

Proposal Assignment: Analyzing Density Dependence and Egg to Fledgling Rates  
Significance of Female Sex Ratio on Survival Rates

EEB313

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### **Hypothesis and Predictions**

We have chosen to complete a hypothesis-driven project based on the study, Density Dependence and Egg to Fledgling Rates. The study was testing for optimal life-history theory within spotless starling, *Sturnus unicolor*, populations. They tested for clutch size and sex ratio at different density locations over 5 years. Spotless starlings are sexually dimorphic, polygynous, and are capable of altering sex ratios. They predict that in high-density locations, daughters will be overproduced due to daughters depending less on their state as a fledgling for their lifetime reproductive success and having further dispersal compared to sons. The results of this study showed that density has a large impact on brood sex ratios; higher female sex ratios occur in high-density locations when compared to low-density locations with high statistical significance. It was found that clutch sizes varied among HD and LD locations but also varied among years. For the most part, clutch sizes decreased in HD locations and increased in LD locations, however, some variation between years was present. It was found that fledgling weight did not vary a significant amount among HD and LD locations. Therefore, this study concluded a strong correlation between sex ratios and density.

It is predicted that in high-density brooding locations, the rate of successful egg to fledgling will be lower. In high-density locations, there will be more competition for resources, and thus the rate of successful egg to fledgling will be lower when compared to low-density locations. In addition, high-density locations yield a higher female-to-male ratio. Since competition is increased in high-density locations, and a high female sex ratio is correlated with high-density locations, we can predict that a higher female sex ratio is a response to increased competition. We can then predict that broods with a higher female sex ratio will experience higher survival rates than those with higher male sex ratios, per density location.

Our hypothesis states that the female sex ratio affects egg-to-fledgling survival rates in populations. We state the female sex ratio as the independent variable and egg-to-fledgling survival rates as the dependent variable.

We predict the female sex ratio and egg-to-fledgling survival to be positively correlated and the male sex ratio and egg-to-fledgling survival to be negatively correlated. It is expected that as the female sex ratio increases, survival rates will increase and as the male sex ratio increases, survival rates will decrease. This will be a method to test if high female sex ratios lead to higher survival rates and are an effective response to increased competition. We predict that female sex ratio has an impact on fitness. We can also see how the female sex ratio impacts birth rates, we

predict that with more females there will be higher birth rates, due to low cost males and high cost females.

## Data

The data in this study was collected by using nest boxes on a colony of starlings in Manzanares el Real, in Madrid Spain. The 48 nest boxes were scattered among trees in the flat pastureland. It was shown that as the number of nest boxes increased the reproductive success of mothers decreased. High-density locations used 4 groups of 6 nest-boxes within approx. 10 m of each other. Low-density locations spread the 24 boxes approx. 58 m apart from each other. It has been observed that starlings will nest from 2m to 70m apart. After sexing, counting, and weighing the broods, the data was analyzed using linear models to determine the effects of breeding density on brood sex ratios. They also analyzed the effects of breeding density on clutch size and fledgling body mass using mixed-effect modelling.

The data collected in this study includes 11 columns: Mother ID (mother), treatment (low density vs high density), year, clutch order (1st vs 2nd clutch), hatching date, nest ID, number of eggs (eggs), number of hatched eggs (hatchlings), number of fledglings 16 days after hatching (fledglings), number of male hatchlings (males), numbers of female hatchlings (females). Mother and Treatment are given as character values. Year, Clutch, Date, Nest, Eggs, Hatchlings, Fledglings, Males, and Females are given numerically. There are 210 rows of collected data.

The main columns we will be looking at are Eggs, Hatchlings, Fledglings, Males, Females and additionally Treatment, Year and Weight as random effects. To analyze deaths, we will have to look at survival from eggs to fledglings. This can be done by simply subtracting fledgling numbers from egg numbers and producing the variable "deaths". To calculate "Survival Rates" we will divide Fledglings by Eggs. We can also look at survival rates from eggs to hatchlings and from hatchlings to fledglings. This may uncover other potential influences on survival. This can be done using the same subtraction and division methods as seen in Fledgling Survival and Survival. We will also need to produce the variable "Female Ratio". This will be done by dividing the variable Females by the variable Males. We will also be looking at the effects of females' percentage on successful eggs to fledgling with Treatment. This data will be derived from the columns  $\text{"Females"/("Males" + "Females")}$ . We also need to create the variable of "Births" this can be done by categorizing the number of eggs produced by year, using the select function. We will be able to calculate birth and death rates of this population. We will also need to account for treatments to properly provide evidence that a high female ratio results in higher survival. We must select these data and categorize them by HD and LD to see if HD and LD have a more substantial effect than the female ratio. In addition to see the impacts of the female ratio per treatment. We will use select to create separate groups to analyze.

From a quick analysis in R, we can see that deaths and the female ratio have a negative correlation trend, that being as the female ratio increases, deaths

decrease. We can also see that as female ratio and survival have a positive correlation trend, as female ratio increases, survival rates increase. What is interesting to note is there appears to be a sudden decrease in survival/increase in death rates once the female ratio is greater than 5. This is interesting to note that there are limitations on the effectiveness of high female sex ratio, and this will be interesting to explore further. We can also see that the majority of deaths occur from the eggs to hatchling period than in the hatchling to fledgling period. With a total of 119 hatchling deaths, and a total of 77 fledgling deaths, over the 5-year period. In total there were 196 deaths. Over the 5-year period, there were a total of 434 females and 412 males, 846 subjects overall. The overall female to male ratio is 1.053398. Based on the data in this study we calculated that the mortality rate in females was 45.2% and the mortality rate in males 47.6%. The overall mortality rate in the entire population is 23.2%.

Our analysis plan is to use both linear models and mixed-effect models. Linear models can be used to directly compare sex with death rates or survival rates. We can also use this to look at the impacts of female sex ratio on birth rates. In a mixed model, we are looking to find a correlation between increased survival, death rates, and sex, with sex as a fixed effect. We must account for random effects such as year, treatment (HD and LD), and weight, which may impact survival rates. If year, treatment and weight account for high amounts of variance we can understand that survival rates may be more dependent on environmental circumstances than sex predisposition. We will use t-values to detect the significance of fixed effects and use p-values to reject or accept the validity of these results. We also will analyze survival from egg to hatchling and survival from hatchling to fledgling. We want to find if there is a stronger correlation in sex ratio with hatchling survival or fledglings survival, or if they are evenly impacted. There may be additional factors impacting this, or sex may play a stronger role in one over the other. If we divide deaths by hatchling and fledgling deaths and see the correlation of each with sex, year, and treatment, we can determine if sex has a larger impact on a particular stage of life for starlings. In a linear mixed model we have the assumptions of normality of the errors, homogeneity of error variance and independence of observations. We will use the `comodel` function to produce normality graphs to determine normality. We will use residual vs fitted and scale-location graphs to determine homogeneity of variance. We can also assume there are independence of observations due to the methods of data collection in this study.

To further our analysis and provide more insight into the field we can use survival and reproduction in this population as a measure on fitness. Since eggs are categorized by year, we can determine how many eggs are produced in each generation since starlings produce a new clutch on a yearly basis. We also have survival data based on egg to fledgling data. From there we can measure fitness in terms of survival (number of deaths) and reproduction (number of eggs) in each generation. We will be able to produce a measure of fitness in this population for the trait of sex ratio. From there we can conduct a regression on this trait to see how it will evolve. We can make predictions on how sex ratio will change in this population.

The assumptions of a linear regression are linearity, which we can see in the relationship between survival and female sex ratios. Independence which is assumed from the methods of this study. Normality will be analyzed, but is assumed. We assume no multicollinearity at this point in the study but may occur when considering year and treatment as random effects. We assume no endogeneity, and we assume homoscedasticity in our data, but will do further analysis to verify.

## **Citation**

Rubalcaba, J.G. and Polo, V. (2022), Density dependence of clutch size and offspring sex ratio in starling colonies. J Avian Biol, 2022: e02993. <https://doi.org/10.1111/jav.02993>

Dataset: <https://zenodo.org/record/6525052>