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Radleigh Herschel

Virginia Commonwealth University, herschelrl@vcu.edu

Anthony G. Kouri

Virginia Commonwealth University, kouriag@vcu.edu

Rebecca Vareed

Virginia Commonwealth University, vareedrd@vcu.edu

Stephanie Warshawsky

Virginia Commonwealth University, warshawskysj2@vcu.edu

Matthew DeSaix

Virginia Commonwealth University, desaixmg@vcu.edu

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# Offspring Sex Ratio in Double Brooding Prothonotary Warblers

Radleigh Herschel<sup>1</sup>, Gregory Kouri<sup>2</sup>, Rebecca Vareed<sup>1</sup>, Stephanie Warshawsky<sup>1</sup>, Matthew DeSaix<sup>2</sup>

<sup>1</sup>Department of Biology, Virginia Commonwealth University, 1000 West Cary Street, Richmond, VA 23284

<sup>2</sup>Center for Environmental Studies, Virginia Commonwealth University, 1000 West Cary Street, Richmond, VA 23284



### Introduction

Prothonotary warblers are bright, golden birds who, with their loud calls, make themselves known in wetland habitats in the spring after returning from their winter homes in the Neotropics to breed. This migratory species is important to study because of their need for these habitats and are declining in population due to the degradation of wetland environments across the western hemisphere.

VCU started a project in 1987 to study prothonotary warblers including population genetics, breeding biology, and migration ecology. Since then, with the help of Richmond Audubon Society, the project has erected over 600 nesting boxes along the James River contributing to a database going back 30 years. This makes them an accessible bird to study and, with the collected information, help to better understand the causes of their decline.

## Background

- Females in better condition have higher ratios of male offspring.
   (Trivers and Willard, 1973)
- Females who produce second broods are often in better body condition. (Whittingham et al. 2002)
- The probability of female prothonotary warblers double brooding is positively correlated with age. (Bulluck *et al.* 2013)

### Methods

- Brachial venipuncture blood collection (Tucker et. al 2016)
- DNA extraction with DNAzol washes (Tucker et. al 2016)
- PCR reaction with P2/P8 primers
- Goodness-of-fit tests





### Results





Figure 1: PCR-amplified blood samples were run in a 3% agarose gel with a 100 bp ladder. The number of bands indicates sex; one band signifies male Z chromosomes, two bands signifies female Z and W chromosomes.

# Fig. 2 Percentages of all clutches \*\*Males\*\* \*\*Females\*\*

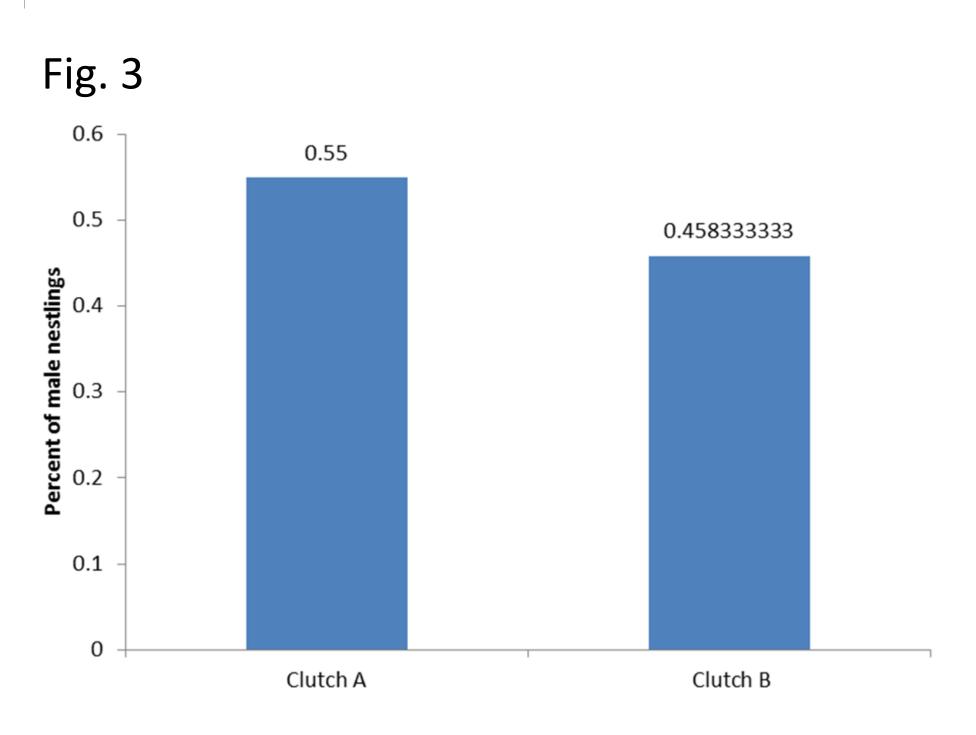


Figure 2 & 3: Among all nestlings, there was a 50.0% (22/44;  $X^2 = 0$ , df = 1, p-value = 1) ratio of male to female offspring. When separated into clutch A and clutch B, slight inequalities in sex ratios arose. Clutch A had a ratio of 55.0% (11/20;  $X^2 = .2$ , df = 1, p-value = .65); clutch B had a ratio of 45.8% (11/24;  $X^2 = .17$ , df = 1, p-value = .68).

### Conclusions

We hypothesized that clutch A would show a higher ratio of male nestlings than clutch B. Analysis of our data supports this hypothesis; however, our data is not significant. If the trend in our preliminary data persists with a larger sample size and shows significance, this would support our hypothesis. We can further hypothesize that since females who double brood are in better body condition, they are more likely to produce more male offspring than females in poor condition. After having a successful clutch, females may put less energy into males in their second brood. Future experiments should be conducted with larger sample sizes, taking into consideration other variables such as females who don't have a successful first brood, females who nest in natural cavities and females who single brood.

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