Athens Dengue Data

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This document goes over the main results from the 2016 Athens Dengue Field Project.

Infection Dynamics

Methods Overview: Mosquitoes were reared at 9 sites (3 rural, 3 suburban, 3 urban) and brought back to the lab to infect with dengue. Mosquitoes were reared in four trays at each site, but pooled by site for infections because there were not enough numbers. At 21 days post infection, they were processed to test for infection (body), dissemination (head), and infectiousness (saliva). This was repeated in the fall and summer. Climate data reported was averaged at the site level over the larval rearing period, and weighted to account for the amount of time mosquitoes spent in each tray.

Infection by Class and Season

The first analysis was to determine how infection dynamics differed by class (rural, suburban, or urban) & season (summer or fall).
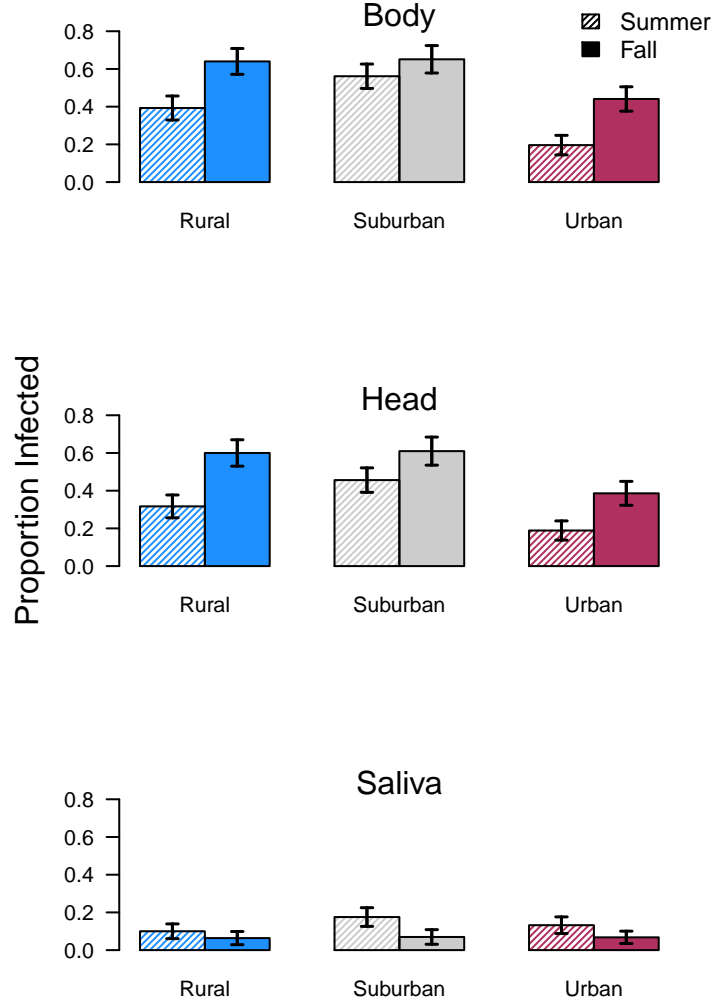
A binomial generalized linear mixed-effects model was fit to the data, with class and season and their interaction as fixed effects and site as a random effect. To test for significance, we calculated an analysis of variance table using a Type II Wald chi square test for the fixed effects. Both class and season had significant effects on body and head infections, however there was no effect of either on saliva infection.

We found that infection in the body and head was significantly lower in the summer than the fall. Although there was a trend towards higher infection in the saliva in the summer, this was not significant (p=0.05676). Land class also influence infection of both the body and head, with urban mosquitoes having lower rates of infection than rural and suburban mosquitoes. Saliva infections were unaffected by land class, however.

Table 1: Mixed model statistics for body level infections.

	Chisq	Df	Pr(>Chisq)
class	18.73327	2	8.55e-05
block	12.60856	1	3.84e-04

Table 2: Mixed model statistics for head infections.

	Chisq	Df	Pr(>Chisq)
class	14.20765	2	0.0008220
block	15.05016	1	0.0001047

As both the summer season and urban land classes had the highest temperatures in their respective groups, this suggests that these differences in vector competence may be due to differences in temperature in the larval environment.

Infection Efficiency

We can also look at infection efficiency. This is similar to what proportion of mosquitoes proceed to having head infections, out of those that have body infections.
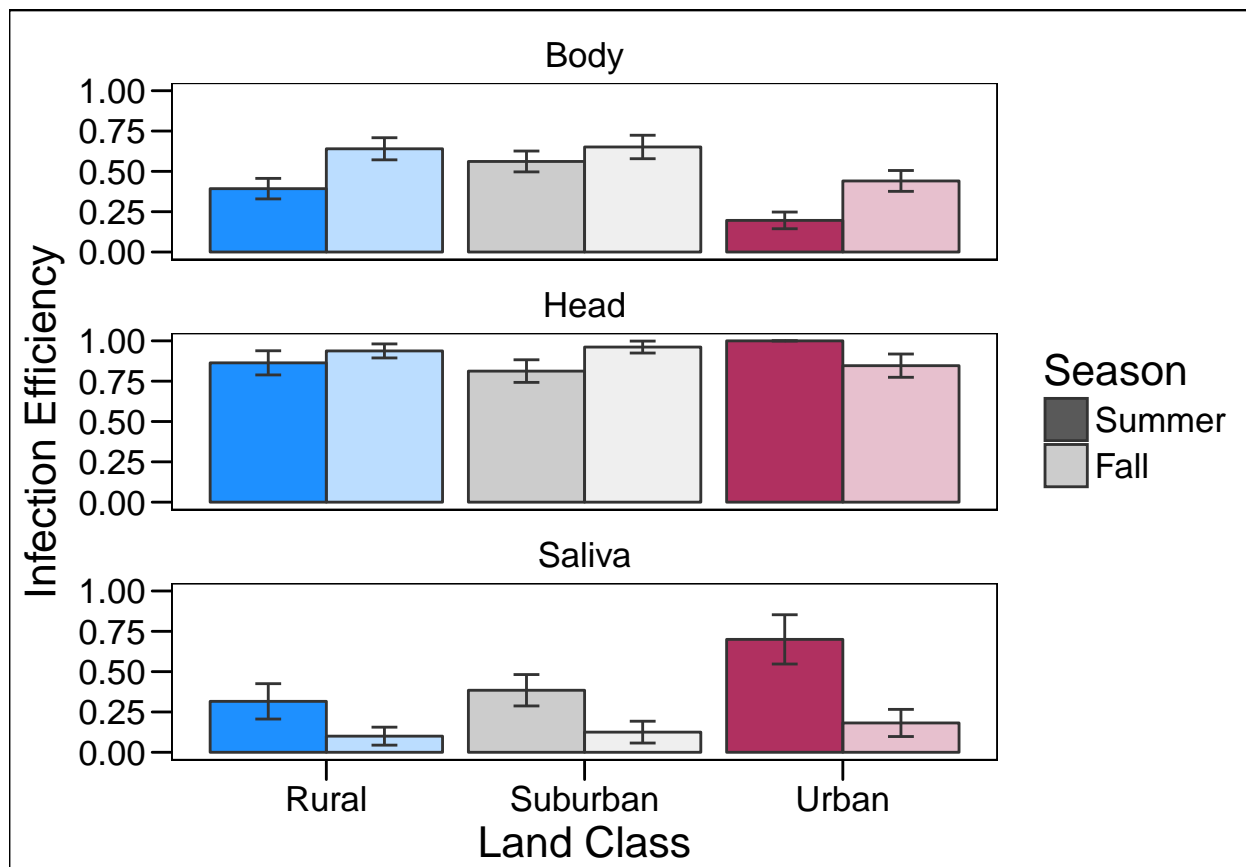

Table 3: Significance of block and season on infection efficiency using a mixed effect model. This is identical to body infection above.

	Chisq	Df	Pr(>Chisq)
class	18.73327	2	8.55e-05
block	12.60856	1	3.84e-04

Table 4: Significance of block and season on dissemination efficiency using a mixed effect model.

	Chisq	Df	Pr(>Chisq)
class	0.1887234	2	0.9099536
block	1.1667504	1	0.2800700

Table 5: Significance of block and season on efficiency of salivary gland infections, using a mixed effect model.

	Chisq	Df	Pr(>Chisq)
class	4.113711	2	0.1278554
block	13.658935	1	0.0002192

The two major obstacles to infectiousness are at the level of the body and the saliva. Nearly all mosquitoes that have body infections also have disseminated infections, suggesting that midgut escape is relatively uncontrolled. However, of the disseminated infections, not all result in salivary gland infections, suggesting there is a mechanism blocking the virus from spreading to the salivary glands. The summer has a significantly higher efficiency than the fall season with regards to saliva, however, there is no difference in progression to disseminated infections.

Infection and Microclimate

Based on our findings above, we wanted to investigate if these patterns across season and class could be driven by microclimate, and how.

Because our experimental design was somewhat limited by the number of mosquitoes emerging, we grouped infections by site, rather than tray. This means that the microclimate data used is the same for all mosquitoes across sites, and that we cannot use it to predict individual level infections in a meaningful way, as we did for land class and season. Therefore, our response variable in this analysis is the proportion of infected mosquitoes by site. This greatly reduces our sample size to 18, and therefore our statistical power.

The original starting set of variables were the average daily mean, minimum, and maximum temperature and relative humidity per site, daily temperature range, and the average number of hours below 16C and above 31C. These temperature values were chosen based on thresholds from the Mordecai et al 2017 model of temperature dependence of dengue infections. Because maximum relative humidity is 100% for all sites, this variable was dropped. This resulted in eight total environmental covariates. Because temperature and relative humidity are so dependent on each other, this resulted in high correlations amongst the covariates.

To select covariates, I used a lasso regression in the `glmnet` package to conduct a step wise regression selection process based on the RMSE. At each step, the variable whose omission resulted in the greatest drop in RMSE was dropped from the model. As the selection process continued, previously dropped variables were again included in the model at each step to determine any effect on RMSE, and if this increased the error, were left out of the final model. Variable selection ended when the dropping of any of the included variables did not decrease the RMSE of the overall model.

Because most of these variables were still highly correlated ($\rho > 0.9$), I then further selected models to eliminate collinearity amongst covariates. For this stage, I chose to use a linear mixed model because many of the exploratory visualizations were clearly linear, but with a strong random effect of season, which I could include in a mixed model. I modeled all variables individually and with an interaction term between another, if the correlation between the variables was less than 0.75. Models were chosen based on that which minimized the AICc and maximized the marginal r^2.

Body Infection

LASSO regression identified mean, minimum, and maximum temperatures and daily temperature range as important variables, with mean temperature the most important.

The final linear mixed model included mean temperature as a fixed effect. It had a marginal r^2 of 0.442. It also had the lowest AIC value out of all models.

The relationship between mean temperature and body infection is negative (β=-0.086) and significant ($p < 0.05$). Higher temperatures seem to be leading to lower body infection rates. Additionally, the relationship seems qualitatively different over the seasons, with the season as a random intercept explaining nearly as much of the variance as the mean temperature ($r^2_{GLMM_c}$=0.885). This may be because the summer temperatures are near the thermal optimum, where the effect of temperature is more pronounced.
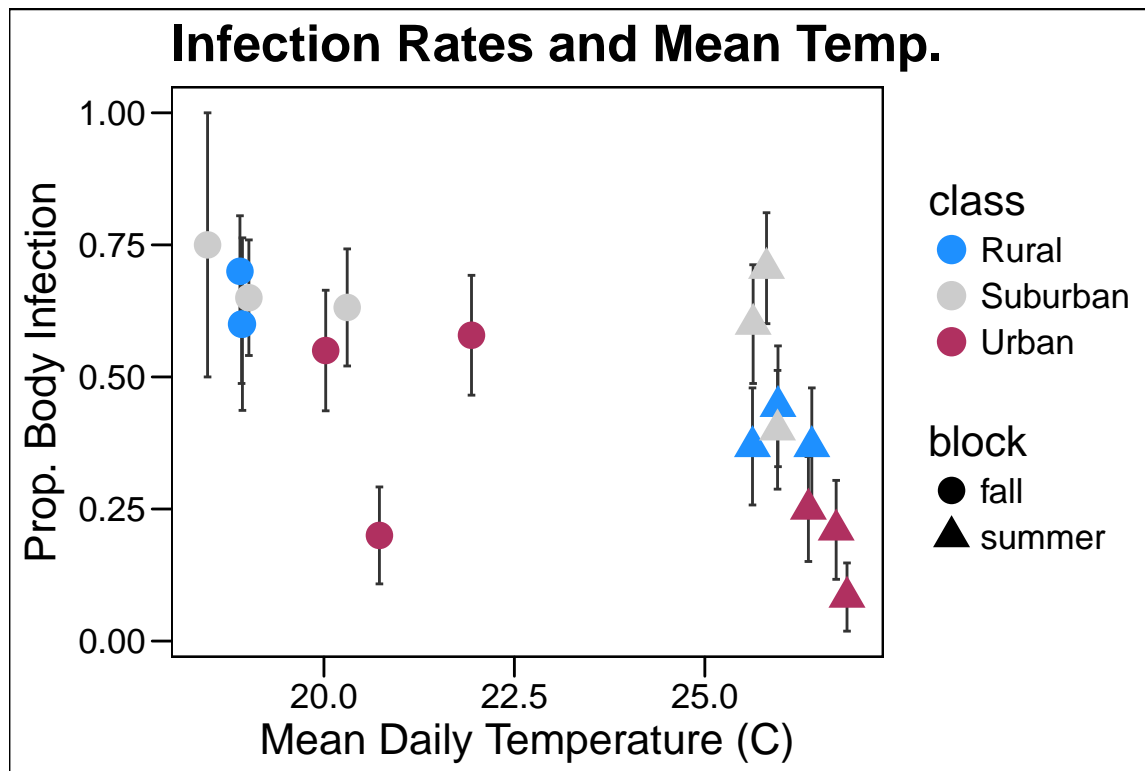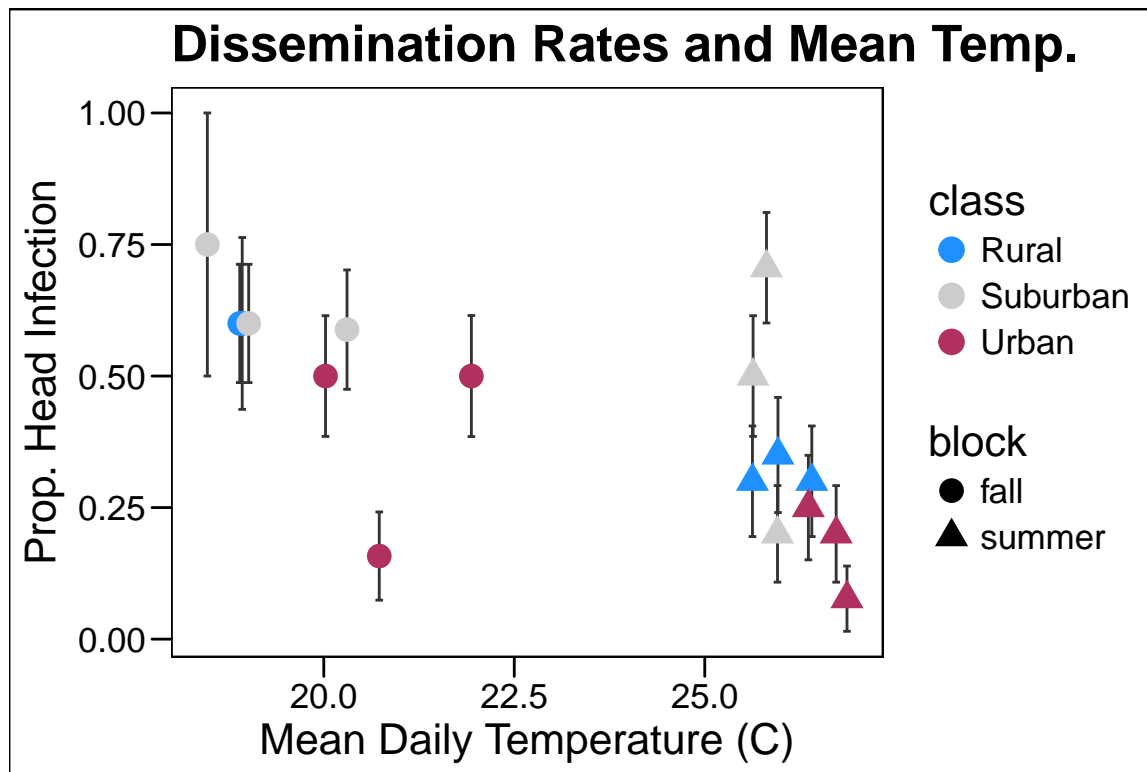

Head Infection

LASSO regression identified maximum temperature, mean temperature, and mean relative humidity as important variables.

The final linear mixed model included mean temperature as a fixed effect. It had a marginal r^2 of 0.463. It only had the second lowest AIC value out of the models, however the marginal r^2 of the model with the lowest AIC, which included mean temperature and relative humidity and their interaction, was much lower than that of the final model, and included additional variables.

Similar to body infections, the relationship between mean temperature and head infection rates was negative (β=-0.09). The effect of mean temperature was significant ($p < 0.05$), as measured using a Type II Wald chi square test. Again, much of the variation was explained by the random effect of season, resulting in a conditional r^2 of 0.893.

Dissemination Rates and Mean Temp.

Saliva Infection

As noted above, there was no significant difference in saliva infection across land class or season. I went through similar steps as above to investigate the effect of microclimate on saliva infection. Although the best fitting linear model did include maximum temperature, it was not significant. Plots of saliva infection across different environmental variables show no clear trends.

Infectious Rates and Mean Temp.
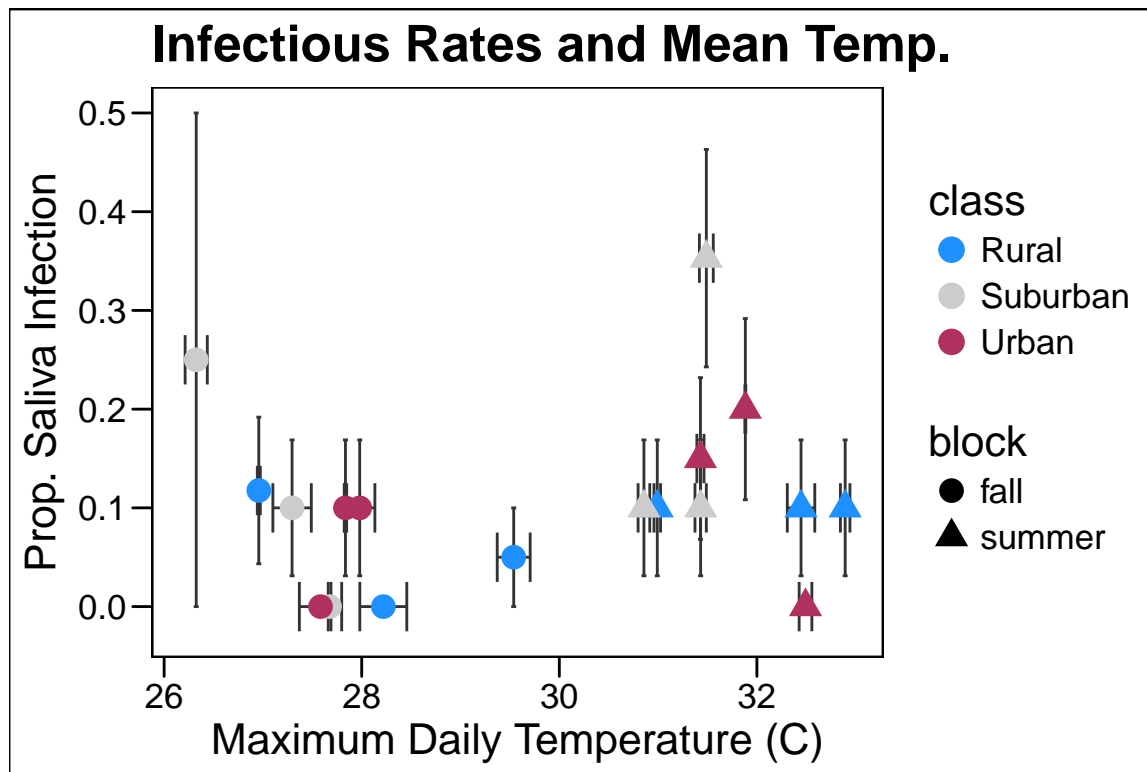
Emergence and Survival Results

In general, we found that larval emergence and survival differed significantly across season, but not across land class.

Female Larval Emergence
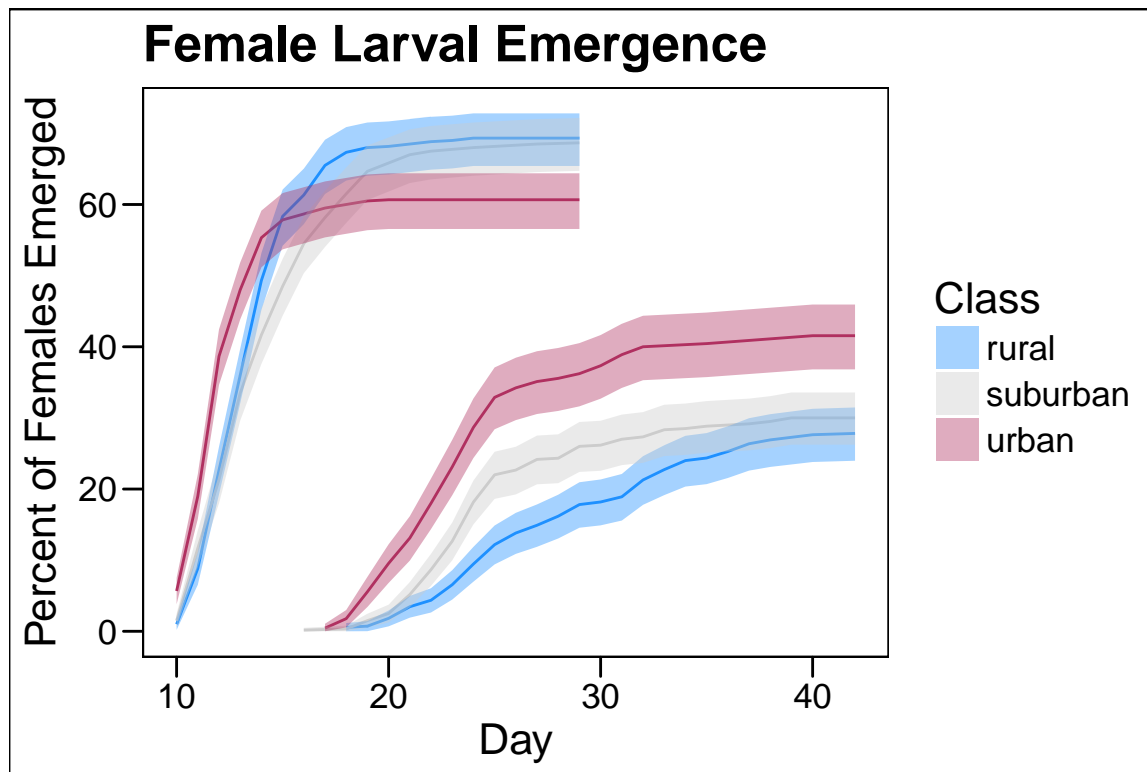
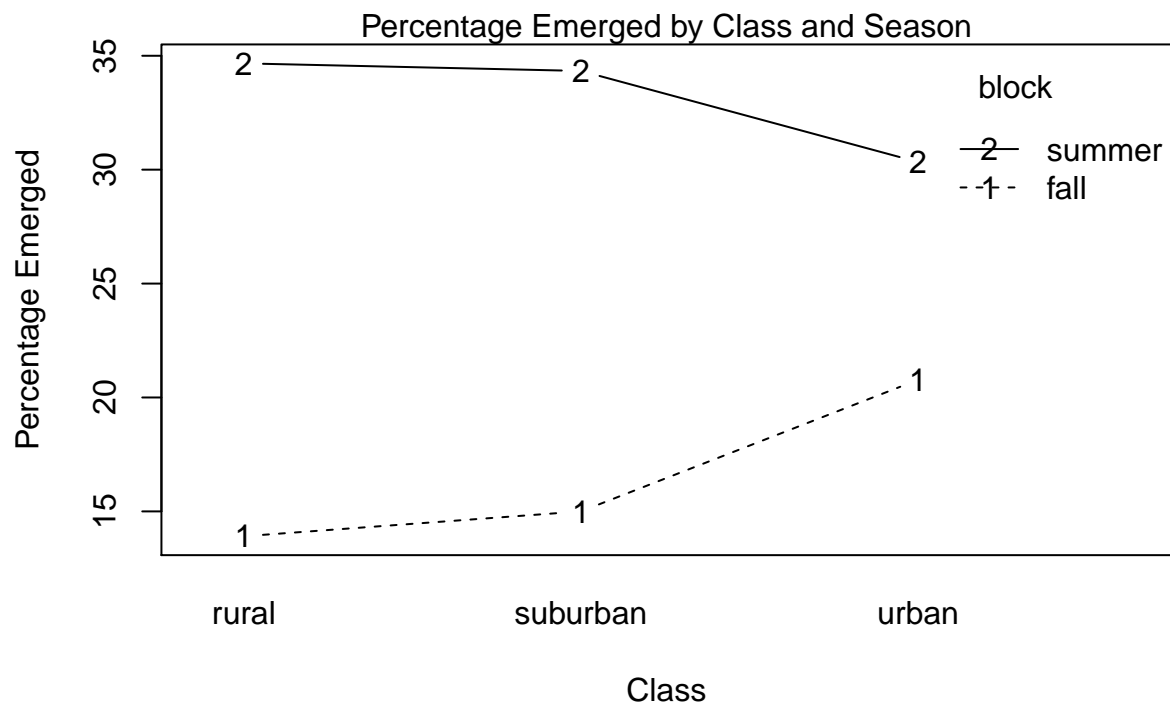This resulted in a difference in both the average time to emergence and survival across season, but not land class.

Percentage Emerged by Class and Season

Table 6: LMEM of class * block on tray level emergence with site as a random effect

	F	Df	Df.res	Pr(>F)
Class	0.0039292	2	5.987797	0.9960811
block	73.9394752	1	56.246604	0.0000000
Class:block	2.6202328	2	56.274644	0.0816501

Day of Emergence by Site
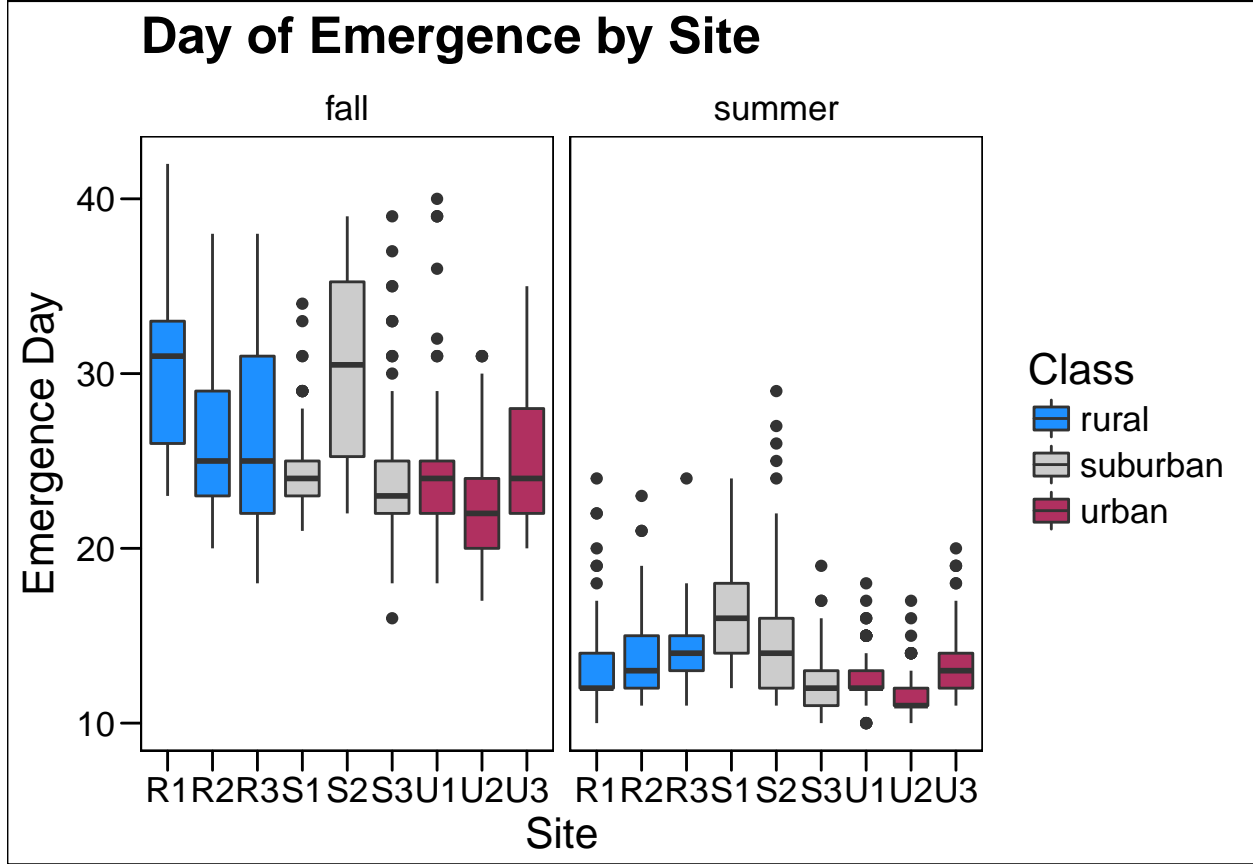
Table 7: Results from Mixed model on the effect of class and block on time to emergence.

	Chisq	Df	Pr(>Chisq)
Class	5.719409	2	0.0572857
block	5155.375909	1	0.0000000
Class:block	58.159814	2	0.0000000

The day of emergence differed significantly across block (and nearly class). Mosquitoes in the summer emerged on average 14 days sooner than those in the fall.

Body Size