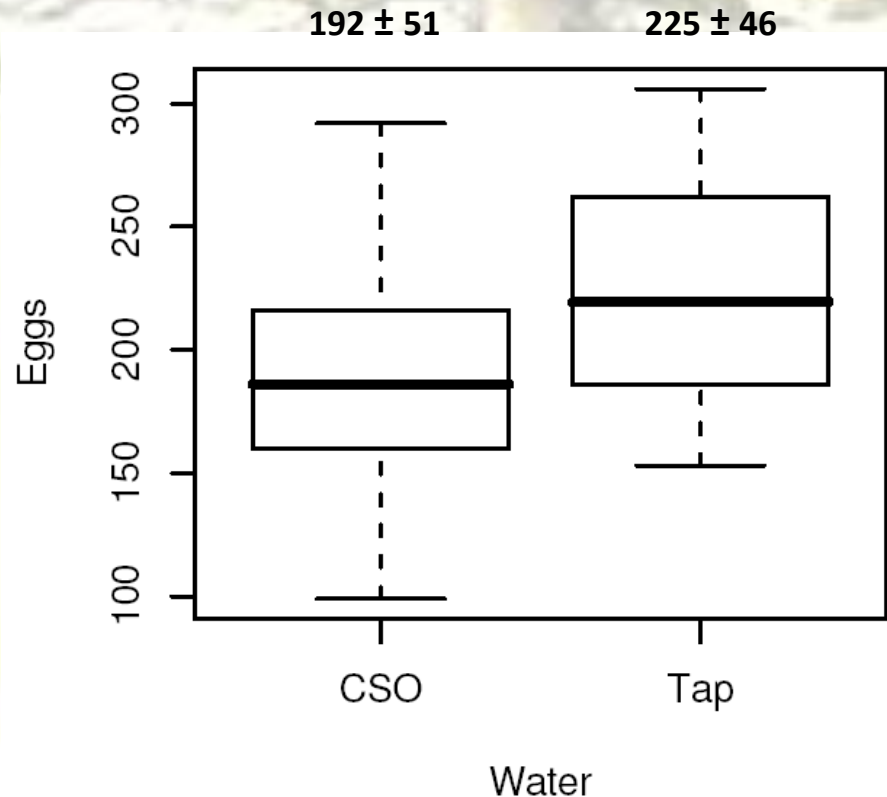
Counted eggs in 15 egg rafts from CSO+  
14 egg rafts from tap+

Compared means with students *t*-test

### Results

No significant difference between # eggs per raft in CSO versus tap ( $t=1.80$ ,  $df=27$ ,  $P>0.083$ )

number of eggs per raft can vary seasonally, so this result is only relevant for rafts collected at the same time



## Experiment 2: Spatial grain of oviposition preferences

- Is the influence of habitat quality on oviposition choices scale dependent?
- How does the attractiveness of each media vary in the presence vs absence of conspecifics?



# Semi-natural study sites

Environmental Studies

Baker woodland



4

3

2

1



© Google maps

# Spatial Grain: methods

- 4 clusters of 10 5gal Rubbermaid containers with 3gal of water spaced >75m along a transect following the stream
- Experimental design: 2 factors by 2 levels controlling for the effect of cluster on oviposition preference
- Same 4 treatments as with the previous experiment
- total amount of CSO and tap as well as nutrients or no-nutrient treatments was consistent across clusters

Tap	Tap	Tap	Tap	Tap +
-----	-----	-----	-----	-------

CSO	CSO +	CSO +	CSO +	CSO +
-----	-------	-------	-------	-------

Tap	Tap	Tap	Tap +	Tap +
-----	-----	-----	-------	-------

CSO	CSO	CSO +	CSO +	CSO +
-----	-----	-------	-------	-------

Tap	Tap	Tap +	Tap +	Tap +
-----	-----	-------	-------	-------

CSO	CSO	CSO	CSO +	CSO +
-----	-----	-----	-------	-------

Tap	Tap +	Tap +	Tap +	Tap +
-----	-------	-------	-------	-------

CSO	CSO	CSO	CSO	CSO +
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## Data collection:

- Counted egg rafts daily for 8-day periods
  - 1<sup>st</sup> 8-day period (July 11-18): egg rafts were **kept** in the containers but sequestered to avoid double counting
  - 2<sup>nd</sup> 8-day period (July 19-26): egg rafts were **removed** daily
- 25 larvae from CSO and tap treatments kept and identified at 4<sup>th</sup> instar
- In raft-removal period, 5 rafts kept and hatched



# Spatial Grain: methods

Tap	Tap	Tap	Tap	Tap +
CSO	CSO +	CSO +	CSO +	CSO +
Tap	Tap	Tap	Tap +	Tap +
CSO	CSO	CSO +	CSO +	CSO +
Tap	Tap	Tap +	Tap +	Tap +
CSO	CSO	CSO	CSO +	CSO +
Tap	Tap +	Tap +	Tap +	Tap +
CSO	CSO	CSO	CSO	CSO +

## Data analysis:

Number of rafts oviposited per container in the last 6 days of each trail were summed (no oviposition in the first 2 days of either trial)

## Split-plot linear mixed effects model

- cluster and error as random factors
- interactions between nutrients, water quality, and number of replicates per cluster as fixed factors
- model parameters selected using backwards elimination, based on Akaike information criterion
- models were fit using a restricted maximum likelihood method

# Spatial Grain: results when rafts are kept

Keeping or removing egg rafts had an effect on the relative importance of parameters selected for the models

Model selected for non-raft removal (1<sup>st</sup> period)

$$Rafts_{ijkl} = \mu + \alpha_i(\text{number}) + \beta_j(\text{nutrients}) + \alpha \times \beta_{ij}(\text{number} \times \text{nutrients}) + \sigma_k + \varepsilon_{ijkl} \quad [1]$$

where  $\mu$  = mean number of rafts

$\sigma$  = cluster variability

$\varepsilon$  = individual container variability

Parameter	No raft removal	
	Estimate	95% CI
Tap ( $\hat{\mu}$ )	3.973	-14.594 to 22.446
No. containers ( $\hat{\alpha}$ )	-0.925	-6.367 to 4.705
CSO ( $\hat{\gamma}$ )	—	—
Nutrients ( $\hat{\beta}$ )	12.550	-11.699 to 36.522
Containers $\times$ nutrients ( $\hat{\alpha} \times \hat{\beta}$ )	8.300	0.692-15.977 <sup>a</sup>
Water $\times$ nutrients ( $\hat{\gamma} \times \hat{\beta}$ )	—	—
Cluster variability VAR( $\hat{\sigma}$ )	10.982	—
Container variability VAR( $\hat{\varepsilon}$ )	140.124	—

Findings:

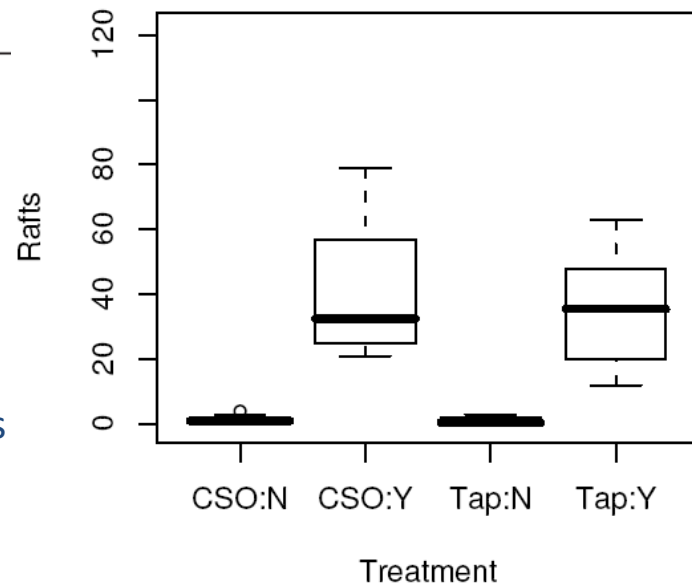
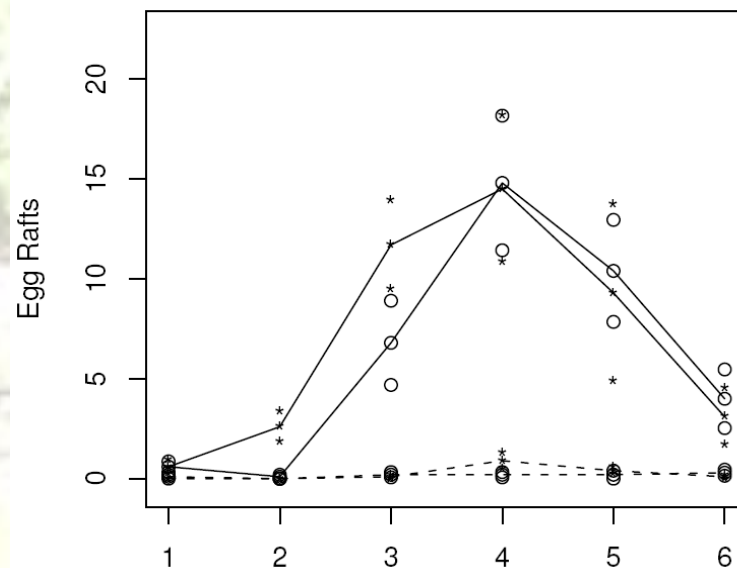
- there is some importance of the interaction between nutrients and quantity, that is more important in presence of conspecifics
- greatest variability is at the scale of the individual container

\* = CSO

o = tap

solid = + nutrients

dashed = - nutrients



# Spatial Grain: daily egg raft removal results

With raft removal (2<sup>nd</sup> period):

$$Rafts_{ijkl} = \mu + \beta_j(nutrients) + \gamma_i(water) + \gamma \\ \times \beta_{ij}(water \times nutrients) + \sigma_k + \varepsilon_{ijkl} \quad [2]$$

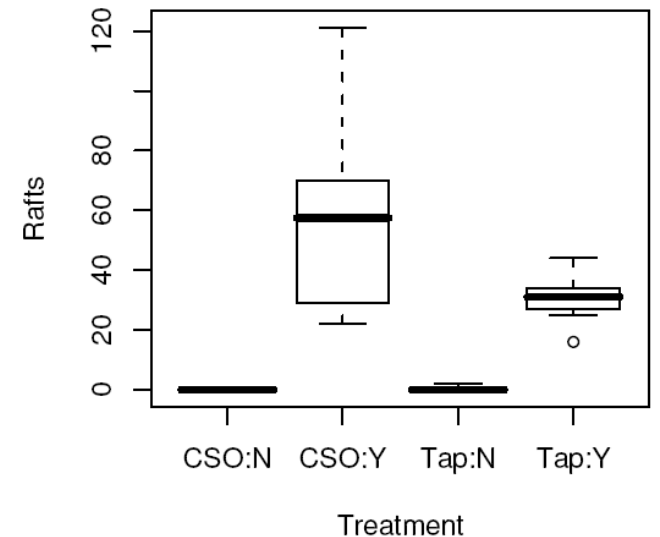
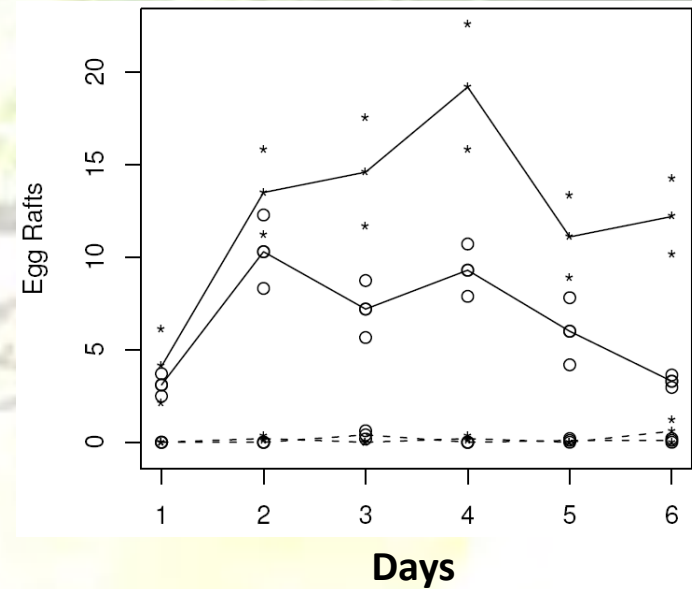
where  $\mu$  = mean number of rafts  
 $\sigma$  = cluster variability  
 $\varepsilon$  = individual container variability

Parameter	Daily raft removal	
	Estimate	95% CI
Tap ( $\hat{\mu}$ )	1.019	-11.668 to 14.417
No. containers ( $\hat{\alpha}$ )	—	—
CSO ( $\hat{\gamma}$ )	-1.238	-15.458 to 12.909
Nutrients ( $\hat{\beta}$ )	29.462	15.643-44.006 <sup>a</sup>
Containers $\times$ nutrients ( $\hat{\alpha} \times \hat{\beta}$ )	—	—
Water $\times$ nutrients ( $\hat{\gamma} \times \hat{\beta}$ )	27.176	6.337-47.586 <sup>a</sup>
Cluster variability VAR( $\hat{\sigma}$ )	28.208	—
Container variability VAR( $\hat{\varepsilon}$ )	225.569	—

## Findings:

- importance of water quantity is decrease, and now the water type and nutrients are most important
- greatest variability is again at the scale of the individual container

\* = CSO      solid = +nutrients  
 ○ = tap      dashed = - nutrients



# Overall conclusions

- Nutrient availability was most important factor enhancing oviposition (consistent with other studies)
  - Significant effect of interaction of nutrients and CSO water
  - Largest variability in oviposition choice was at the level of individual containers
    - Local differences in oviposition medium are most important factor governing oviposition choices
  - No trade off in number of eggs deposited in CSO versus tap
- CSO nutrient pulses and flushing events may alter vector population dynamics by concentrating oviposition and production in CSO streams
- Preference for CSO streams may confer overall fitness advantages, but may involve trade-offs at different life stages

BEHAVIOR, CHEMICAL ECOLOGY

## **Combined Sewage Overflow Enhances Oviposition of *Culex quinquefasciatus* (Diptera: Culicidae) in Urban Areas**

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# Current and future studies

- Effect of CSO larval habitat on
  - Survival
  - Time to emergence
  - Body size
  - Sex ratio
- Density interactions of CSO vs non-CSO larval habitats
- Significant effect of interaction of nutrients and CSO - what's the mechanism?
  - bacterial communities and/or nutrient processing?
- Interactions with other stream inverts

# Thanks to:

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An Nguyen  
Greg Decker  
Andy Nguyen  
Will Galvin  
Alex Vannostrand  
Aubrey Dennis-King



And thanks to GMCA for the  
opportunity to present this study!

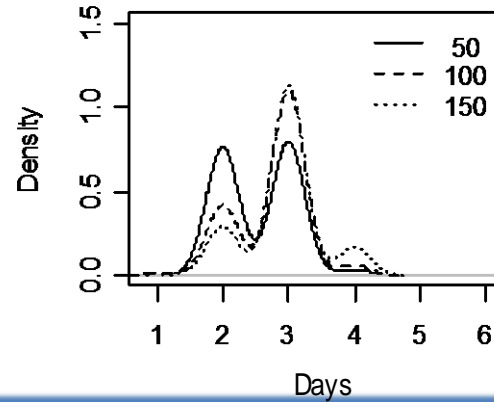
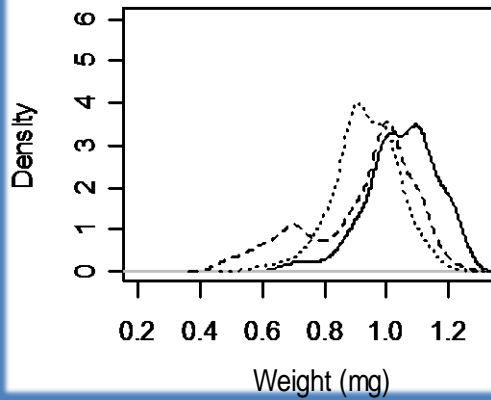
# Effect of Density on CSO vs tap survival, sex ratio, and size

- Does development in the CSO larval habitat affect larvae survival, body size, or sex ratio?
- Does larval density affect “?”

Density dependence: preliminary results  
Effects of density on weight and time to emergence of:

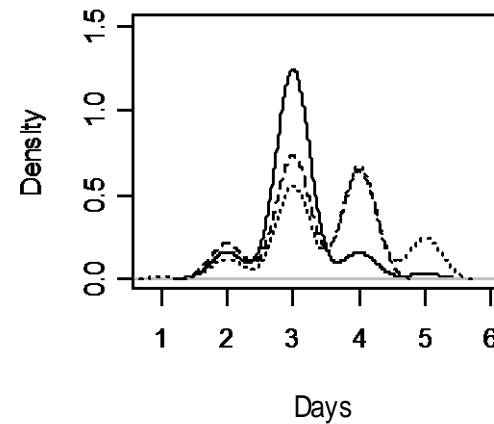
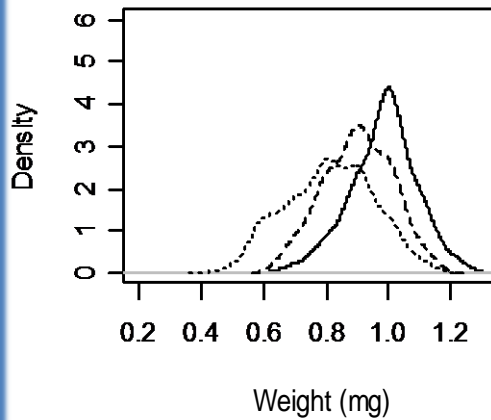
CSO

Females in CSO water



Tap

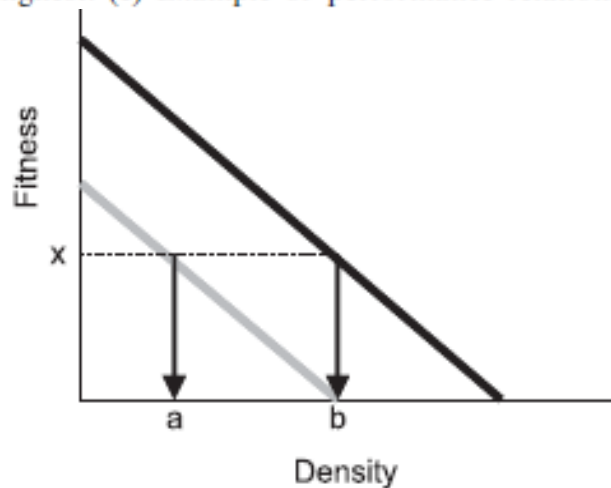
Females in tap water





# Ideal Free distribution and mosquito oviposition choice

**Fig. 1.** (a) Hypothetical example of ideal free distribution. In this example there should be a density of  $a$  in habitat 1 (grey line) and  $b$  in habitat 2 (black line) to achieve average fitness of  $x$  in both habitats. (b) Hypothetical example of the preference–performance hypothesis. Here there is no density dependence, and females should place all offspring in habitat 2 (black line) where offspring performance is always highest. (c) Example of performance relationships for



Habitat 1

Habitat 2

