

Introduction

- West Nile Virus is virus that can cause encephalitis in humans and horses
- *Culex tarsalis* is a vector for West Nile Virus that is found across Montana
- Differences in pigmentation – some lighter and some darker
- Color variation among species could be geographically correlated
- Distinct populations could be linked to differences in pigmentation
- A described gene associated with pigmentation is the Ebony gene
- **Hypothesis:** There is significant differences in the expression of the Ebony gene in *Cx. tarsalis* within the drainages of Montana.

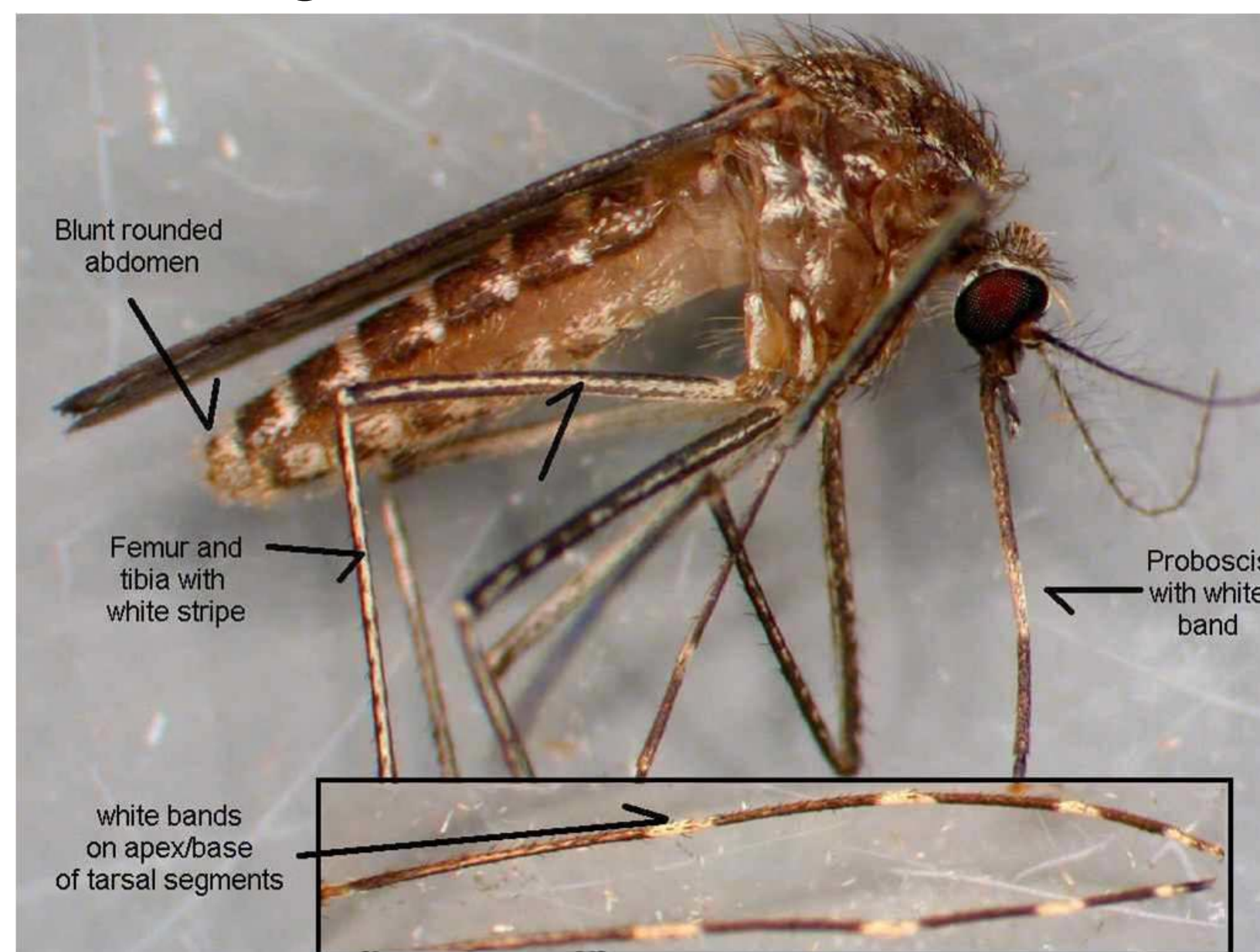


Figure 1. *Cx. tarsalis* is the primary vector for West Nile Virus in Montana.

Methods

- Mosquitoes were trapped where *Cx. tarsalis* is present
- RNA was extracted from *Cx. tarsalis*
- RT-PCR was performed on the RNA extractions analyzing the expression of the Ebony gene with the use of Actin as a normalizing gene
- The Cq values were transformed into RQ values
- Comparative statistics were used to analyze drainages and sites

Results

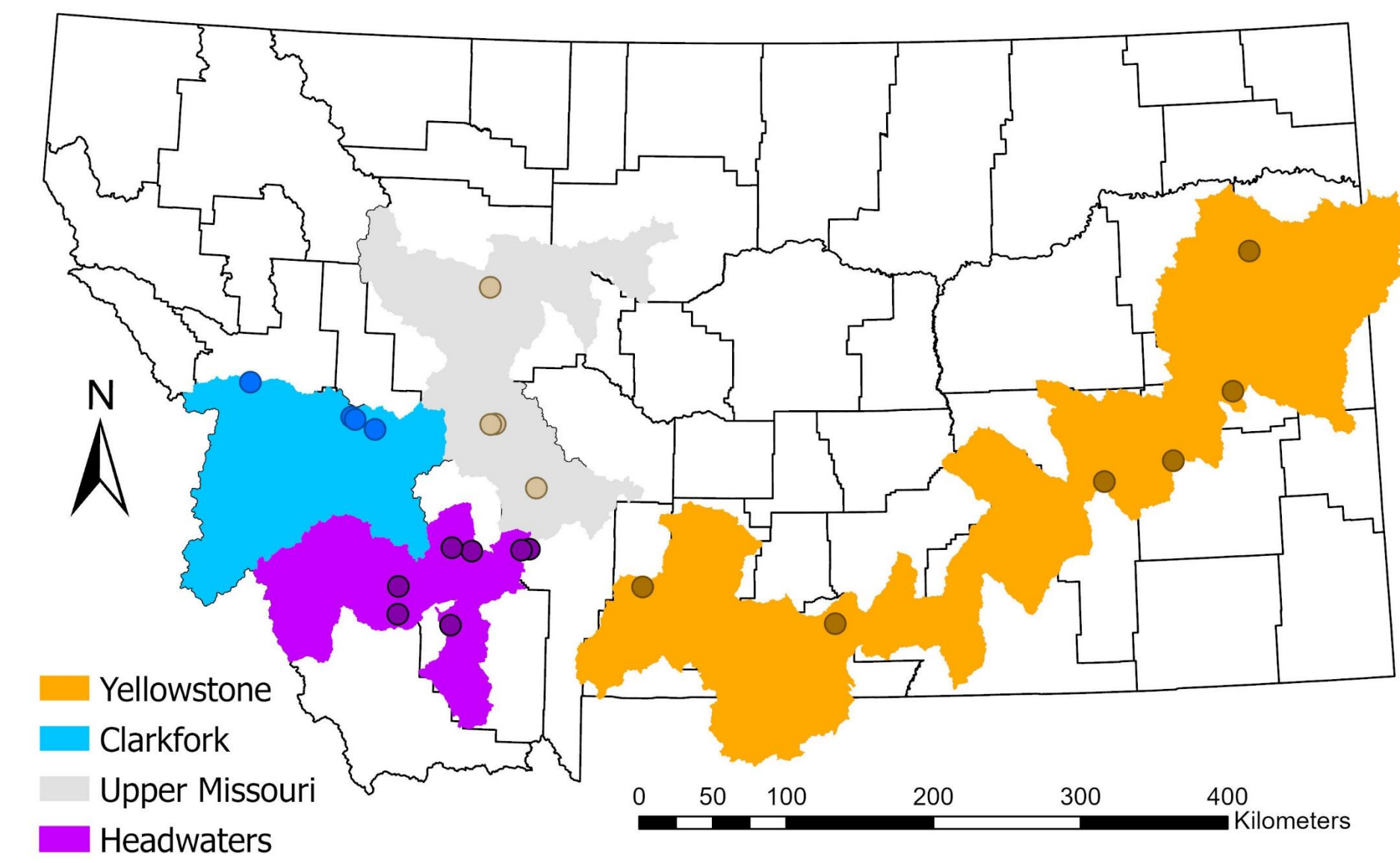


Figure 2. A map of Montana with the Yellowstone, Clark Fork, Upper Missouri, and Headwaters drainages highlighted as well as markers for each collection site within these four drainages.

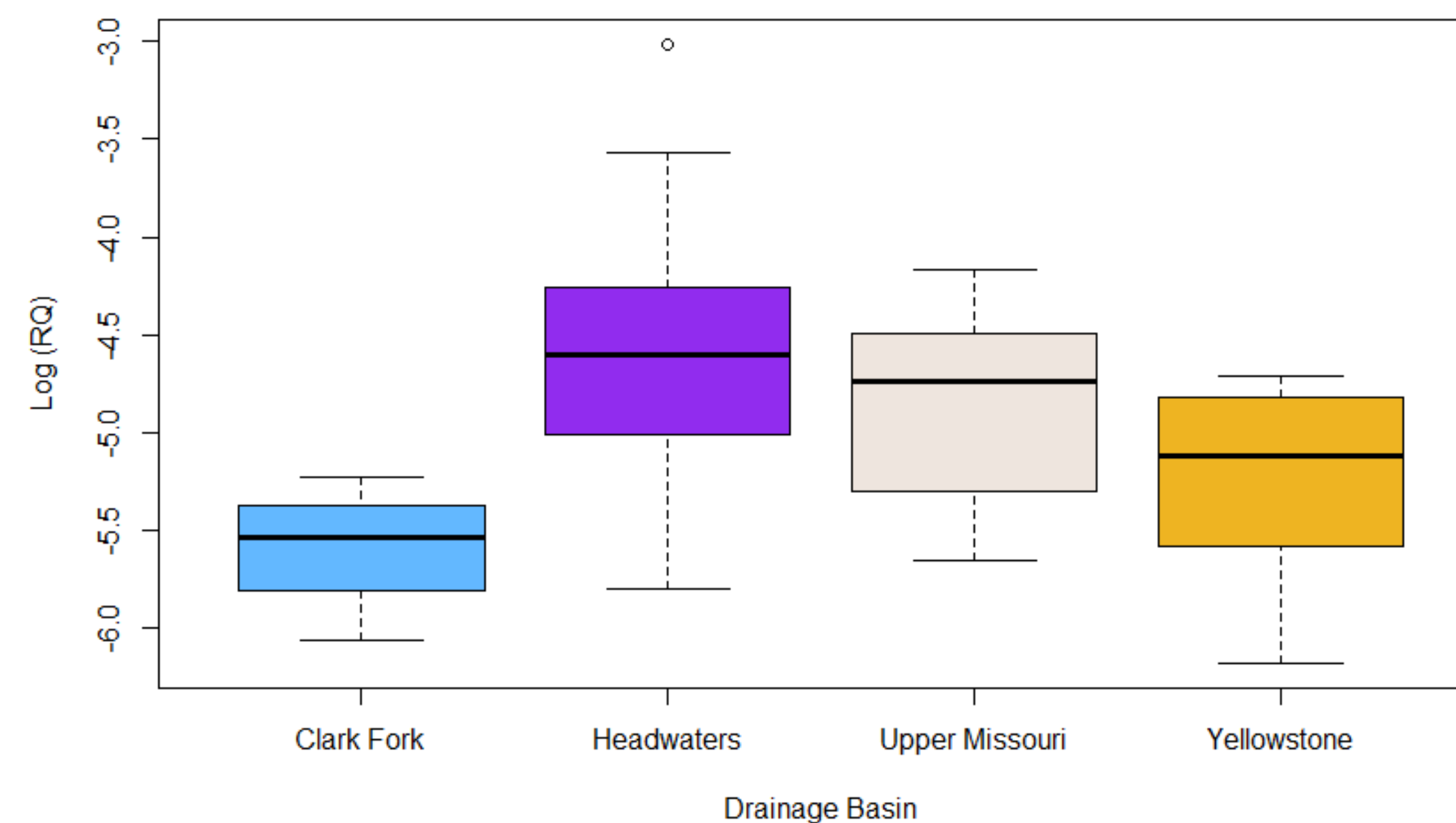


Figure 3. A boxplot showing the Log transformed RQ values for each drainage.

Drainage Comparison	P-Value
Clark Fork-Headwaters	0.0281*
Clark Fork-Upper Missouri	0.0859
Headwaters-Upper Missouri	0.4242
Clark Fork-Yellowstone	0.3732
Headwaters-Yellowstone	0.0497*
Upper Missouri-Yellowstone	0.2537

Table 1. Results of the Kruskal-Wallis non-parametric test between the drainages (chi-squared=11.257, df=3, & p-value=0.01041) showed significance of the RQ values between drainages. The Dunn Test results are shown above with the significant values bolded and marked with an asterisk.

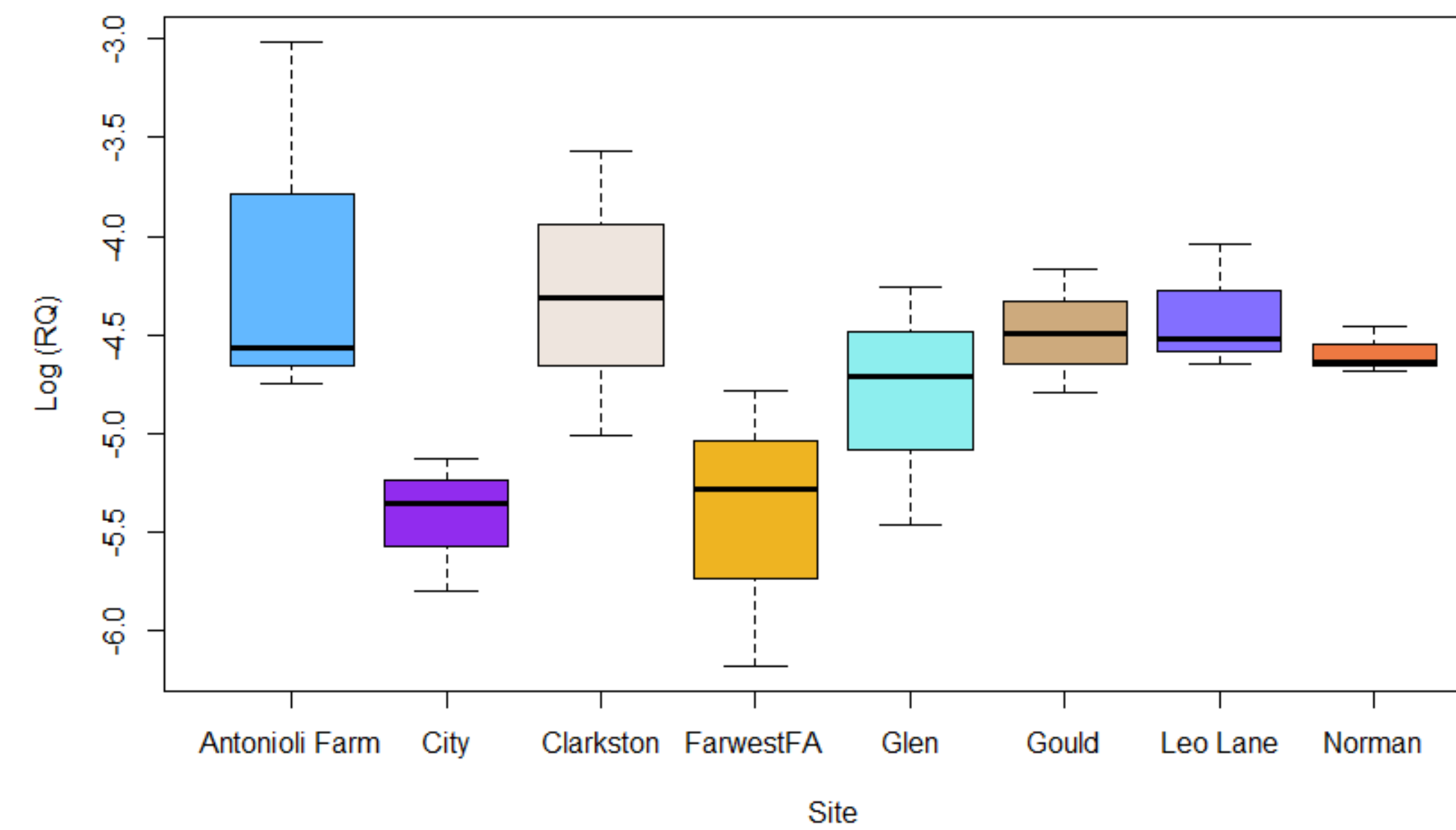


Figure 4. A boxplot showing the Log transformed RQ values for each site that had sufficient biological replicates.

Site Comparison	P-Value
City-Antonioli Farm	0.1608
Clarkston-Antonioli Farm	0.9999
FarwestFA-Antonioli Farm	0.1647
Glen-Antonioli Farm	0.8005
Gould-Antonioli Farm	0.9901
Leo Lane-Antonioli Farm	0.9978
Norman-Antonioli Farm	0.9627
Clarkston-City	0.3008
FarwestFA-City	1.0000
Glen-City	0.8806
Gould-City	0.5106
Leo Lane-City	0.4114
Norman-City	0.6413
FarwestFA-Clarkston	0.3071
Glen-Clarkston	0.9493
Gould-Clarkston	0.9999
Leo Lane-Clarkston	1.0000
Norman-Clarkston	0.9978
Glen-FarwestFA	0.8861
Gould-FarwestFA	0.5189
Leo Lane-FarwestFA	0.4190
Norman-FarwestFA	0.6498
Gould-Glen	0.9963
Leo Lane-Glen	0.9854
Norman-Glen	0.9997
Leo Lane-Gould	1.0000
Norman-Gould	1.0000
Norman-Leo Lane	0.9999

Table 2. The ANOVA test performed on the Log transformed RQ data for the sites (F value=2.223 & p-value=0.088) showed no significant differences between the sites. The Tukey's HSD test results are shown in the table above.

Conclusion

- Significant differences between the Headwaters and Clark Fork and the Headwaters and Yellowstone drainages
- No significant differences between individuals sites
- Headwaters and Clark Fork and the Headwaters and Yellowstone drainages could be significantly different due to selective pressures within the drainages

Future Directions

- The addition of the Flathead, Marias, Lower Missouri, Milk River, and Musselshell drainages
- The addition of biological replicates to increase site level analysis
- Targeting of the significantly different drainages for future population genetics studies
- The use of SAGE to examine the gene expression
- The investigation of additional pigmentation genes

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