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Traffic Noise and Sexual Selection: Studies of Anthropogenic Impact on Bird Songs and Undergraduate Student Reasoning of Evolutionary Mechanisms

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TRAFFIC NOISE AND SEXUAL SELECTION: STUDIES OF ANTHROPOGENIC IMPACT ON BIRD SONGS AND UNDERGRADUATE STUDENT REASONING OF EVOLUTIONARY MECHANISMS

by

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TRAFFIC NOISE AND SEXUAL SELECTION: STUDIES OF ANTHROPOGENIC IMPACT ON BIRD SONGS AND UNDERGRADUATE STUDENT REASONING OF EVOLUTIONARY MECHANISMS

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Humans have transformed much of the natural landscape and are continuing to do so at an accelerated rate, compromising natural areas that serve as important habitat for many species. Roads impact much of the environment as they fragment habitat and introduce traffic noise into the acoustic environment, deferentially affecting wildlife in roadside habitat. I explored how traffic noise affects the detection of birds based on whether their vocalizations were masked by traffic noise. Masked species detection was not affected by an increase in traffic noise amplitude, while there was a negative effect of traffic noise amplitude on unmasked species detection, an unexpected result. Conducting more experiments on individual species detection will help ecologists better understand the changes in behavior that influence detection. The effect of human activity on the environment should be better understood by more than just ecologists. Yet, people in the United States fall behind other developed countries in their understanding of many scientific processes, such as evolution. Improved evolutionary knowledge leads people to have a higher acceptance of evolution, and biology educators are responsible for improving evolution education to promote more acceptance. For example, biology students seem committed to survival-based reasoning of evolution, but there are other important evolutionary forces to consider, such as sexual selection. Multiple selection pressures can act on a species, including pressures that select for traits that are

maladaptive for survival. Through interviews, we explored how selection for the same and different trait variants affected student reasoning of evolution. When asked to describe evolution in a scenario where selection favored the same variant of a trait, students relied on survival-based reasoning. When students were presented with a scenario where different selection pressures selected for different trait variants, most students described how sexual selection acted on the traits of the population and included reproductive potential as a component of fitness and inheritance in their descriptions of evolution. Teaching examples with scenarios where different selection pressures are selecting for different traits may improve student ability to reason about the role of sexual selection in evolution and the role of reproductive potential in fitness, improving overall understanding of evolution.

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CHAPTER 1: INTRODUCTION AND STUDY OBJECTIVES

Humans have transformed much of the natural landscape and are continuing to do so at an accelerated rate, compromising natural areas that serve as important habitat for many species (Mittermeier et al., 2003; Watson et al., 2016). Humans are imposing large-scale changes on the environment that have led to major ecological consequences like increased greenhouse gas emissions, pollution, and landscape transformation (Steffen et al., 2015). The patterns of global change are very reflective of the patterns of human behavior, occurring at a similar rate and during the same time period. As humans continue to change the land and cause habitat fragmentation, biodiversity will be threatened (Haddad et al., 2015). Collectively, us humans can change our behavior to reduce our negative impact on the environment. We are responsible for deciding whether to maintain ecosystems or to continue to degrade them, making our understanding of the natural world incredibly important (Sanderson et al., 2002).

Human use of roads is a prime example of how we are introducing several negative stimuli into the environment. Roads increase habitat fragmentation and introduce light and sound pollution into the environment, which affects about 20% of land in the United States (Forman, 2000). The biggest impact of roads is the introduction of loud traffic noise into the acoustic environment (Barber et al., 2010). Traffic noise alone has a direct effect on wildlife and the habitat in which they reside. Road developers implement noise mitigation plans into road development projects (Coffin, 2007), but roads continue to introduce unnaturally loud traffic noise into the environment (Pijanowski et al., 2011).

Many organisms rely on sound for communication, and in areas with loud traffic noise, communication can be compromised (Reijnen & Foppen, 2006). Birds are

especially affected by traffic noise due to their reliance on vocal communication to attract mates (Kight & Swaddle, 2011). Traffic noise affects an important component of avian reproduction and could cause evolutionary change in birds over many generations. The behavioral response of birds to traffic noise is an example of one of the many ecological effects caused by humans. While the scientific community is still determining the specific effects of traffic noise, it is well established by ecologists that traffic noise has an effect on wildlife (Shannon et al., 2017).

To better understand how humans are impacting the environment, we explored how traffic noise is affects which species are detected in roadside habitat. Based on vocalization characteristics, birds can respond different to traffic noise (Shannon et al., 2017). For example, some species have vocalizations that overlap traffic noise in frequency, while others do not, leading to different responses. Furthermore, individual species with similar vocalization frequencies can respond differently based on other aspects of their biology. The ecological study in this thesis (Chapter 2) provides an example of different species' responses to traffic noise.

Science is our tool for explaining the natural world, yet the opinion of the general public does not align with information presented by scientists. There is a disconnect between scientific consensus and the popular belief of the public (Ding et al., 2011). For example, around 2/3 of people in the United States believe in climate change, while almost all climate scientists support that climate change is occurring and caused by human activity (National Science Board, 2018). Therefore, people will not be as apt to support environmental policies to mitigate climate change, especially if they require significant personal trade-offs. This creates a challenge for scientists and environmental

policymakers that are trying to gain support for policy changes that benefit the environment.

Compared to other developed countries, there is low scientific acceptance in the United States. For example, there is a lower proportion of people in the United States that believe in evolution than 33 other developed countries, despite scientific consensus in support of evolution (Miller et al., 2006). It is critical for the public to have awareness of evolutionary mechanisms as they can impact our daily lives. For example, the general public lacks knowledge about antibiotic resistance, but people make decisions about whether to use antibiotics and expect them to be used, even for acute illnesses (McCullough et al., 2015). Additionally, the changes we induce on the landscape have evolutionary implications. It is important for people to understand how their behaviors may affect the evolution of other organisms through our impact on the environment and artificial selection.

The more knowledge a person has of evolution, the more likely they are to accept evolution (Weisberg et al., 2018). Since knowledge of evolution increases acceptance of evolution, there needs to be more efforts to improve instruction of evolution to improve the evolutionary knowledge of the public (Miller et al., 2006). The general public's knowledge of scientific topics overall has not really changed over the last twenty years (National Science Board, 2018). There should be efforts to not only improve student knowledge of important science concepts, but also providing opportunities for students to gain the problem solving and critical thinking skills necessary to for scientific reasoning.

Engagement in Science, Technology, Engineering, and Math increases evolutionary acceptance (Heddy & Nadelson, 2013). It has been found that The United

States is falling behind other countries in the retention of students in STEM (science, technology, engineering, and math), and consequently, the number of US citizens contributing to STEM work in the United States (Dugger, 2010; National Science Board, 2018). STEM jobs pay more and people with STEM degrees have lower unemployment rates. Low interest in STEM fields where there are plenty of jobs available suggests that people perceive science as more difficult and do not want to pursue subjects with higher risk for failure (Sithole et al., 2017). There seems to be a bit of resistance to learning science, leading people to have less knowledge of the way in which we are impacting the environment. Therefore, improvements in science education may help increase public acceptance of evolution.

In the field of biology education research, there are many studies dedicated to student reasoning of evolution as evolution is a particularly difficult topic for undergraduate biology students (Ziadie et al., 2018). Evolutionary concepts challenge students to apply concepts to evaluate and predict how evolution may act on a population. Students carry many misconceptions about evolution, which may affect their ability to think critically about potential evolutionary implications of human behaviors (Bishop & Anderson, 2002). Improving baseline knowledge of evolution is not sufficient for improving evolutionary acceptance. Students must learn how to think critically and apply their knowledge to novel situations (Sinatra & Hofer, 2016). To train students to think critically, it is important to understand how they are reasoning about important scientific concepts like evolution.

Helping students think critically about different components of evolution and different selection forces can improve evolutionary knowledge (Nehm & Reilly, 2007;

Price & Perez, 2016). However, students tend to relate much of evolutionary change to natural selection alone, leaving other important evolutionary forces, such as sexual selection, out of their evolutionary reasoning. Sexual selection can oppose natural selection, leading to different evolutionary trajectories (Chenoweth et al., 2015). The education study (Chapter 3), is a biology education research project where I explored how students are reasoning about evolution when presented with different interactions between sexual selection and natural selection. Providing students with scenarios where sexual selection opposes natural selection may guide students to include more important evolutionary components in their explanations of evolutionary change.

Studying the aspects of evolutionary reasoning is just one way to explore how to improve science education. Continued efforts to improve people's scientific reasoning and acceptance are necessary to gain public support of science-based policy changes. It is important for humans to understand the extent of our impact on the planet, which begins with education and continues with well-adapted management plans that benefit not only humans, but the other organisms that call Earth home.

CHAPTER 2: THE IMPACT OF TRAFFIC NOISE ON AVIAN VOCALIZATON DETECTION IN ROADSIDE HABITAT

ABSTRACT

Traffic noise may vary due to variation in human behavior and changes the acoustic environment of roadside habitat. Songbirds living in roadside habitat may be affected by traffic noise due to their dependence on vocal communication for important behaviors such as mating song. We explored whether the difference in dawn traffic amplitude on weekends versus weekdays had a differential effect on detection of songbird vocalizations. There was no difference in the number of species detected on weekends (median = 5) versus weekdays (median = 5) at 06:00 when the difference in traffic amplitude was greatest (5 dB) and at 09:00 when the difference in traffic amplitude was the least. Different species can have different responses to traffic noise based on whether their vocalizations are masked by traffic noise and their persistence in urban areas, affecting observable trends in community-level analyses. To address species-specific response, we explored how increasing traffic noise affected masked and unmasked species detection and specifically explored the behavior of three songbird species. American Robins and Northern Cardinals have masked vocalizations and were detected more as traffic noise increased, while the Red-winged Blackbirds have unmasked vocalizations and were detected less as traffic noise increased. These results opposed expectations and suggest other behavioral acclimations may impact detection of these species. Conducting more experiments on individual species detection and behavioral response will help ecologists understand mechanisms behind community-level trends in detection. Increased knowledge of bird behavior in roadside habitat will better inform management of traffic noise in areas with sensitive species.

INTRODUCTION

Humans are altering the landscape at an accelerated rate. From 1970 to 2000, 58,000 km^2 (~36,039 mi^2) of rural land area had been converted to urban use, with the highest rate of urban expansion occurring in North America. Urban expansion is exceeding the rate of urban population growth, suggesting that cities are also becoming more spread out (Seto et al., 2011). As cities continue to spread, humans will continue to alter the landscape to meet their needs. For example, humans have a high demand for roads, so as urban areas expand, roadways will also expand. The rate of the expansion of roadways has exceeded the rate of population growth by ten times (Barber et al., 2010). Over 20% of the land in the continental United States is affected by noise coming from traffic, and that number will increase as urbanization increases. Roads cover about 1% of U.S. land, but the ecological effects of roads span to about 15-20% of land due to light and noise coming from traffic (Forman, 2000). The impacts of traffic noise are not limited to urban areas. Even protected areas such as national parks are subject to anthropogenic noise exposure (Barber et al., 2011).

Transportation networks are primarily responsible for human-induced change on the acoustic environment (Barber et al., 2010). Traffic noise has such an impact on the soundscape that road development projects include extensive noise mitigation plans to reduce the negative impact of traffic noise on both humans and wildlife (Coffin, 2007). Despite these efforts, roads are introducing unnaturally loud noise into the soundscape, generating challenges for wildlife in roadside habitat (Pijanowski et al., 2011). Traffic noise itself is more of a disturbance than other factors from traffic as it introduces loud, constant, low-frequency noise into the environment that degrades roadside habitat

(Reijnen et al., 1995; Parris & Schneider, 2009; Ware et al., 2015). Many studies have isolated traffic noise from other ecological factors as having a direct effect on wildlife behavior, physiology, and habitat quality (Reviewed in Barber et al., 2010; Dowling et al., 2011; Shannon et al., 2016).

Traffic noise fluctuates over different times of day and across different days, leading to differential effects of traffic noise on roadside habitat based on day and time of day. For example, weekday traffic is typically louder than weekend traffic, and they have different timing of peak activity (NDOT, 2017). The difference in traffic activity between weekends to weekdays may generate very different soundscapes that can affect wildlife populations near roads by disrupting their communication (Halfwerk et al., 2011). For example, there is evidence of reduced raptor abundance near roads on weekdays compared to weekends, suggesting that some bird species avoid roads when they are louder (Bautista et al., 2004). While some species avoid habitat near roads altogether, (Reijnen et al., 1995), many bird species must acclimate to noisy conditions because suitable habitat may be limited to vegetation near roads, creating a trade-off between suitable habitat and a suitable acoustic environment (Warren et al., 2006; Parris and Schneider, 2009).

Birds are subject to many road ecology studies due to their reliance on vocal communication (Shannon et al., 2016). Overall, bird abundance decreases near roads, although population changes may differ based on the characteristics of the species within the community (Rheindt, 2003; Goodwin & Shriver, 2011). Traffic noise can affect age structure of the population because hatch year individuals avoid roads more than adults, and more experienced males can better perform behavioral responses to overcome traffic

noise (LaZerte, 2016; McClure et al., 2017). Some species that are more successful near roads may have greater reproductive success because there are typically fewer predators (Francis et al., 2011). However, species can also have reduced reproductive success when songs are distorted by traffic noise because female songbirds use vocalizations to assess quality of potential mates (Christie et al., 2004; Patricelli & Blickley, 2006; Swaddle & Page, 2007; Halfwerk et al., 2011). For example, birds in rural environments typically sing at a higher frequency than their urban counterparts, (Hu & Cardoso, 2009) and could be perceived differently by females, isolating populations if vocalizations differ too much. Whether traffic noise leads to evolutionary changes will depend on the magnitude of the impact on reproductive potential.

Loud traffic noise can cause *acoustic masking*, when one sound (in this case, a vocalization) is covered by another (traffic noise), reducing the detection of the masked sound (Rheindt, 2003). The higher the amplitude of traffic noise (i.e., louder traffic), the greater the effect of acoustic masking. Acoustic masking impacts wildlife residing in roadside habitat as it can disrupt communication by reducing the signal transmission of vocalizations (Reijnen & Foppen, 2006; Shannon et al., 2016). Birds are affected by acoustic masking from traffic noise because they rely on acoustic communication for behaviors such as alarm calls, mating songs, defending territory, and resource communication (Slabbekoorn & Ripmeester, 2008). Acoustic masking may affect species differently based on the frequency of their vocalizations. Traffic noise delivers constant, low-frequency noise into the environment, and many species have low-frequency vocalizations that overlap the frequency of traffic noise (Coffin, 2007, Halfwerk et al., 2011). Avian species with song frequencies that are low and overlap the frequency of

traffic noise are considered to have “masked” vocalizations, and birds with song frequencies that do not overlap traffic noise have “unmasked” vocalizations. Traffic noise can impact species with masked and unmasked vocalizations differently because of the overlap in frequency causing a greater effect of acoustic masking on low-frequency, masked vocalizations (Slabbekoorn & Peet, 2003; Hu & Cardoso, 2009; Halfwerk et al., 2011; Goodwin & Shriver, 2011). Bird species with songs that are a higher frequency may even be preadapted to urban areas because the frequency of their vocalizations does not overlap with the frequency of traffic noise (Hu & Cardoso, 2009).

The idea of differential effects on species based on frequency has led to many studies of the behavioral changes performed by masked species. Many bird species adjust their vocal behavior to overcome acoustic masking and communicate in habitat near roads with loud traffic noise. There is evidence of birds performing several types of short-term adjustments to traffic noise to overcome acoustic masking such as increasing vocalization frequency (pitch) (Gross et al., 2011; Oden et al., 2015), singing louder (increasing amplitude) Slabbekoorn & Peet, 2003; Brumm, 2004; Patricelli & Blickley, 2006), altering the rate of vocal signals performed (Diaz et al., 2010; Gross et al., 2010), and changing the timing of their vocalizations (Arroyo-Solis et al., 2013; Fuller et al., 2007). Birds with lower frequency vocalizations have a larger shift in song frequency in response to traffic noise (Parris & Schneider, 2009; Dowling et al., 2011). Birds with low-frequency vocalizations may have a greater short-term behavioral response than species with high-pitched vocalizations. However, it is unclear whether changes in frequency are effective enough for improving communication to be an adaptive response to high-amplitude traffic noise (Brumm & Zollinger, 2013). Low-frequency sound travels

a greater distance, and expending energy to sing at a higher frequency may make it difficult to maintain other vocalization characteristics such as song rate, the number of songs vocalized during a given amount of time (Gross et al., 2011).

More recent studies have provided evidence that frequency changes may not be the most adaptive strategy for birds to overcome loud traffic noise. Changes in amplitude may have more of an effect on avian success. Frequency changes as a behavioral adjustment in birds may be a byproduct to changes in amplitude due to the Lombard effect (an increase in frequency associated with an increase in amplitude) (Nemeth & Brumm, 2010). Birds that have low-frequency vocalizations must sing louder to overcome traffic noise, more so than unmasked species. Therefore, a change in frequency as a byproduct of amplitude changes may be the reason species with low-frequency vocalizations are observed making greater changes in frequency.

Persistence in urban areas may be another explanation behind the roadside success of certain individuals and species. The persistence of bird species in urban areas has a large effect on how they respond to increasing traffic noise (Gross et al., 2011). Generalist species, like American Robins are successful in urban environments (Evans et al., 2015). American Robins have masked vocalizations, but their success may be due to their high vocal plasticity, the ability to adjust vocalizations (Dowling et al., 2011) Species that are generally well-equipped for urban areas may be able to overcome the limitations imposed by having a low-frequency vocalization. Furthermore, individuals with more experience residing in loud areas are better able to adjust their vocalizations (LaZerte et al., 2016). Birds with high urban persistence may benefit from traffic noise reducing the number of predators and competitors in the area, which in turn can improve

reproductive success (Stone, 2000; Francis et al., 2011). Therefore, some birds may take advantage of their ability to adjust to roadside conditions and remain near roads, even roads with loud amplitudes.

The extent to which different species change behavior in response to traffic noise may be based on a species behavioral plasticity and vocalization characteristics. Furthermore, the impacts of traffic noise on avian communities can be very complex due to the non-uniform introduction of noise into the environment, leading to many studies exploring impacts on wildlife and behavioral responses (Shannon et al., 2016). To explore how differential traffic noise affect species with varying vocal frequencies, birds were recorded during the morning at sites near roads that have varying levels of traffic noise. Bird species counts were conducted to 1) determine if weekend and weekday soundscapes generate different avian vocalization detectability 2) observe how a gradient of traffic noise affects the detection of species with masked and unmasked vocalizations. I also provide a case study of three different commonly detected species (two masked and one unmasked). I predicted the number of species detected to decrease as traffic noise increased and there to be a significant difference between species detection on weekends versus weekdays. Additionally, I predicted masked vocalization detection would be more negatively affected by traffic noise than unmasked vocalization detection because traffic noise frequency overlaps the frequency of masked vocalizations.

METHODS

Data Collection

There were 28 study sites located near roads in Cass, Douglas, Lancaster, Sarpy, Saunders, Seward, and York counties in eastern Nebraska, USA [APPENDIX A]. Roads next to study sites carried traffic ranging from 5,000 to about 100,000 vehicles per day (NDOT, 2017). Study sites ranged from urban to rural habitat with varying habitat types. Sites also varied in habitat type with wetlands, small woodlots, grasslands, and urban habitats represented. Study sites were located at least 500 meters apart to avoid recording the same individuals simultaneously. Recorders were placed between 50 and 300 meters from roads to capture the soundscape of roadside habitat (Grade and Sieving, 2016).

Songbird vocalizations were recorded using wildlife audio recorders (Song Meter SM2; Wildlife Acoustics Inc., Maynard, MA; www.wildlifeacoustics.com). SM2 recorders were left at study sites to record automatically. Recordings were taken during the morning from April to June, when male songbirds were actively singing to attract mates. Recordings were taken on two weekend days and two weekdays at 06:00, 07:00, 08:00, and 09:00, each lasting 20 minutes. The largest volume of vehicle traffic in the morning occurred around 07:00 on weekdays and around 09:00 on weekends in Nebraska (Nebraska Department of Roads, 2017).

Data Analysis

Traffic Amplitude

The difference between weekend and weekday traffic noise amplitude was measured to determine the extent to which days carrying different loads of traffic generated different acoustic environments. Noise produced by traffic typically falls below a frequency of 2 kHz (Warren et al., 2006). Therefore, the amplitude of each recording

was set at the 0-2 kHz bandwidth to isolate noise produced by traffic from other ambient sounds. The average amplitude of traffic noise for each recording was measured using Raven Pro Software (version 1.5; Cornell Lab of Ornithology, Ithica, NY, USA, 2018), a wildlife vocalization analysis software. Using Raven Pro Software, we measured the average amplitude of traffic noise over each 20-minute recording. Using the selection tool, we framed the 0-2 kHz bandwidth of each recording to measure the traffic noise amplitude, other background noise being produced at higher frequencies than traffic noise.

The mean traffic noise for both weekends and weekdays was calculated from the for each recording (06:00, 07:00, 08:00, and 09:00) on two consecutive days (total of 8 recordings for weekends and 8 recordings for weekdays). Weekends and weekdays were then compared for each of the times. For example, the 06:00 weekend noise conditions were compared to the 06:00 weekday noise conditions for the same location. The traffic noise amplitudes for each recording time were compared between weekends and weekdays using a 2-tailed, paired t-test with $\alpha = 0.05$.

Species Detection

Species were detected by vocalization in each recording both visually and aurally. Each 20-minute recording was analyzed using Audacity (version 2.1.3; Audacity, Carnegie Mellon University, Pittsburgh, PA, USA). Audacity is a free software that allows for visual analysis of sound, and can be used to analyze wildlife vocalizations. Songbird vocalizations were visualized using the spectrogram view that displays vocalization frequency and amplitude. The spectrogram tool uses frequency and amplitude of each vocalization to generate an “image” of the sound, allowing for detection and identification of individual songs. The number of species at each location

was recorded using a presence/absence table. Species were marked with a 0 or a 1 to indicate whether each species had been detected in each 20-minute recording.

We compared the number of species detected in weekend versus weekday recordings by counting the total number of species in each recording. Then, an average of the two weekend and two weekday recordings was calculated for each location. Weekend and weekday counts were then compared at 06:00, 07:00, 08:00, and 09:00. For example, the number of species counted at 06:00 on weekends was compared to the number of species counted at 06:00 on weekends for one location. The number of species in each recording time were compared between weekends and weekdays using a 2-tailed, paired t-test with $\alpha = 0.05$.

The effect of traffic noise amplitude on the number of species detected was also determined in this study. To reduce pseudo-replication of individual males, only recordings at 06:00 on weekdays were used. Using multiple recordings from the same day may cause the same male to be counted multiple times. Using the 06:00, weekday data, we ran a generalized linear model with Poisson distribution using R Software to determine the effect of traffic noise amplitude on the number of species detected in a recording.

Masked vs. Unmasked Species

Masked and unmasked species were isolated based on whether their song frequency overlapped with the frequency of traffic noise, 0-2 kHz. If a species' song had any portion below 2 kHz, it was considered a species with a "masked" vocalization. If a species' song had a frequency that was completely above 2 kHz, it was considered "unmasked" (Figure 3.1). We compared the detection of masked and unmasked species in increasing traffic noise to determine the effect of traffic noise amplitude on birds with

songs that do or do not overlap traffic noise in frequency. Using the 06:00, weekday data, we ran a generalized linear model with Poisson distribution using R Software to determine the effect of traffic noise amplitude on the number of masked and unmasked species detected in a recording. The relationship between traffic noise amplitude was compared between the two song types.

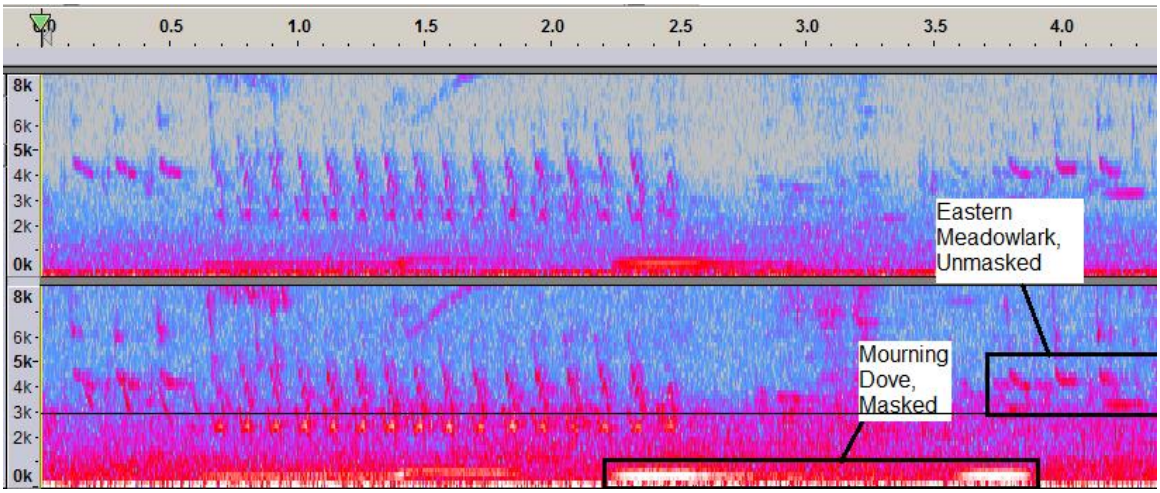


Figure 2.1) A spectrogram of songbird vocalizations and traffic noise, showing an example of a masked and unmasked songbird vocalization.

Individual Species Behavior

The five most detected species for each song type were determined based on the number of recordings in which each species was detected (Table 2.1).

Table 2.1) The five most detected unmasked species and the five most detected masked species.

UNMASKED	MASKED
Red-winged Blackbird (Agelaius phoeniceus)	American Robin *(Turdus migratorius)*
Common Yellowthroat (Geothlypis trichas)	Northern Cardinal (*Cardinalis cardinalis)*
Eastern Meadowlark (Sturnella magna)	Common Grackle *(Quiscalus quiscula)*
Yellow Warbler (Setophaga petechia)	Blue Jay *(Cyanocitta cristata)*
Eastern Kingbird (Tyrannus tyrannus)	Mourning Dove *(Zenaida macroura)*

Then, three individual species were selected, the two most detected masked species, American Robin and Northern Cardinal, and the most detected unmasked species, Red-

winged Blackbird. Each of these species were selected based on their commonality among study sites. Additionally, these three species have all been previously studied in traffic ecology literature, providing some explanations for the behavioral responses observed.

The relationship between individual species detection and the amplitude of traffic noise at the sites was assessed using a logistic regression model. We isolated 06:00, weekday recordings to avoid pseudo-replication, as the males of many species sing over multiple hours of the morning. Using the fitted model, the probability of species detection was predicted for the 75 dB and 80 dB amplitude levels, the average 06:00 noise levels for weekend and weekday traffic.

RESULTS

Traffic Noise and Species Detection

Weekday traffic noise amplitude measurements ranged from 80-82 dB, and weekend traffic noise amplitude measurements ranged from 75-80 dB. Weekdays had less variation in traffic noise across the morning, with a range of 2 dB, and all times were equally loud. Weekend traffic noise had a 5 dB increase from 06:00 to 09:00. Weekdays were consistently louder across the morning based on traffic noise amplitude data collected from recordings (Fig 2.2). Traffic noise measurements from this study were reflective of state traffic volume estimates that showed higher volumes of traffic on weekdays than weekends and a steady increase in traffic volume across weekend mornings (NE Department of Roads, 2017).

The largest difference in traffic noise between weekends and weekdays was at 06:00 with a 4.8 dB difference (p value < 0.05; t = 6.14, df = 43). This was the only recording time with significantly different traffic noise between weekends and weekdays. The smallest difference in traffic noise was at 09:00, with a difference in amplitude of 1.27 decibels (p value = 0.296, t = 1.056, df = 45), which was similar to the other recording times after 06:00. While weekends start out quieter than weekdays, by 07:00 the difference was not significantly different at the study locations. Traffic volume ranged from <1000 to 100,245 vehicles per day (NDOT, 2017), and traffic noise ranged from 52.1 decibels to 100 decibels across all sites.

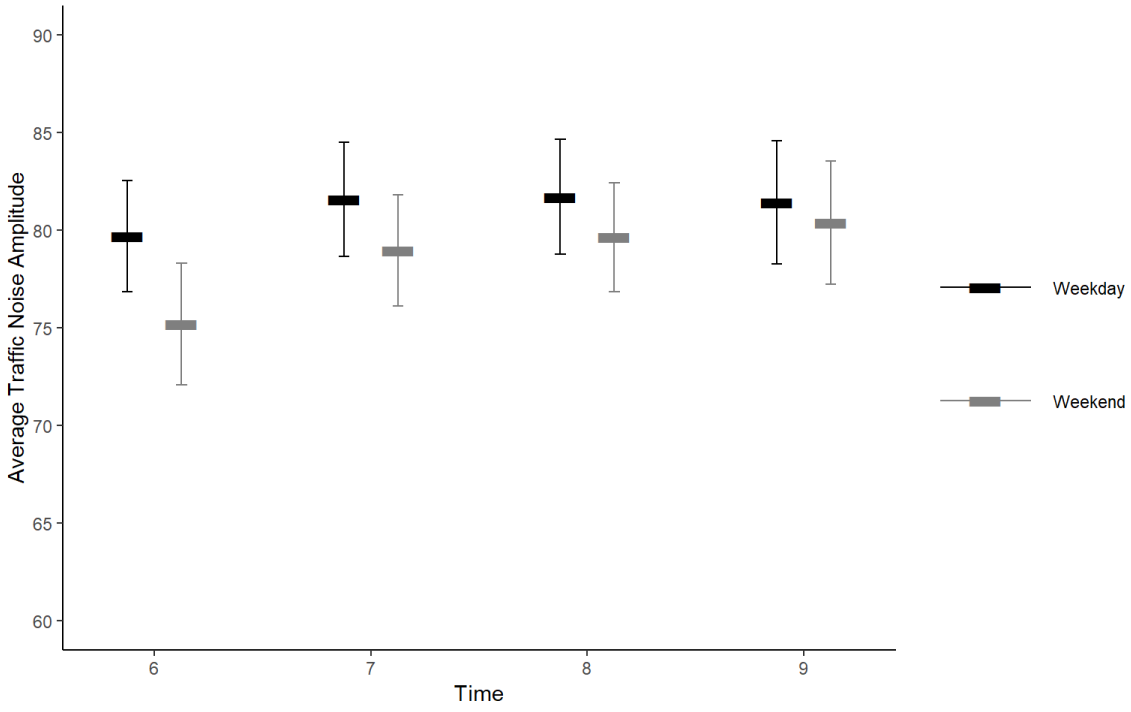


Figure 2.2) Traffic noise amplitude values for weekends and weekdays at each of the recording times (06:00, 07:00, 08:00, and 09:00).

The difference in species detection between weekends and weekdays was not significant for any time across the morning, even at 06:00 when the difference in traffic

noise between weekends and weekdays was significantly different. Typically, louder, more urban sites had fewer total species detected.

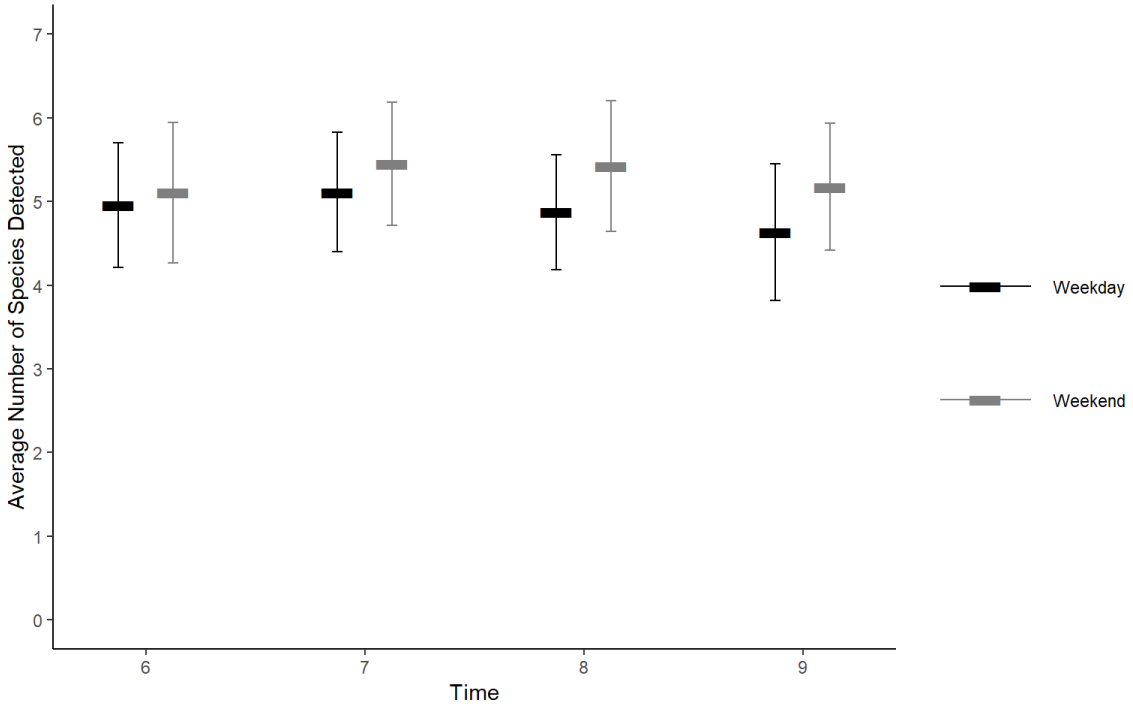


Figure 2.3) The average number of species detected at the four recording times (06:00, 07:00, 08:00, and 09:00) on weekends and weekdays.

There was a significant, negative relationship between traffic noise and species detection overall ($p = 0.02$; $F = 5.86$; $R^2 = 0.06$); (Figure 2.4). The R-squared value suggests that the data is not sufficient to account for the variation in number of species detected overall. We observed 40 different species across the 23 sites. Sites varied in species richness, ranging from 7-19 species detected. The quietest site (Riverside) had the greatest number of species detected, while the loudest site (Oak Hills Central 2017) had the fewest number of species detected. For mean weekend traffic noise amplitude (75 dB) there was an average of 5.2 species detected, and for mean weekday traffic noise amplitude (80 dB) there was an average of 4.7 species detected. Between the minimum

and maximum measurements of traffic noise, a range of 50 dB, the average number of species detected ranged from 6.7 to 3.8.

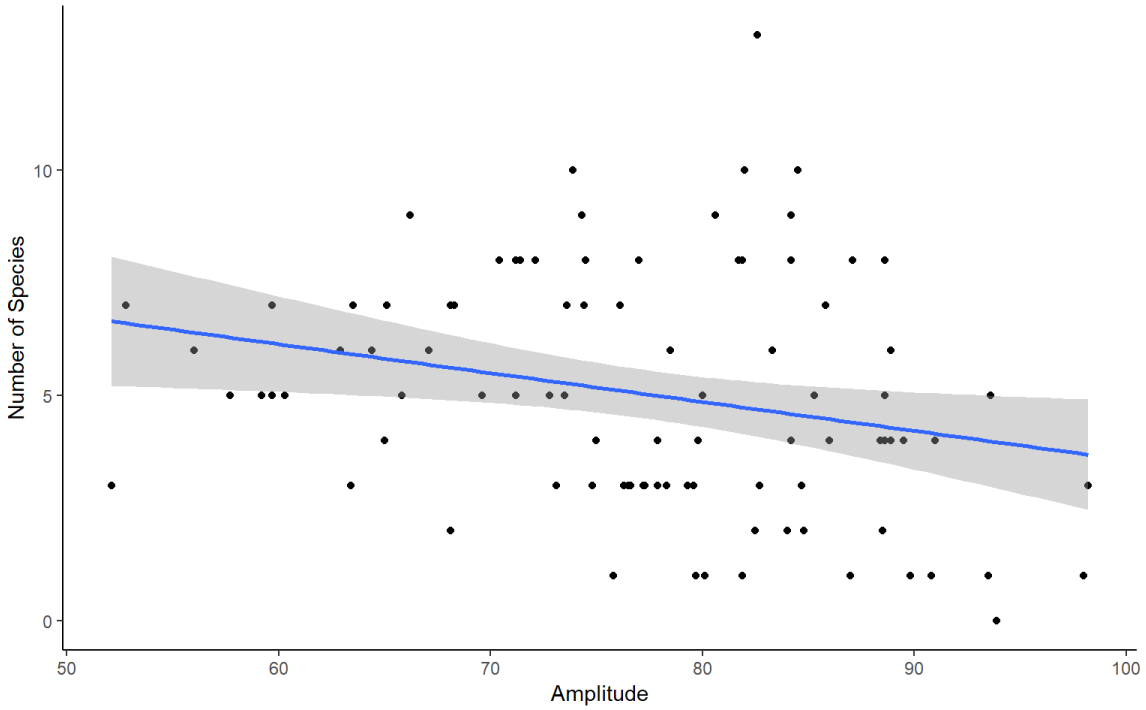


Figure 2.4) The relationship between the amplitude of traffic noise and the average number of species detected in a 06:00 recording A 5 dB increase in traffic noise results in a 0.4 decrease in the average number of species.

Masked and Unmasked Species

The relationship between traffic noise and species detection was different for masked versus unmasked species (Figure 2.5). There was a non-significant, positive relationship between traffic noise and the number of masked species detected ($p = 0.86$; $F = 0.032$; $R^2 < 0.01$) and there was a significant negative relationship between traffic noise and the number of unmasked species detected ($p < 0.01$; $F = 10.50$; $R^2 = 0.11$). The R-squared value indicates that the data may not account for all of the variation present. Traffic noise may affect unmasked species, species with vocalization frequencies that do not overlap the frequency of traffic noise.

At the average weekend traffic noise amplitude (75 dB), 1.9 masked species were detected and 1.3 unmasked species were detected, and at the average weekday traffic noise amplitude (80 dB), 2.1 masked species were detected and 0.9 unmasked species were detected. Masked species had a smaller difference in detection on weekends versus weekdays than unmasked species (Figures 3.5; APPENDIX F). For the range of traffic noise amplitudes recorded in the study (50-100 dB), the average number of masked species detected ranged from 1.7 to 2.3 species, and the average number of unmasked species detected ranged from 2.2 to 0.1 species. When the amplitude of traffic noise was higher, the difference between masked and unmasked species detection was greater.

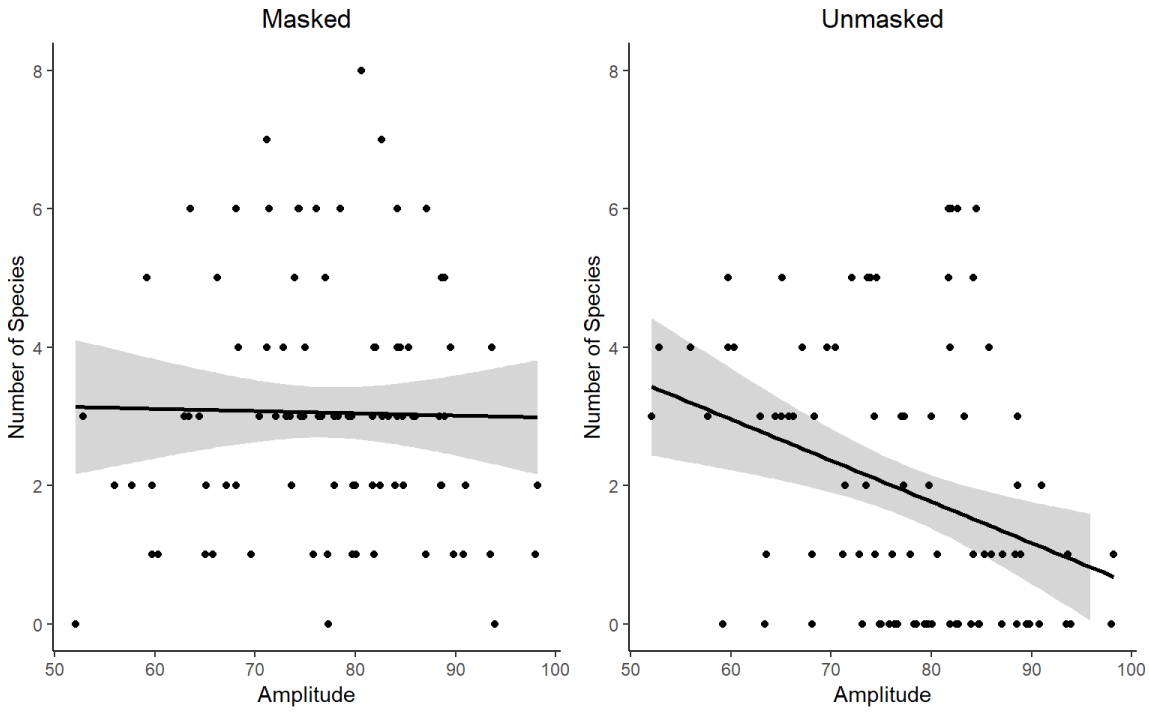


Figure 2.5) The relationship between the amplitude of traffic noise and the average number of masked species and unmasked species detected in a recording. The number of masked species detected increased as traffic noise increased ($p = 0.86$; $F = 0.032$; $R^2 < 0.01$), and the number of unmasked species detected decreased significantly as traffic noise increased ($p = 0.18$; $F = 1.89$; $R^2 = 0.01$).

Table 2.2) The probability of detection based on the fitted curves from logistic regressions of the five most detected masked and unmasked species at the average amplitude for weekend (75 dB) and weekday (80 dB) traffic noise.

	Species	Weekday	Weekend	Difference
Masked	**American Robin**	**74%**	**69%**	**5%**
	Common Grackle	33%	29%	4%
	Northern Cardinal	**39%**	**36%**	**3%**
	Blue Jay	17%	18%	-1%
	Mourning Dove	21%	24%	-3%
Unmasked	Common Yellowthroat	23%	33%	-10%
	Yellow Warbler	16%	23%	-7%
	Red-winged Blackbird	**25%**	**34%**	**-9%**
	Dickcissel	30%	31%	-1%
	Eastern Meadowlark	16%	22%	-6%

Individual Species

American Robin – Masked

American Robins were commonly detected at each recording time at all but two sites and were detected similarly on weekends and weekdays. At the average weekend traffic noise level (75 dB), the probability of detecting an American Robin vocalization was 67%, and at the average weekday traffic noise level (80 dB), the probability increased to 74% (Table 2.2). As traffic noise amplitude increased, American Robin detection increased (p = 0.153; z = 1.430), despite American Robin vocalizations being masked. There were more American Robins detected at louder sites than quiet sites, and robin singing persisted through all morning recording times.

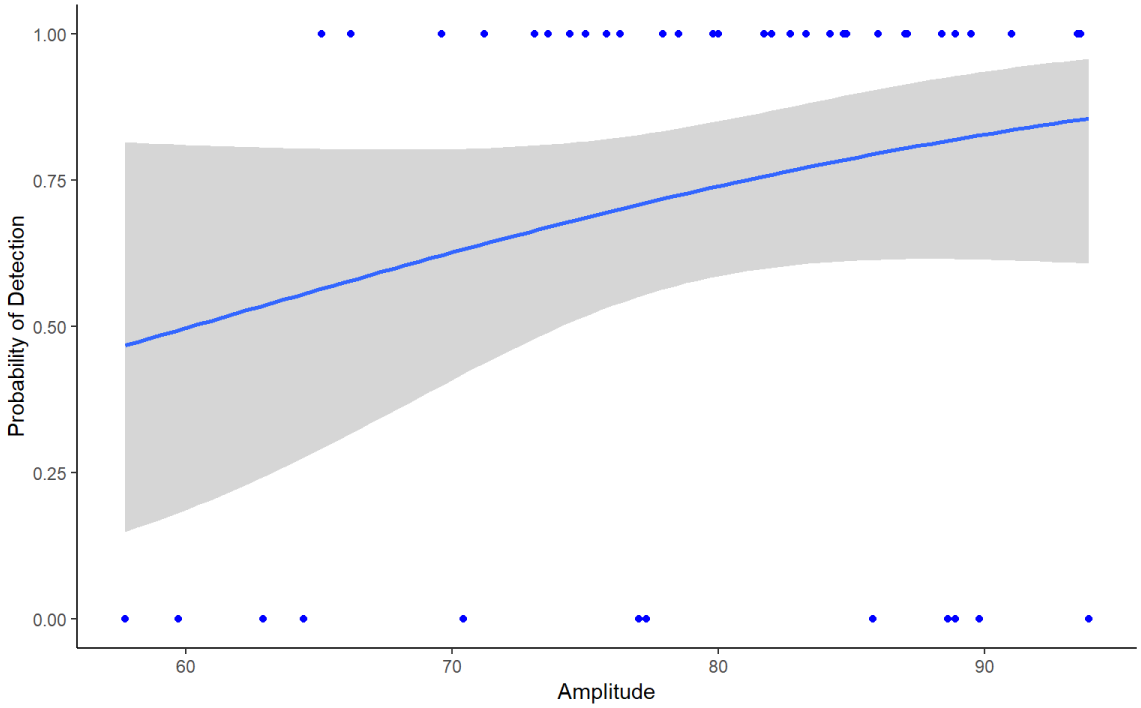


Figure 2.6a) American Robin detection probability increased with traffic noise amplitude (p = 0.153; z = 1.430). The solid line represents the fitted logistic regression and gray shading represents the 95% confidence interval. Traffic noise values were the mean of weekday 06:00 sampling times.

Northern Cardinal – Masked

Northern Cardinals were detected at 24 of 28 sites, across all recording times, and on both weekends and weekdays. As traffic noise amplitude increased, there was no change in the detection of Northern Cardinal vocalizations (p = 0.388; z = 0.8634). Northern Cardinal vocalization frequency overlapped traffic noise frequency but increasing traffic noise amplitude did not affect the probability of a Cardinal vocalization being detected. At the average weekend traffic noise level (75 dB), the probability of detecting a Northern Cardinal vocalization was 39% At the average weekday traffic noise level (80 dB), the probability decreased to 36%. Northern Cardinals seem to sing similarly on weekends and weekdays, and across a wide range of traffic noise.

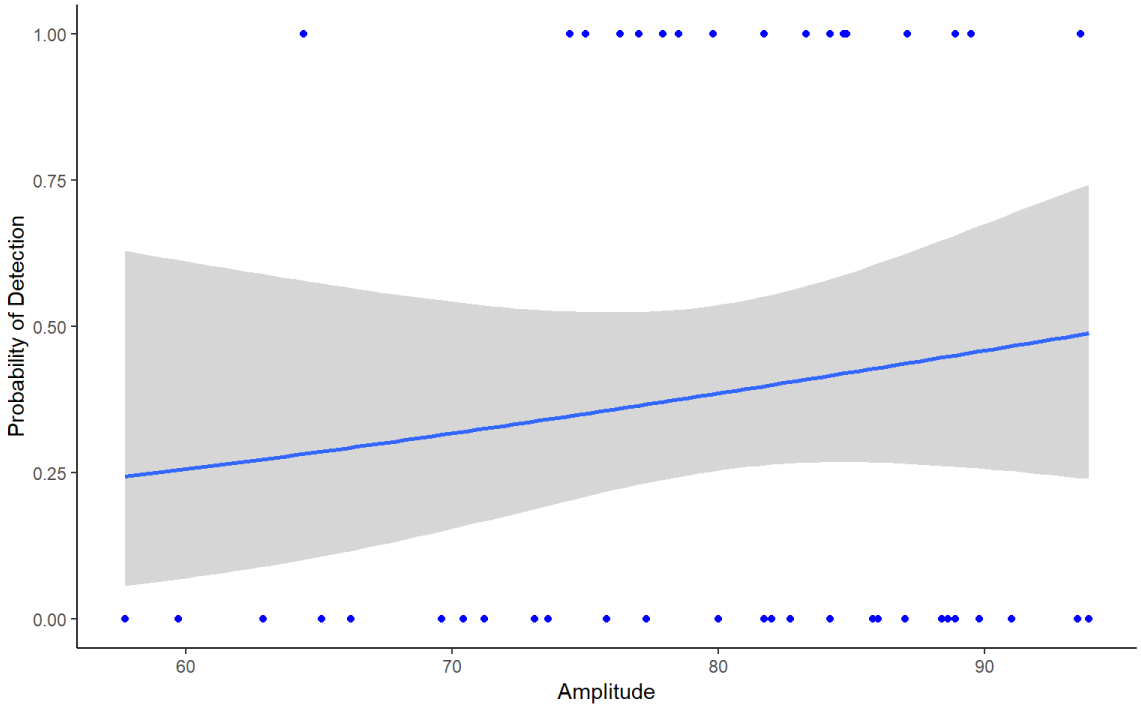


Figure 3.6b) The effect of traffic noise on Northern Cardinal detection was unclear (p = 0.388; z = 0.8634). The solid line represents the fitted logistic regression and gray shading represents the 95% confidence interval. Traffic noise values were the mean of weekday 06:00 sampling times.

Red-winged Blackbird - Unmasked

Red-winged Blackbirds were detected at 13 out of 28 sites and detected across all morning recording times. Their songs were detected when traffic noise was below 85 dB. As traffic noise increased, the likelihood of detecting a Red-winged Blackbird decreased (p = 0.023, z = -2.266). At the average weekend traffic noise level 75 dB, Red-winged Blackbirds were detected 34% of the time. At the average weekday traffic noise level 80 dB, Red-winged Blackbirds were detected 25% of the time.

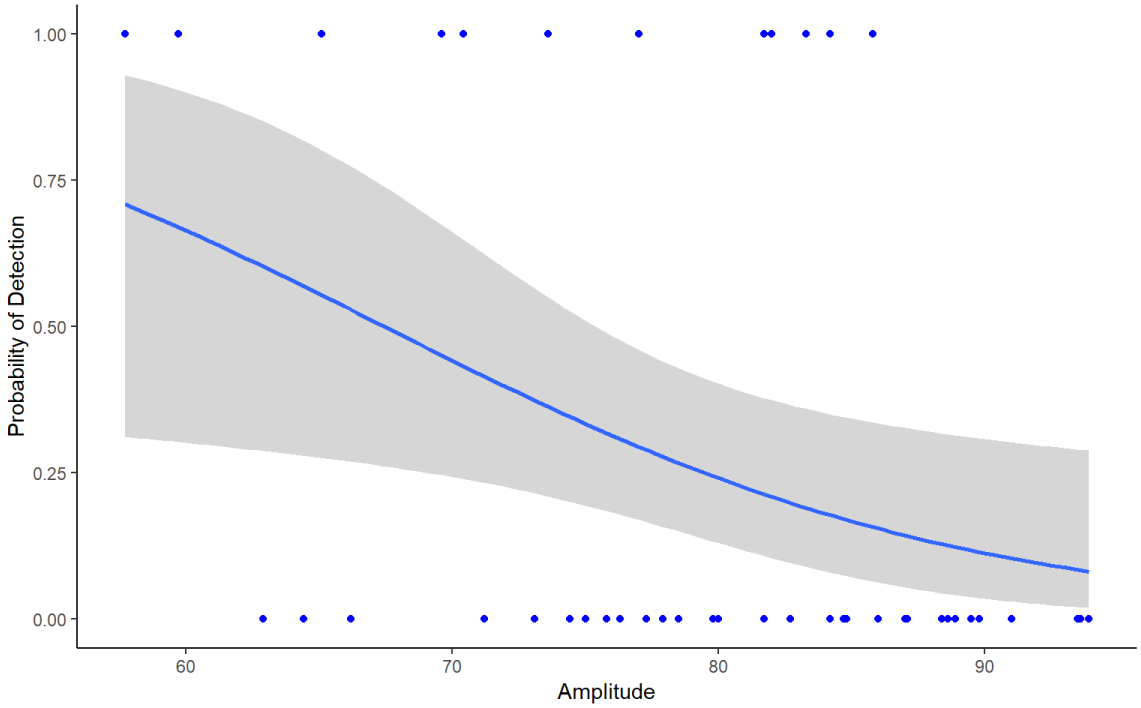


Figure 3.6c.) Red-winged Blackbirds were detected significantly less as traffic noise increased (p = 0.023, z = -2.266). The solid line represents the fitted logistic regression and gray shading represents the 95% confidence interval. Traffic noise values were the mean of weekday 06:00 sampling times.

DISCUSSION

Traffic Noise and Species Detection

Overall, the number of species detected decreased significantly as traffic noise increased (Figure 2.4), but the difference in traffic noise between weekends and weekdays (Figure 2.2) was not enough to cause a significant change in detection for most species (Figure 2.3). The amplitude of traffic noise in this study ranged from 50 to 100 dB (mean = 75-80 dB), and the amplitude where traffic noise begins to affect bird behavior is approximately 45 dB (Shannon et al., 2017). The minimal difference in number of species detected on weekends and weekdays (Figure 2.2) may be due to 4.8

dB being a relatively small change when the amplitude is high. All observations in this study occurred when traffic noise was above the amplitude where behavior change in birds has occurred. A previous study that found significantly lower abundance of birds near roads on weekdays when traffic load on weekdays was double of that on weekends (Bautista et al., 2002). The difference in traffic noise between weekends and weekdays was much less in our study. Traffic noise difference may need to be greater than 4.8 dB to have a significantly different effect on detection.

Masked vs. Unmasked Species

Masked species were detected more as traffic noise amplitude increased, and traffic noise amplitude did not affect the detection of species with masked vocalizations in this study (Figure 2.5). Since unmasked vocalizations are a higher frequency than traffic noise, they can be detected on a spectrogram even when the amplitude of the vocalization is not greater than the amplitude of traffic noise. The decrease in unmasked species detection with increasing traffic noise amplitude suggests a behavioral change. Unmasked species may not have sung as much or avoided roadside habitat when traffic noise was loud. It has been previously found that birds with masked vocalizations are typically detected less near roads (Coffin, 2007, Dowling et al., 2011), and birds with unmasked vocalizations are more successful in communication and have higher relative abundance (Arroyo et al., 2013). Therefore, I expected birds with masked vocalizations to be detected less in loud traffic noise than unmasked species, since traffic noise directly overlapped their song. However, the results of this study were opposite of the expected outcome.

One reason for this unexpected result may be due to the variation in traffic across and within sites (Patricelli & Blickley, 2006). The average traffic noise was different for

each site, and the changes in traffic noise across the hours of the morning and days of the week were also inconsistent. In a study of the effect of traffic noise on the Eastern Peewee, an unmasked species, they changed the tonality of their vocalizations in response to the traffic noise amplitude at the time of their songs, rather than overall traffic noise at the site. The fluctuations in traffic noise may have more of an effect than the average background noise at a location (Gentry et al., 2018). Therefore, the average traffic noise of the recording may not explain the effect of traffic noise as well as traffic noise at the time of each vocalization.

Birds have peak vocalization times just as there are peak times in traffic noise, and the relationship between these two peak times may have an effect on detection. Therefore, when a bird sings may be just as important as how a bird sings in loud roadside habitat. Some species may change the timing of vocalizations rather than changing the characteristics of their song (Hanna et al., 2011; Arroyo-Solis et al., 2013). If the species changed the timing of vocalizations and sang during quiet moments throughout the day, it may be the reason they were not detected during times with loud traffic noise. However, determining if species changes the timing of vocalizations or characteristics of their song will require monitoring individual birds or populations rather than community-level detection. The timing of the dawn chorus differs for each species, and some species may sing at times where traffic noise is relatively louder. If species near roads have peak singing times that overlap with peak traffic times, such as Robins, there may be more artificial selection pressure for them to adjust their vocalizations to communicate in loud traffic noise.

It is possible that the masked species in this study changed their vocalization behavior to overcome loud traffic noise, leading to the result that there was no effect of traffic noise on the detection of masked vocalizations. Masked species may have increased their singing rate. If an individual was singing more, the possibility of detecting that individual would increase simply because there were more opportunities for the amplitude of the song to overcome the amplitude of traffic noise and for the song to be detected. Another possibility is that the masked species may have increased the amplitude of their vocalizations. Since traffic noise can fluctuate, a masked vocalization may be eventually detected in a recording because the bird sang at an amplitude higher than the traffic noise at that time (Slabbekoorn & Peet, 2003). Both of these responses may not ensure that every vocalization is detected, but increasing song rate or amplitude will increase the probability of detection, improving signal transmission.

While many studies have explored the effect of frequency on how birds respond to traffic noise, recent evidence suggests that frequency changes may not increase signal detection as well as amplitude changes. Vocal plasticity, the ability to adjust vocalizations, may be a better explanation as birds with high vocal plasticity are more persistent in urban areas (Gross et al., 2011). Birds that can adjust their vocalizations enough to overcome traffic noise may be more successful than birds that sing at high frequency, but cannot adjust characteristics of their song. The masked species in this study may be more vocally flexible, altering song characteristics such as amplitude, allowing them to persist in noisy roadside habitat (Nemeth & Brumm, 2010; Francis et al., 2011). However, this behavioral response may not sufficient for some species. For example, Great Tits (*Parus major*), an unmasked species, adjust the amplitude of their

vocalizations in response to traffic noise, but the increase in amplitude does not improve the signal transmission enough to communicate as effectively as in quiet environments (Templeton et al., 2016). Perhaps the common masked species had higher vocal plasticity on average than the common unmasked species.

In addition to variation in when a bird sings, there is also variation in where songs are coming from. Birds that reside further from the forest edge typically sing at a lower frequency than those at forest edge and in grassland habitat (Ryan & Brenowitz, 1985). In a study that explored the effects of traffic and proximity to forest edge on bird occupancy, it was found that for low-frequency vocalizations, traffic noise had the greatest effect on occupancy, while distance from forest edge had a greater effect on birds with unmasked vocalizations (Goodwin & Shriver, 2011). Therefore, the habitat of the study sites may have an effect on whether masked or unmasked species were present and detected, as study sites varied in habitat type.

Individual Species

When and how a bird changes behavior in response to traffic noise is species-dependent, whether that is some sort of temporal change or a change in a song characteristic. The two most detected species in this study were American Robin and Northern Cardinal, both of which are masked species. None of the five most detected masked species had a significant decrease in detection as traffic noise increased (Table 2.1). In fact, two masked species, the Northern Cardinal and American Robin, had increased detection as traffic noise increased (Figures 3.7a and 3.7b). They were detected at almost all sites and in various traffic noise amplitudes at each site. Unmasked species like the Red-winged Blackbird and Common Yellowthroat were detected often when traffic noise was around the mean but were detected less often at sites much louder than

the mean. In contrast, four of the five unmasked species, Red-winged Blackbird, Common Yellowthroat, Eastern Meadowlark, and Yellow Warbler, had a significant negative response to traffic noise (Table 2.1). Red-winged Blackbirds served as a representative for these species since the four species had similar changes in detection with increasing traffic noise, as the Red-winged Blackbird was most commonly detected in the study.

American Robin and Northern Cardinal (Masked)

The probability of detecting an American Robin increased significantly as traffic noise increased, (Figure 2.6a) and Northern Cardinal detection was not affected by traffic noise (Figure 2.6b). Both American Robins and Northern Cardinals are generalist species that are more successful in urban environments (Evans et al., 2015). The two species have masked vocalizations, but their success may be due to their high vocal plasticity, the ability to adjust vocalizations (Dowling et al., 2011; Francis et al., 2011), allowing them to persist in noisy roadside habitat (Figures 2.6a; 2.6b). Based on their persistence in urban areas, American Robins may be able to alter their songs enough to continue to effectively communicate in noisy conditions, as high vocal plasticity has a large impact on persistence (Gross et al., 2011), and their ability to tolerate loud areas may be an explanation for a greater probability of detecting American Robins in increasing traffic noise. Their persistence gives them an advantage in areas where traffic noise gets too loud for other species.

Northern Cardinals are known to be persistent in urban areas as well (Leston & Rodewald, 2006). In this study it was found that the probability of detecting a cardinal vocalization was similar for all traffic noise levels. Cardinals do not change singing rate or song length with increasing traffic noise (Seger-Fullam et al., 2011). Like robins,

cardinals change their vocal frequency as noise level increases (Seger-Fullam et al., 2011). Their vocal plasticity allows for them to continue to sing in a loud acoustic environment (Francis et al., 2011). Cardinals also have similar reproductive rate and survival rates in urban and rural areas, further supporting that cardinals are less affected by urban activity than other species (Leston & Rodewald, 2006).

Red-winged Blackbird

Red-winged Blackbirds have an unmasked song, but the probability of detecting a Red-winged Blackbird decreased as traffic noise increased (Figure 2.6c). From the 80 dB weekday average amplitude compared to the 75 dB weekend average amplitude, there was a 9% decrease in the probability of detecting a Red-winged Blackbird. The difference between weekend and weekday morning traffic noise may be enough to generate a change in Red-winged Blackbird singing behavior.

Vocalization characteristics are important for Red-winged Blackbird success. Females select mates based on territories that are established by males. Male Red-winged Blackbirds with larger song repertoires are perceived by females as more experienced and have an advantage in competition for territories (Yasukawa et al., 1980). A change in song characteristics may alter female perception of male quality. If traffic noise amplitude reaches a point to where it affects the transmission of male vocalizations by masking the vocalization, portions of a male's repertoire may not be heard by females, or the female may perceive the male as less fit because his entire repertoire was not transmitted. Females also use introductory syllables for individual recognition and males near loud traffic noise reduce the number of syllables in the introductory notes of their song (Cartwright et al., 2014). Therefore, female perception of male fitness may be

affected if males change their repertoire too much, which may reduce reproductive success for the species.

A previous study found that Red-winged Blackbirds increased their vocalization rates during midday to avoid overlapping the loudest traffic activity (Cartwright et al., 2014). It is possible that the Red-winged Blackbirds were detected less because they were singing less when the traffic noise amplitude was high. There is evidence that Red-winged Blackbirds increased the tonality of their vocalizations (compressing to increase amplitude) during quiet periods near roads that can be particularly noisy (Hanna et al., 2011). Red-winged blackbirds may sing more often and louder at quieter times to acclimate to habitat near loud traffic noise. Timing changes may be the strategy that has led to the most success in this species, explaining why they were detected less as traffic noise increased. Red-winged Blackbirds seem to cope well in the traffic noise captured by this study, but as traffic noise increases in amplitude at all parts of the day, it may reach a threshold where changing the timing of singing behaviors is no longer effective.

Conclusions

As roads continue to expand and traffic volume increases, traffic noise will increase and impact more ecosystems. Some species' coping strategies may not be effective in mitigating the effect of traffic noise if the noise gets too loud. For example, a finch species sang more in loud traffic noise until the noise reached 70 dB, then dramatically decreased its vocal activity, suggesting that coping mechanisms may only work when traffic noise does not exceed a certain amplitude (Diaz et al., 2011). This threshold will differ across species. It is important to understand individual species' response to different levels of traffic to determine how the species will be affected by the future expansion of roads and the increase of traffic volume on those roads.

Birds are making a variety of behavioral changes, such as altering timing or characteristics of vocalizations, to respond to traffic noise. The results of this study support the notion that species may increase or decrease singing behavior as traffic noise increases. Vocally flexible species like the Northern Cardinal and American Robin persisted in loud areas, as vocal flexibility increases urban success (Dowling et al., 2011; Gross et al., 2011). However, major behavioral changes can have consequences for other aspects of avian behavior. The extent to which a species is able to change its vocalization along with the increasing pressure of traffic noise may lead to lower persistence in species that are typically successful in urban areas.

If the changes in detection observed in this study indicate a change in behavior, traffic noise may cause changes in roadside bird populations. Background noise is an impactful habitat characteristic and when isolated from other urban disturbances, there is evidence that it reduces individual fitness in songbirds (Habib et al., 2007). For birds specifically, noise has the potential to affect important behaviors such as antipredator response and mating songs. Traffic noise may lead to major fitness consequences as female songbirds rely on vocalizations to assess male quality, as observed in Red-winged Blackbirds (Kight and Swaddle, 2011; Cartwright et al., 2014). Additionally, noisy roads may weaken communication at the population and community level by impairing species' ability to communicate important survival cues such as alarm calls (Grade & Sieving, 2016). Both the reproductive and survival components of fitness are affected by traffic noise.

Traffic noise has a negative effect on reproductive success of some avian species. Traffic noise may reduce songbird mating success because females may respond

negatively to a distorted song, interpreting a male as unfit. Further, they may be unable to determine the individual associated with the song. This has negative implications for reproductive success because mating pairs may rely on song for pair bonding (Swaddle & Page, 2007). There is evidence of females laying smaller clutch sizes near louder roads (Halfwerk et al., 2011), meaning other elements of avian life outside of communication may be affected by the noise as well. The most abundant species in this study may not be facing major fitness consequences yet, but some species may be facing them already.

Roads and traffic noise do not have the same effect on all birds since each species has their own complex behaviors and vocalization characteristics. Even categorizing birds into groups of similar frequency did not provide a detailed explanation of the mechanisms behind community-level trends. Studying individual species will aid in understanding how traffic noise affects individual species and the fitness consequences for that species. It is difficult to make community-level assessments based on detection alone and studying individual species in a variety of road noise conditions provide more information about how the behavior of individuals affect patterns observed in communities near roads.

CHAPTER 3: SELECTION PRESSURES AND STUDENT REASONING OF EVOLUTION AND FITNESS

ABSTRACT

There is an emphasis on survival-based natural selection in biology education that can allow students to neglect other important evolutionary forces such as genetic drift and sexual selection. Different selection forces can select for the same variant of a trait or they can oppose one another, and sexual selection can lead to the selection of trait variants that are maladaptive for survival. Using examples that provide a discrepant event contradicting survival-based reasoning may challenge students to consider other potential selection pressures. In semi-structured interviews with undergraduate biology students (n=12), I explored how the interactions of different types of selection affected student reasoning of evolution. When asked to define evolution, many students equated evolution to natural selection and no students included other evolutionary forces. In scenarios where sexual selection and survivability favored the same variant of a trait, students emphasized survival in their reasoning. When students were presented with a scenario where sexual selection selected for trait variants that were maladaptive for survival, more students described how two different selection forces contributed to evolutionary outcomes, described reproductive potential as a part of fitness, and included inheritance in their descriptions of evolution. Scenarios where sexual selection and natural selection select for different variations of a trait improved student ability to reason about how factors other than survival can impact evolutionary change. When instructors include examples where selection is based exclusively on survival, they miss an opportunity to determine how students reason how multiple selection forces may act differently on a trait.

INTRODUCTION

Evolution is a particularly difficult topic for undergraduate biology students. Evolutionary concepts challenge students to evaluate how populations change over time and make predictions about how different evolutionary forces like natural selection, genetic drift, and gene flow will impact future populations (AAAS, 2011). It is difficult for students to conceptualize how the small genetic changes that occur within each generation become observable changes in traits over time. There have been increased efforts to research how students learn about evolution due to the conceptual challenges associated with evolutionary reasoning (Nehm & Reilly, 2007; Nehm & Ha, 2011; Ziadie et al., 2018).

Students' prior knowledge and ideas about evolution plays a major role in how students learn about evolution. Prior evolutionary "knowledge" may come from formal or informal learning of evolutionary ideas. The informal introductions to evolutionary ideas, such as everyday experiences and verbiage, may contradict accurate evolutionary reasoning, leading students to have misconceptions about evolution (Alters and Nelson, 2002; Coley & Tanner, 2012). For example, students may use the word "adapt" to describe a change in individual behavior in response to environmental stimuli, which is actually describing the term "acclimation." An adaptation is a change in the population that occurs over many generations. Students may also hear or use the word "fitness" in application to physical well-being or strength, which is a common use of the word in non-evolutionary contexts. However, in terms of evolution, fitness is the ability to successfully pass on genetic information to the next generation, meaning the ability to attract mates is also important to fitness. The textbook *Campbell Biology in Focus* states

that ‘struggle for existence’ and ‘survival of the fittest’ are commonly used to describe natural selection, but these expressions are misleading if taken to mean direct competitive contests among individuals, instead of populations” (Urry, 2014, p. 411). Each of these terms relates fitness to survival when reproduction is also important to consider. The common use of the term fitness contributes to the emphasis of survival-based reasoning when students are reasoning about evolution (Gregory, 2009).

Despite there being multiple evolutionary mechanisms that can act on a population, university biology students tend to focus on natural selection based on the ability of individuals to survive when describing evolution (Andrews, 2012; Hiatt et al., 2013; Perez et al., 2013). Their current understanding likely builds on natural selection instruction in high school evolution education where survival dominates natural selection examples, even when mating success may be a better mechanistic explanation (Price & Perez, 2016). For example, many male birds have large ornamental tails that increase their success in attracting females, and having a large tail reduces survivability because it is easier for predators to capture individuals with long tails (Loyau et al., 2005). Long tails can still be observed in a population because those males were successful in mating and passing on their genes. Therefore, multiple selection pressures, survival AND mating success, affect tail length in these birds.

The use of discrepant events, or events that contradict inaccurate student conceptions, may help students think about selection differently (Anggoro et al., 2019). For example, if a student describes evolutionary change only in terms of survival, the student could be presented with an example where survival cannot be the only explanation for the change, like the peacock example. Since selection of peacock tails is

based on female mate choice, the student may recognize that survivability isn't always the only explanation behind evolutionary change. Instruction that emphasizes other selection forces, especially those that contradict survival-based reasoning, may guide students to incorporate these forces into their evolutionary reasoning and improve their ability to provide holistic evolutionary descriptions (Price & Perez, 2016). Students' reasoning may be limited by their tendency to describe selection based on survival alone while they have the knowledge and ability to consider multiple selection pressures.

Components of Selection

Previous studies have identified components necessary for a complete description of natural selection: 1) sources of phenotypic variation, 2) heritability of phenotypic variation, 3) reproductive potential, 4) limited resources, 5) competition/limited survival, 6) selection of heritable traits, and 7) change in distribution of individuals with different traits (Mayr 1982; Nehm & Reilly, 2007). Another study divided natural selection concepts into the phenotypic and genotypic aspects of four components: variation, inheritance, fitness, and change over time (generations) (Salter & Momsen, 2018). Both sets of criteria require some sort of variation in the population, inheritance of different traits based on their relative fitness, and inheritance of traits in the population leads to a change over time. Students typically do not include all the components when reasoning about natural selection, even when a majority reported already learning natural selection in high school (Nehm & Reilly, 2007).

There is intraspecific variation in populations, meaning individuals of the same species vary genetically, and in turn, phenotypically. The inclusion of variation is required for describing evolutionary change, as selection acts upon the variation of a trait in a population. Students that reason about evolution without including variation may

describe evolutionary change as a gradual change in the entire population or describe the characteristics of individuals rather than addressing the entire population (Coley & Tanner, 2012; Alred et al., 2019). Students who receive instruction about variation are better able to describe evolutionary forces acting upon the variation in a population (Settlage, 1994; Nehm & Reilly, 2007). Furthermore, providing students with examples that have population-level variation may provide students with a context to describe how selection may be acting on this variation (Alred et al., 2019).

Traits in the population will vary across individuals, and those with traits that have higher relative fitness will pass on traits more often than those with less fit traits, leading to changes in the frequency of certain traits in the entire population. Students commonly describe something as fit when it benefits survival, such as suitability to the environment, strength, health, speed, or size, (Alters & Nelson, 2002; Bishop & Anderson, 2002). In terms of evolution, fitness is the ability of an organism to pass on its genetic information to subsequent generations. Therefore, being "fit" is also based on an individual's reproductive potential, not just survival. Survival is an important component of fitness, but it does not guarantee survival. Reproduction assumes survival and guarantees higher fitness since genes are being passed on (Hendry et al., 2018). If students are committed to survival-based reasoning, they may only think about how surviving longer provides more opportunities to pass on traits, leaving out the role of attracting mates and reproducing (Bishop & Anderson, 1990; Nehm & Reilly, 2007).

Inheritance is the passing of genes from organisms to their offspring. Traits that benefit survival and reproductive potential will be inherited more often (Hendry et al., 2018). The genes that are inherited more often over generations will become more

prevalent in the population. After many generations, there may be observable changes in the common traits of the population based on the genes that were inherited most often. To have holistic reasoning of selection, students must recognize how reproductive potential affects inheritance and how inheritance affects evolutionary processes (Nehm & Reilly, 2007; Salter & Momsen, 2018).

Sexual Selection Effects on Populations

Sexual selection is a type of natural selection based on the preference of individuals to mate with other individuals with certain traits. Sexual selection impacts evolution because mate preference influences which traits are inherited (Panhuis et al., 2001; Maan & Seehausen, 2011). Sexual selection has a strong effect on phenotype and can lead to evolutionary change based on the mating preferences of a species. Sexual selection can select for the same variant of a trait as survival-based natural selection if a trait is beneficial for survival and also appeals to mates, such as bright skin color in Poison Dart Frog males which deters predators and attracts females. However, it can also select for trait variants that are maladaptive for survival (Ritchie, 2007; Chenoweth et al., 2015). For example, Long-tailed Widowbird (*Euplectes progne*) females are more attracted to males with long tails, but having a shorter tail helps individuals escape predation (Andersson, 1982), leading to the most common tail length in the population being a medium-length tail.

The potential for sexual selection to act against survival seems to be overlooked by students, and student reasoning of sexual selection has not been addressed by biology education literature. While there are many papers about student reasoning about natural selection, a review paper aimed at identifying gaps in evolution education literature found zero papers about student reasoning of sexual selection specifically (Ziadie & Andrews,

2018). Studying how students incorporate sexual selection into their evolutionary reasoning is an opportunity to better understand how students reason about evolution. It is important to explore this gap in the literature as sexual selection is an important selection type that can lead to evolutionary outcomes that are not beneficial for survival.

The scenarios used in assessment questions can elicit different responses from students based on the context of evolutionary change. For example, students have better evolutionary reasoning when they reason about a trait gain rather than a trait loss (Nehm & Ha, 2011; Nehm et al., 2012). The context of different types of selection pressures and their interactions has yet to be explored by biology education research. It is unknown how students reason about different selection pressures acting on a single trait. Students may respond differently when presented with scenarios where selection pressures are selecting for the same or different variants of a trait. Changing the context of selection type in scenarios may cause students to reason differently about evolution and provide more insight on student reasoning of how different selection forces act on a population. Providing students with scenarios where survival is not the only mechanism behind evolutionary change is an opportunity to present a student with a discrepant event that contradicts reasoning based only on survival. Using discrepant selection forces may provide a learning opportunity for students to consider multiple selection pressures and how they act upon a trait.

I conducted interviews to assess how students reasoned about individual components of evolution (variation, fitness, inheritance, and change over time) when presented with scenarios with different interactions between natural and sexual selection pressures. Students were asked to describe the evolutionary implications of four different

scenarios where: selection was based on survival only, two selection forces selected for the same variant of a trait, two selection forces selected for different variants of a trait, and selection acted on traits differently in different environments. Responses were analyzed to determine 1) how the context of different selection forces may affect student reasoning of variation, inheritance, fitness, and change over time, and 2) what sort of scenario contexts lead students to consider sexual selection as an evolutionary force. I predicted that scenarios where there is selection based on survival and mating success would result in evolutionary descriptions that included more components of evolution, specifically components related to reproductive potential and inheritance. I also predicted that scenarios where sexual selection selected for a trait that was maladaptive for survival would help students recognize sexual selection pressures acting on a population, as that type of scenario highlights how two selection forces can oppose one another.

METHODS

Participants were selected from an introductory biology course at a large Midwestern university. The course was the second of a two-course introductory biology series intended for students majoring in Life Sciences. Most students enrolled in the course were in their first or second year of their undergraduate program. The course description states "A systems-based phylogenetic approach to the study of organisms considering their morphology, life histories, physiology and ecology. The nature and evolution of biological diversity and how that diversity is studied." The course is delivered in five sections of 150-250 students each year. The students were from three

different sections with different instructors, one during the fall term and two from the spring term, all from the same academic year. At the time of the interview, participants had previous instruction of evolutionary concepts such as selection pressures, fitness, inheritance, variation, and changes in the population over time.

After students had completed their evolution unit, lecture sections were visited by the researcher and informed of the research participation opportunity [APPENDIX A]. Those interested in being a participant were contacted via email. All students who expressed interest within two weeks of the announcement were put into a pool and participants were randomly selected. A total of 15 students were selected, 3 from the Fall 2017 term and 12 from the Spring 2018 term. There were 11 female and 4 male participants.

Participants took a written pre-test and were interviewed to explore student reasoning of evolution. Immediately following the pre-test, I conducted one-on-one interviews to assess student reasoning of evolution. Interviews occurred within three weeks after the participants were selected and took place in a small, private interview room on the university campus during normal business hours and lasted 30-45 minutes. The entire interview was recorded using a handheld recorder and was transcribed using Temi software (Temi, 2018).

Interviews

The pre-test was adapted from the Concept Inventory of Natural Selection (CINS), an assessment created by Anderson and colleagues (2002). Its ability to accurately assess natural selection knowledge has been verified by comparisons to semi-structured interviews and implementation on many groups of students (Anderson et al., 2002). This assessment was selected because it is both research-based and allows for a

quick, reliable look into students' natural selection background knowledge. The multiple-choice assessment is based off real scientific studies of natural selection and has two questions about ten different components that are important to natural selection such as variation, limited survival, population stability, and reproductive potential. The adapted pre-test included the eight Galapagos Finch questions from the CINS, plus two other questions from the lizard context (Questions 18 and 20) that were adapted to the Galapagos Finch context. I chose the finch context since the first context used in the interview portion was also related to the Galapagos Finches and would allow for CINS responses to be compared to interview responses about a similar scenario.

To explore how the context of selection pressure affected student reasoning of evolution, I presented students with scenarios that differed in how one or multiple selection forces acts in a system. I organized the interview questions in the order of increasing complexity in terms of selection pressures acting on a population, 1) a single selection force, 2) two selection forces selecting for the same variant of a trait, 3) two selection forces selecting for different variants of a trait, and 4) selection forces selecting for different variants of traits in different environmental conditions. The progression of this interview allowed me to observe if students changed their evolutionary reasoning as they were presented with different types of selection pressure interactions and whether a discrepant event would lead students to describe other selection forces besides selection based on survival. For each scenario, students were provided background of the ecological system and then asked about the evolutionary implications. Questions followed evolutionary stories of the following organisms: Darwin's Finches (subfamily *Geospizinae*), Strawberry Poison Dart Frogs (*Oophaga pumilio*), Long-tailed

Widowbirds (*Euplectes progne*), and Black-capped Chickadees (*Poecile atricapillus*). The full scenario descriptions can be found in APPENDIX E.

Darwin's Finches

Darwin's Finches of the Galapagos Islands provide an example of evolution due to geographic isolation. An ancestral species of finch immigrated from the South American mainland to occupy several nearby islands that had different major food sources. Populations of finch were isolated from one another for many generations, enough time for reproductive isolation and eventual speciation. Natural selection pressure from the food sources favored beaks that were shaped in a way that was advantageous for reaching a certain food type (Grant, 1999). In this scenario, I looked at the Large Ground Finch (*Geospiza magnirostris*), which evolved a larger beak than that of the ancestral species over many generations. Large Ground Finches are found on most of the Galapagos Islands and have a diet composed primarily of large seeds (Schulenberg, 2018).

Darwin's Finches were chosen as an interview context because they are widely used as a classic example of natural selection acting on a trait in a population. The finches' beaks were selected upon based on their ability to obtain food. This scenario allows for the assessment of student reasoning of evolution when there is one selection force based on survival alone. The Darwin Finch example shows how a trait's effect on survivability can lead to evolutionary change in a population, but it does not have any other clear selection forces. Using this example allowed for the assessment of student reasoning when survival was the only selection force.

Poison Dart Frogs

Strawberry Poison Dart Frogs are a rainforest species that secrete poison from their skin to deter predators. Their red skin color deters predators, and brighter red frogs have higher survivability (Noonan & Comeault, 2008). Strawberry Poison Dart Frogs are sexually dimorphic; males and females have different forms of the skin color trait. In this case, female skin is a lighter shade of red than that of males. Females prefer males with brighter red color, imposing sexual selection pressure on males for brighter red skin color. Strawberry Poison Dart Frogs provide an example where one variant of a trait benefits both mating success and survivability (Maan & Cummings, 2009). The Poison Dart Frog scenario allowed for the assessment of student reasoning when two different selection forces was present, and selected for the same variant of the trait.

Long-tailed Widowbirds

Long-tailed Widowbirds are a grassland bird species found in many parts of southern Africa (Craig, 2018). The birds have clear sexual dimorphism in color and tail length. Females are brown with short tails, and they are a very similar color to their grassland surroundings. Males are black with yellow and red markings on their wings and have extremely long tail-feathers. Female Widowbirds are more attracted to males with longer tails, but large tails reduce their ability to escape predation (Andersson, 1982). The trade-off between survival-based selection pressure from predators and sexual selection from females has led to the medium-sized tail length being the most prevalent among males in the population (Andersson, 1982). There are two selection forces that select for different variants of a trait, and lead to evolutionary change that is maladaptive for survival.

Black-capped Chickadees

Black-capped Chickadees use vocalizations for mating purposes. Males sing to females and the females prefer males with a low-pitch song (Christie et al., 2004). In rural areas, males with the lowest pitches would be perceived as the most attractive by females. However, in urban areas, males have been shown to increase the pitch of their vocalizations to be heard over loud traffic (Oden et al., 2015; Christie et al., 2004). Individuals residing near roads may need to sing at a higher pitch to be heard, which comes as a cost if females perceive the higher pitched song as less attractive (Swaddle & Page, 2007). This provides an example of selection pressures that change across space, based on the acoustic environment. The type of selection pressure will that differ based on proximity to loud traffic noise.

Both urban noise and mate choice may affect how chickadees evolve. The heritable components of singing adapt over generations, but the acclimation to loud traffic noise during an individual's lifetime would not be inherited. This was an opportunity to test how students' reason about adaptation (change in traits through evolution) and acclimation (a change in behavior in response to environmental conditions). I explored how students used these terms in their responses to this scenario.

Interview Questions

First, students were asked to define "fitness" and "the process of evolution" to get students' personal definitions of the terms. Following the general questions, students answered questions about four different scenarios [APPENDIX D]. Students were introduced to scenarios one at a time, and the scenarios were presented in the same order in each interview. For each scenario they were asked 1) how characteristics of the trait affected the fitness of the organism, 2) the evolutionary implications of having a certain

trait. Students were asked the same questions for each scenario so responses could be compared based on the selection pressures interactions occurring in the scenarios.

In the frog and widowbird scenario, students were shown a picture of a male and female next to one another and asked to observe the differences between the two sexes to determine if students recognized sexual dimorphism. Then, students were asked about how a trait affected fitness in males. When students were asked about the evolutionary implications of the variation in the trait on the population, they were presented with a population of males that showed variation in the traits of interest. The populations of males were created in Adobe Photoshop by slightly changing the color of individual frogs or altering tail length and adding many unique males to one population (Adobe Photoshop, 2018).

In the final scenario students were told that Black-capped Chickadee males sing higher near loud traffic and that females preferred males with lower songs. Students were told of the mate choice in this final scenario, informing them of the sexual selection in the system. Students were also asked about each evolutionary component (variation, inheritance, fitness, and change over time) directly to observe how students described the role of each component in evolutionary change. I directly asked students about the implications of traffic noise on fitness for urban and rural populations. To explore student reasoning of adaptation vs. acclimation, I asked students if behaviors learned in a lifetime could be inherited and examined the language they use when describing these phenomena. Students were also directly asked about the variation in the population and their predictions of change over time. Therefore, if a student left a component out of their

reasoning in prior scenarios, there was an opportunity for the student to describe that component.

Data Analysis

At the start of the interview, students were asked to provide definitions of "fitness" and the "process of evolution" without a scenario. For the fitness definitions, I coded based whether students included the components reproductive potential and/or survival and how they described each of these components (Table 3.1). For evolution responses, I coded using a rubric adapted from a natural selection rubric by Salter & Momsen to determine if and how students were including the components variation, fitness, inheritance, and change over time (Salter & Momsen, 2018) (Table 3.1). The inclusion of evolutionary components provided me details of how they describe each component in terms of evolutionary change.

Selection

Evolution definitions were also coded for description of selection forces and whether they were based on survival or mate choice (Table 3.1). Responses that describe selection based on survival may describe a change in the population in response to food availability, predation, or other impacts on survivability. Responses that describe selection based on mate choice may include that mate preference of a certain trait drives changes in that trait in the population. I analyzed student descriptions of evolution acting on a population to examine whether they included reasoning about each of the four components of interest (Salter & Momsen, 2018):

- Variation
- Inheritance of traits/genes
- Fitness
- Change over time

First, responses were coded simply for presence/absence of each component. Then, to uncover more qualitative details of student reasoning, responses were coded based on the details included about each component (Table 3.1).

Table 3.1: The coding rubric that was used to assess student responses to interview questions.

	Coding Instructions	Code	Code Descrption
Selection	Code for the inclusion of selection occurring based on survival (NS) and/or selection occurring based on attracting mates (SS). Some responses may include no selection force (N) or both types of selection forces (B).	NS	Natural Selection: The student describes the process of natural selection, or selection based on survival or a change in the environment. Including the term "natural selection" without describing the process is not sufficient.
		SS	Sexual Selection: The student describes an evolutionary change based on mate preference or reproductive success
		N	The response does not include any selection force.
		B	The student describes selection based on survival and attracting mates.
Variation	Code for the inclusion of a source of variation and whether they describe variation in the population. There will be two codes for each response, one for source of variation followed by one for variation in the population. Example: V, 0 if the student described variation in the population but did not include a source of variation.	M	The response includes a source of variation, and the source is a mutation.
		S	The response includes a source of variation that is not a mutation.
		V	The student describes variation in the population.
		0	The response does not include variation in a population or a source of variation (0,0 if none for both).
Fitness	Code for the use of survival and reproductive potential in student responses when they are directly asked about fitness. Responses that include how both survival and reproduction affect fitness	S	The student describes fitness as the ability to survive or bases fitness on survival alone.
		R	The student describes fitness as reproductive potential alone.

	should be labelled "SR." If only survival or reproduction is used alone, label "S" or "R."	SR	The student includes both survival and reproductive potential in their description of fitness.
Inheritance	Code for whether responses include inheritance. Responses marked "I" may include the term inheritance directly or the student may describe the passing on of genes or traits to the next generation. If student responses do not directly include inheritance but it can be inferred, code as "P." If there is no mention of inheritance, label "0."	I	The student clearly describes the passing on of genes or traits; includes the terms “inheritance” or “passed on.”
		P	The student describes reproduction and connects it to change over time, and it can be inferred that the student is describing inheritance, but the student does not directly include inheritance.
		0	The response does not include inheritance.
Change over time	Code for whether responses include a description of a change in the population over time. Responses may describe change over many generations (MG) or one generation (OG).	MG	The student describes a change in the population and includes that it occurs over multiple generations.
		OG	The student describes a change in the population in a single generation.
		0	No mention of a change in the population over time.

Variation

Responses were coded for the inclusion of a source of variation and whether the students described a source of variation [APPENDIX F]. Sources of variation could be coded as included a source of variation in the population that is not a mutation (S). For example, *“Even the population that was present with this common ancestor, at least a couple of them had to have had larger beaks”* (Student 644). The response could also be coded as mutation as the only source of variation (M), *“I think there'd have to be a*

mutation for a larger beak size." (Student 929) or no source of variation mentioned (0) (Table 3.1). Variation in the population was coded for whether they address variation of individuals within the population (V) or not (0) (Table 3.1). An example where a student included variation would be, *"There was a different variation of the red in these frogs"* (Student 563), as the response directly addressed differences of individuals within the population.

Fitness

Student responses to all questions about fitness were coded based on whether they included survival, reproduction, or both in their descriptions of fitness. Students frequently included only reproduction or only survival (Table 3.1). Students who described how survivability and reproductive potential affect fitness had the most accurate reasoning and were coded for both (Table 3.1). For example, *"it probably helps them get more mates because it's attractive and for probably female birds, but it probably also decreases their chances for survival"* (Student 712). More representative quotes for each can be found in APPENDIX F.

Inheritance

Responses were coded for inclusion of inheritance when students described the passing on of genes, traits, or characteristics. Responses where the student directly mentioned inheritance or the passing on of genes were marked as complete inclusion of inheritance. The students needed to include the passing down or inheritance of genes or traits to the next generation (e.g., "*as reproduction happened, there were more of the, like red frogs available to reproduce and pass on their bright red to their offspring. And so eventually we have more bright red frog surviving and less of the dull color*" Student 270, Frogs) for it to be clear that they were describing the role of inheritance in evolution.

Some responses included reproduction and change over time, and it could be inferred that students were describing inheritance as the link between the two. For example, *"I see more red than the orange. I guess that the red frogs were more successful in having offspring, so that caused the population to have a change in the alleles so that more of the frogs nowadays are red than they were in the past"* (Student 818). Responses where inheritance can be inferred were coded as a partial response [APPENDIX F].

Change Over Time

The ways in which students described the evolutionary change over time were coded based on if a student described a change in the population in one generation (OG), or described the change over many generations (MG) (Table 3.1). For example, a student may only describe the change in a population without including generations "*The population probably changed due to chance or mutation. One ended up with the different sized tail and if it was more beneficial for them to have that sized tail, they would probably reproduce more so there would be more of them*" (Student 499). Therefore, was difficult to determine of the student was describing a change in the frequency of a trait in the current population or if they were describing an evolutionary change over generations, the more accurate response. A complete response would have included generations, for example*, "you're going to get higher allele frequencies of the bright red as more of these are able to develop because they have the right alleles. So once the next generation of these survivors are going to have higher concentration of the, the bright red allele, and then you're going to see a decline in the orange allele, in the lighter allele"* (Student 644) [APPENDIX F].

RESULTS

Pre-test

The students in this study (n =12) performed above average on the pre-test adapted from the Concept Inventory of Natural Selection with an average score of 85% (Bishop & Anderson, 2002). Scores ranged from 30% to 100%, with six students answering all questions correctly. Individual student scores provided a quantitative representation of baseline knowledge that could be compared to their interview performance. The number of students answering a single question incorrectly ranged from one to three students.

Student Definitions of Fitness and Evolution

Natural selection dominated students' general definitions of evolution. For example, Student 499 described evolution as *"the whole series of how natural selection occurs and how animals have grown and adapted to their environments and how all of them came to be where they are now."* Seven out of twelve students directly included the term "natural selection" in their personal definitions, typically equating evolution and natural selection. Some students described natural selection acting over the entire population, "*Evolution occurs when natural selection occurs...within the whole population.*" (Student 270), or that natural selection is small-scale evolution, "*the big picture version of natural selection, adaptation"* (Student 712). None of the students included any other evolutionary force, such as sexual selection, gene flow, or genetic drift.

Four students included all four components in their general definitions of evolution. For example, Student 644 stated, "*Evolution is the process where natural selection is acting on it, a population, and the traits that are helping them survive are*

being inherited and reproduced way more than traits that aren't working so great. So populations gonna gear more towards whatever's leading the organisms to be successful." Student 818 provided the definition, *"The process of evolution is the change in allele frequencies over time. It happens through natural selection, the individuals with more relative fitness are going to be able to have more offspring, making their traits be more present in the next generations. And that will lead to the change of alleles in a population."* These students showed the ability to apply all evolutionary components to evolution. They also described natural selection as the mechanism behind evolutionary change. However, a majority of students (n = 8) did not include as much detail as expected.

Many students (n = 8) provided a general definition of fitness that included only one component of fitness, either survival or reproductive potential alone. Students described fitness as "*reproductive success whoever reproduces the most organisms*" (Student 929), or "*survival of the fittest…the more fit species survive longer*" (Student 615). The students that did include both survival and reproductive potential (n = 4) described fitness as "*the ability to reproduce and survive*" (Student 499) without elaborating about how reproductive potential and survival contributed to fitness. The students provided short definitions without applying the knowledge to any sort of example. Therefore, general definitions of fitness provided very little detail of student reasoning and how students applied aspects of fitness to their assessment of an organism's fitness.

Some students included that evolution must have occurred over many generations. "*Evolution would come not immediately, obviously it will have to happen over multiple*

generations in order for something to happen" (Student 563). However, they did not go on to describe the function of inheritance occurring at each generation that eventually led to an observable change in the proportion of individuals in a population with specific traits.

Selection for a The Same Variant of a Trait

In the finch scenario, the selection force favored a trait that benefitted survival. None of the students described selection based on mating success, as expected. Survival-based natural selection was the only clear selection force in this scenario so survival-based reasoning was accurate. In the frog scenario, two selection forces selected for brighter red skin color because the brighter variant of the trait benefitted both survival and mating success. When students responded to the frog scenario, they were able to describe that female frogs have lighter red skin than males when asked about sexual dimorphism. While all students recognized a difference between the sexes, only two students included sexual selection as the mechanism behind the difference between males and females. Student 929 said "*They must be attracting mates*," and Student 818 said "*the [more] red frogs were more successful in having offspring, so that caused the population to have a change in the alleles*" when describing evolution of the frogs.

Overall, most students addressed variation when selection forces were selecting for the same variant of a trait. Nine students discussed the variation in beak size in the finch scenario, even when they were not shown a population of individuals. They described how the finches vary in beak size, "*some of them had like slightly smaller beaks and some had slightly larger beaks*" (Student 689). Student 644 directly described the slight variation present within a population, *"even the population that was present with this common ancestor, at least a couple of them had to have had larger beaks."* All

students were able to address the variation between individual males in the population of poison dart frogs. The students described how the red skin color varied across the individual males. Students were better able to address variation in response to the frog scenario since they were presented with a visual of the population [APPENDIX D].

Fewer students included reproductive potential in scenarios where multiple selection pressures were selecting for the same variant of a trait. In the finch scenario, students attributed fitness to the ability to access food. Birds that had better access to food lived longer, so they were more fit. For example, Student 818 stated, *"I guess having a larger beak, makes the finch be able to eat from a specific food that is more present in the island, the finch has more fitness because it can more successfully access food through his beak."* For the frog scenario, where two selection forces selected for the same variant of a trait, students mentioned that a brighter color would help frogs avoid predation, which would increase survival and therefore, fitness, *"Brighter color would have a greater fitness then. Because there's a lesser chance that they will be eaten"* (Student 929); *"I would say if you have a brighter color, you have a better chance of surviving because they're not going to eat you because they don't want to get poisoned"* (Student 499). Students described fitness similarly between the two scenarios, even when the frog scenario included two selection forces, the difference between males and females, and the variation of males in the population.

Students included less details about inheritance when selection forces were selecting for one variant of a trait. For the finch scenario, seven students included inheritance, and for the frog scenario, four students included inheritance. Some responses included the passing on of genes, *"when that bird went to have offspring, that mutation*

would have gotten passed down" (Student 615), *"They're going to be able to survive and stay healthy and mate, some of those traits are going to be passed on and those traits are going to take over the population."* (Student 644). Other students included responses where inheritance could be inferred, but it was not directly stated *"I see more red than the orange. I guess that the red frogs were more successful in having offspring, so that caused the population to have a change in the alleles so that more of the frogs nowadays are red than they were in the past"* (Student 818). As compared to when selection forces were acting on the same variant of the trait, students included less inheritance information.

When students described the change in a population over time, it was difficult to determine if students were discussing changes in population frequency over one generation or multiple generations. For example, regarding the finches, Student 270 stated, *"that trait would probably eventually just become common throughout the whole population to get to the large ground finch with a bigger beak."* Even when students included inheritance, the time scale wasn't always clear. In response to the frog scenario, Student 270 states, *"as reproduction happened, there were more of the red frogs available to reproduce and pass on their bright red to their offspring. And so eventually we have more bright red frog surviving and less of the dull color."* It was unclear if the student was describing a change in the current population or a change occurring over many generations. Many students had responses similar to that of student 270, describing how changes occurred in the entire population and that beneficial traits became more prevalent. Many students used terms like "gradual," "eventually," and "slowly" to describe a change in evolutionary time and had a lack of clarity with regards to changes

occurring in the population over generations, "*So then slowly as the lighter ones got preyed upon, there would be less and less of those. And so the brighter ones would reproduce more and more. And so it would, not overtake, but there would be more compared to the lighter ones*" (Student 499).

Selection for Different Variants of a Trait

In the widowbird scenario, selection favored a long tail because it improved mating success, and selection favored a short tail because it benefitted survival. The opposing selection forces led students to provide more details about selection, inheritance, and fitness compared to when there were two selection forces selection for the same variant of a trait. Most students (n = 9) described both selection forces in their reasoning. Seven students included selection based on mate choice for the first time. They described how female preference for tail length influenced the prevalence of middle-length tails in the population. For example, *"the medium sized tail would be able to get away from a predator easier than the ones with the long tail. But then they would be able to find a mate better than the ones with the short tail. So I feel like the medium length tail mutation and gene was passed down more frequently"* (Student 615).

After observing images of the male and female widowbirds, all students recognized that male widowbirds had longer tails and darker plumage than females. All students were successful in explaining that the males in the population of widowbirds varied slightly in tail length. They recognized that the medium tail length was the most prominent in the widowbird population, and they described the cause for the intermediate tail length. In both the frog and widowbird scenario, students were able to describe variation in the population presented to them.

All but two students included both reproductive potential and survival when describing fitness in the widowbird scenario (Fig. 1). Students were able to describe how both survivability and reproductive potential influence fitness, by explaining that having a long tail would attract mates and make it harder to escape predators. For example, *"I feel like it kind of has this kind of like destructive interference where it probably helps them get more mates because it's attractive and for probably female birds, but it probably also decreases their chances for survival at least compared to females because of them taking up more space and making it easier for predators to catch them"* (Student 712). In contrast, they also explained that having a short tail would make them less attractive to females and make escaping predators easier. When selection was based on mate choice and survival, students included how both reproduction and survival affected fitness.

The widowbird scenario led the most students to include inheritance in their responses (n=9). They described how the medium length tail was successful for both survival and reproductive potential, leading to more mating opportunities. For example, when Student 470 included inheritance, they also described change over time well. *"The medium length was more successful so that as it reproduced it, like other males that were born would have the medium length tail and then it would evolve and over the generations would be more prevalent in the medium length than the longer or shorter"* (Student 470). Prior to the widowbird scenario, Student 470 had not described the connection between inheritance and change over time.

Selection Pressure Changing Over Space

For the chickadee scenario, students were informed of female mate preference for males with lower frequency vocalizations and another selection force that selected for higher frequency vocalizations near traffic noise. Eight students included both selection

pressures in their reasoning. The students described how a low-frequency song would be selected for in places without traffic noise because of selection based on mate choice, but if there was traffic noise, singing higher may be favored to overcome traffic noise to be heard. Four students included selection based on mate choice alone in their responses. This was the only scenario more students described selection based on mate choice than selection based on survival.

Student descriptions of fitness were reflective of how they described selection forces, and many students included situational fitness based on whether there was traffic noise. They explained how fitness implications for a certain song pitch may vary based on the amount of noise in the environment. "*I would say the pitches influence fitness because depending on where they are, the more females they'll get, if the pitch is lower, they probably get more in the rural areas. But in the urban areas if their pitches higher, the females will be able to hear them so they'll be able to mate more with the females. So the fitness would depend on how many times they like mated it and stuff. And how like how they could change the pitch to get to the females*" (Student 499). In contrast to the widowbirds, in the chickadee scenario, half of the students (6 out of 12) only described how reproductive potential affected fitness. Students now described the role of reproductive potential well, as there were two selection forces that affected communication with females. *"So if females prefer mates with lower pitch songs, then the males that have the average lower pitch, the rural population would probably have increased fitness because they're more likely to find a female to mate with and reproduce because they prefer lower pitches"* (Student 270). Overall, students recognized that

communication with females was imperative for fitness, and they did not rely on survival alone to describe success in the population.

Students were directly asked if there was variation in song across the individual males in the population. All but one student recognized variation within urban and rural populations. Students were able to describe that there was variation in song, with six students describing a change in song as a behavior, five students describing variation in the behavior and variation in the traits. Student 615 did not describe variation within the urban and rural populations. Instead, she compared urban and rural populations as a whole, not mentioning the variation within those populations, *"I feel like the ones in very heavy traffic have very high pitched songs whereas the ones in the not heavy traffic areas could have a medium or lower pitch song."* When asked about the rural population specifically she responded *"The ones that live in farming areas where there's tractors and combines, they could have like a higher pitch song, but since it is like country and everything, like they probably have a lower pitch song."* This description seems to claim that all individuals in a similar environment will have the same characteristics.

For the chickadee scenario, eleven out of the twelve students explained that learned behaviors were not inherited. Student 615 described inheritance as the song being learned by offspring from listening to parents, *"I just think of me and how I inherited my beliefs and so these birds, they kind of inherited what pitch depending on where their parents are."* She also described change over time occurring by location based on isolation of populations rather than evolution acting on variation within a population *"I would say it could [evolve] because the ones who are in the urban population don't get as many mates as the ones in the rural population. So they might like lower their pitch so*

then they could get a mate and they wouldn't be as susceptible to predators as the ones in the rural community". This student is describing acclimation that occurred if birds changed behavior. Many students described acclimation but used the term adaptation in their descriptions. Although, some students went on to accurately explain that the singing trait could only evolve if it was heritable. For example:

Interviewer: "Singing is a learned behavior in chickadees how would offspring be affected by this change in behavior?

Student 270: "So learned behaviors can't be passed on to offspring. So the offspring would have to learn these behaviors themselves once they were born."

Student 270 explained that learned behaviors cannot be passed onto offspring, but offspring can learn behaviors once they are born.

Trends Across Interview

Selection Forces

Selection based on survival was invoked more often during interviews than sexual selection, in scenarios where a trait was beneficial to both survival and mating. Selection based on survival was the only selection force used in general definitions and in descriptions of evolution in the finch scenario. Two students described how there were two possible mechanisms behind evolution of the poison dart frogs, with brighter red being beneficial to both mating and survival. The widowbird scenario responses included the most detail about how multiple selection forces could be selecting for different traits. In the widowbird scenario, nine students included two selection forces, while three students still persisted in only using survival-based reasoning. In the chickadee scenario, eight students used two selection forces, describing how traffic noise can sometimes

oppose sexual selection. Overall, scenarios where two selection pressures selected for different types of traits led to responses with the most detail sexual selection.

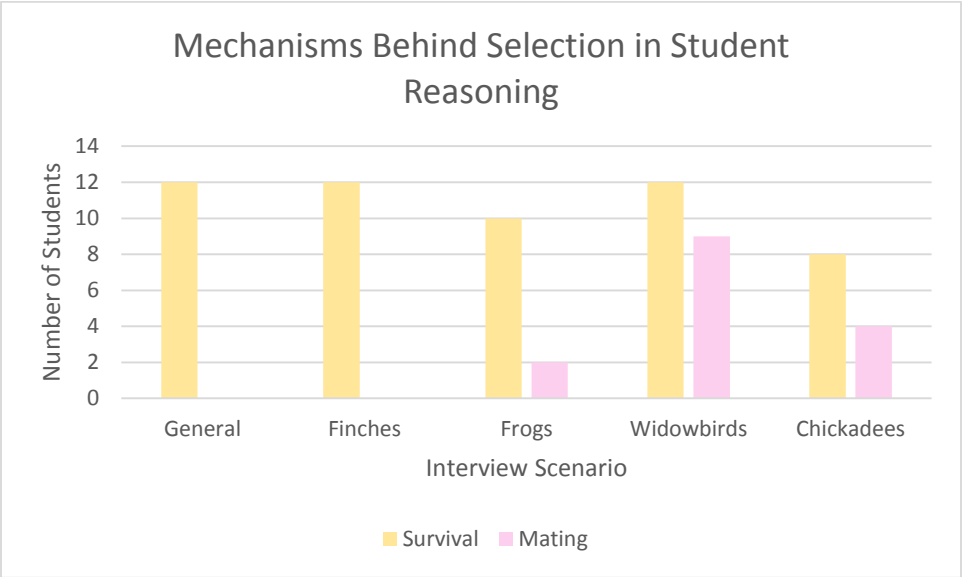


Figure 3.1) The selection pressure(s) included in student reasoning in their responses to each scenario. Selection based on survival was included in 62% of student responses, selection based on mate choice was included in 7% of student responses, and both selection forces were included in 31% of student responses.

Evolutionary Components

When students were asked about fitness, their responses changed across the interview. General responses led to an equal number of responses that included only survival, only reproduction, or both as important to fitness. Survival was used most often in responses to both the finch and frog scenarios, when selection forces all favored the same type of trait (Figure 3.2; Figure 3.3). Students described fitness using both survival and reproductive potential more often when reasoning about the widowbird scenario (Figure 3.2; Figure 3.3). For the chickadee scenario, more students included only reproductive potential than both fitness components (Figure 3.2; Figure 3.3).

None of the students included both the role of survival and reproductive potential in fitness on all the scenarios. Of the eight students that included reproductive potential in their general definitions of fitness, six did not apply the role of reproductive potential in fitness in their responses to the finch scenario (Figure 3.3). Three of the eight students that included reproductive potential in their personal definitions of fitness did not apply it to a scenario until the widowbird scenario. Two students did not include reproductive potential until the chickadee scenario where they were directly told of female preference.

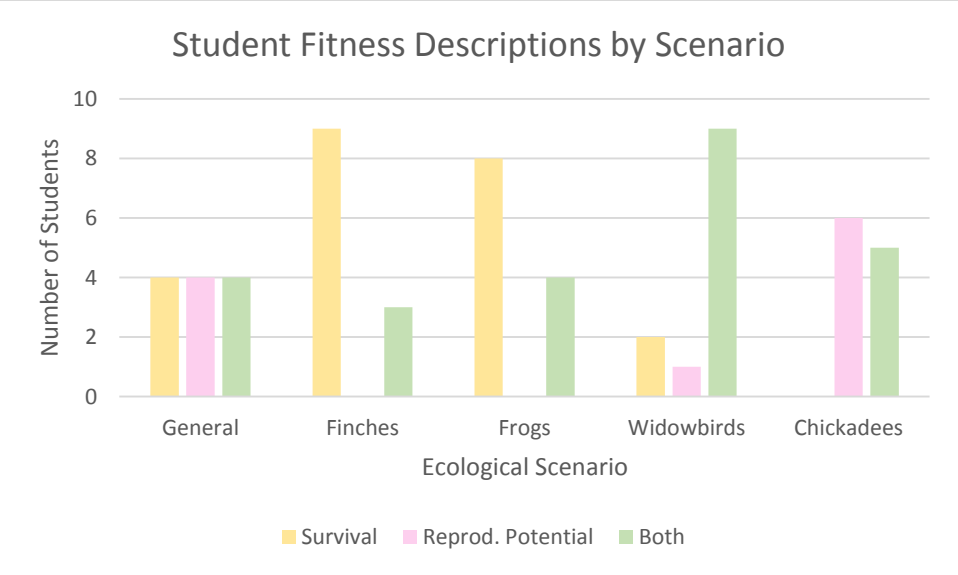


Figure 3.2) The number of students whose fitness responses were coded as "survival" - includes survival alone or "reprod. potential" - includes reproductive potential alone. When sexual selection reinforced natural selection, survival was used by more students, and when sexual selection opposed natural selection, more students included both survival and reproductive potential.

	Student											
	644	929	615	499	818	470	712	270	563	578	689	191
General	B	R	S	B	R	S	B	B	S	R	R	S
Finches	S	S	R	S	S	S	B	S	S	B	S	S
Frogs	B	B	S	S	B	S	S	S	S	R	S	S
Widowbirds	B	B	B	S	B	S	B	B	S	B	B	B
Chickadees	R	R	B	B	B	R	B	R	R		R	S

Figure 3.3) The coding for how students described fitness in each scenario. S represents survival, R represents reproductive potential, and B indicates that the student included both reproductive potential and survival in their description of fitness.

Five of the twelve students included inheritance in their general definitions of evolution. For the finch and frog scenarios, about half of the students included inheritance by describing the passing on of genes or a trait to offspring. Students typically included inheritance in the frog or finch scenario, but not both. For the widowbird scenario, nine students directly included inheritance. The widowbird scenario fostered more responses with an inclusion of inheritance than the other scenarios. When two selection forces were selecting for different variants of traits, students seemed to better include inheritance in their responses.

Student 270 provided an example of the change in reasoning that commonly occurred across different scenarios (Table 3.1). In their finch response, the student mentioned reproduction and not inheritance; student 270 stated *"the finches that had smaller beaks wouldn't survive and be able to reproduce while the finches that had larger beaks would be able to survive and eat the seeds. And then that trait would probably eventually just become common throughout the whole population to get to the large ground finch with a bigger beak."* In the frog scenario, where sexual selection was apparent and reinforcing natural selection, the same student included inheritance. "*As reproduction happened, there were more of the red frogs available to reproduce and pass*

on their bright red to their offspring. And so eventually we have more bright red frog surviving and less of the dull color." In the widowbird scenario the student once again included inheritance. *"There might be less of the short tails because they couldn't find a mate to reproduce. And so they couldn't pass on that short tail and then the long tail, there might be less of them because they were being hunted more often so then they die and can't reproduce… [The males with intermediate tails] were able to mate and reproduce and pass on their trait of having an intermediate sized tail to their offspring that way."* For this student, the presence of sexual selection provided them with a context for reproduction and the inheritance of traits. In the chickadee scenario, where two selection forces selected for different types of traits, mate choice was pointed out, and the student was directly asked about inheritance, Student 270 correctly explained that a learned behavior could not be inherited. This student was able to describe inheritance has a good understanding of inheritance and could apply it well in scenarios where it was clearly applicable.

Student Case Studies

In her personal definition of evolution, Student 929 provided a very broad evolution definition without inclusion of details about evolutionary components and equated evolution to natural selection. *"I would say it's like natural selection and adaptation together. Overtime, allele frequencies will change in a population. The alleles that are more favorable will increase, they will be more frequent over time, per generation time. It's the change, they're changing genes per generation"* (Student 929). The student described change over time, but did not mention the other evolutionary components that were important for describing the mechanisms behind this change over time. For each of the scenarios, she included all evolutionary components. Unlike most

other students, she recognized the multiple selection forces acting on the frog population. She was able to apply sexual selection reasoning when applicable. She also explained that behaviors learned in an individual's lifetime were not inherited. She had good evolutionary reasoning, but simply asking for their personal definition of evolution did not lead the student to apply their knowledge of each evolutionary component to the process of evolution.

Most students did not include selection based on mate choice unless selection based on mate choice opposed survivability. Student 712 equated evolution to natural selection in their definiton of evolution, as many other students did. She stated "*evolution is the big picture version of natural selection, adaptation, and other words we just talked about*" (Student 712). Student 712 typically used most components in her reasoning, but she favored survival-based reasoning when a single type of trait favored both survival and mating success, even when presented with a scenario with sexual dimorphism. She did not apply sexual selection until the selection forces selected for two different variants of a trait, then she described how multiple forces affected evolutionary change. This student was representative of the typical change in reasoning observed in students as nine students described mutliple selection forces when they selected for different types of traits.

Other students were very committed to the use of natural selection reasoning, which has been found in other university biology students (Price & Perez, 2016). Student 470's definition of evolution included "*how a species evolves in the environment that they are put in...over time the genetic setup would change to better benefit them in the environment*." Student 470 applied natural selection throughout the interview even when

sexual selection opposed natural selection. When she described evolution for the widowbird scenario, she relied on natural selection pressure to explain why both very long and very short tails were not as common as medium tails in the population. She explained that a short tail and long tail may be easy to catch by predators, and that females having short tails makes male offspring have shorter tails. It wasn't until the scenario where students were told of female mate choice, that she considered the role of sexual selection.

Interestingly, when Student 470 was told of the mate choice in a scenario, she was able to describe inheritance well.

"Interviewer: Singing is a learned behavior in chickadees. So how would offspring be affected by this change in behavior?

Student 470: Depending on where they're raised, their parents would teach them, is that what you're saying? They learn it from their parents? OK yeah, if they were raised in a city environment, then they would see their parents or their dad singing in a higher pitch and that's just what would seem normal to them, singing in a higher pitch.

Interviewer: Then, are learned behaviors inherited?

Student 470: No.

Interviewer: OK and why not?

Student 470: Well, it was learned you don't inherit it. Same thing with like if my dad was a bodybuilder, I wouldn't come out of the womb with muscles. If I want to be jacked, I would learn how to work out like him and become muscular like him, but my offspring wouldn't be muscular too.

Interviewer: OK and then can singing evolve in chickadees?

Student 470: If it is learned, then no. Like earlier I was saying it was yes an evolution occurrence because of the different environments that they were in, but with the information that they learn it, I would say that they probably don't. That wasn't an evolutionary change, that was something that their species figured out in a different environment.

Large patterns are interesting and individual student trajectories provide additional information by showing when students consider inheritance, reprodction in fitness, and sexual selection. Students that struggled to apply inheritance may be able to better apply the evolutionary component when told of mate choice in the ecological system, as observed in these case studies.

DISCUSSION

This study explored student reasoning of evolution in scenarios when there were one or multiple selection forces and whether those selection forces selected for the same variant of the trait. My hypothesis was that students would include selection based on survival more than other selection forces when they reasoned about evolution in scenarios where there was one selection force, as students are typically committed to survival-based reasoning (Price & Perez, 2016). I predicted that more students would be able to describe mutiple selection forces when those forces were opposing each other and selecting for different types of traits. I observed this pattern as students applied survival-

based reasoning in scenarios where survival-based selection was not contradicted by another selection force. More students described multiple selection forces when the selection forces were opposing one another rather than reinforcing.

Student Use of Survival-based Reasoning

When presented with a scenario where multiple selection forces favored a single trait, most students described how survival influenced evolutionary change. Even in scenarios with sexual dimorphism, when all students recognized the differences between males and females, only two students included sexual selection, the mechanism behind sexual dimorphism (Figure 3.1). Therefore, pointing out sexual dimorphism was insufficient to guide students to consider other selection forces. Instead, students explained that the brighter red frogs were eaten less, and therefore they had more time to reproduce, rather than considering that females preferred brighter red males. The students that used multiple selection forces in their reasoning when they did face a discrepant event could describe how multiple selection forces can occur, but they did not include this information when there was sexual dimorphism and the selection favored the same variant of a trait. The selection forces may need to select for a different outcome than increased survivability for students to recognize another selection pressure acted on the population as well. The students showed commitment to survival-based reasoning. Phrases like “survival of the fittest” and the common use of fitness to describe health or strength may have influenced students to emphasize survival when they described the fitness of a trait or organism (Alters & Nelson, 2002; Price & Perez, 2016). Students may have relied on survival-based reasoning alone because they were not provided with a discrepant event where survival was opposed by another type of selection force.

Several students who included reproductive potential in their personal definitions of fitness did not apply reproductive potential or mate choice in their descriptions of evolution when selection forces selected for the same type of trait (Figure 3.3). The lack of inclusion of reproductive potential when there was one trait being selected for may be because the selection forces led to the same outcome. Therefore, survival-based reasoning could not be contradicted, and students primarily thought about survival when they reasoned about fitness. Fewer students included inheritance in their evolutionary reasoning when selection forces selected for different traits. It was unclear if some students recognized that traits/genes were being passed down in the scenarios where selection forces selected the same trait. The lack of inclusion of reproductive potential and inhertiance may be related due to their similar roles in the evolutionary process. Reproduction must occur for traits to be inherited, and traits must be heritable for them to be passed on to the next generation. The lack of inclusion of inheritance made it difficult to determine how students were reasoning about the component of change over time. Students described changes in the populations, but it was unclear if the change was over generations or a change in the current population during a single generation.

Changing the context of selection pressure did not impact how students described variation and change over time. Students were able to describe intraspecific variation, especially when presented with an image of a population, regardless of the interaction between selection forces. Students varied in how they described change over time, as they described change over one generation or they described change over multiple generations. There was no clear pattern indecating that changing the context of sexual selection affected how they reasoned about change over time.

Selection of Different Variants of a Trait May Elicit Improved Reproductive Reasoning and Application of Sexual Selection

Scenarios with multiple selection pressures acting on a population fostered responses that included more application of sexual selection. When selection pressures selected for the same trait, even when students recognized sexual dimorphism, many students did not incude sexual selection as an important evolutionary force (Figure 3.1). Providing students with examples that have multiple selection pressures selecting for the same variant of a trait may not guide students to recognize there are multiple pressures behind some traits. Scenarios where selection based on mate choice and selection based on survival selected for different variants of a trait provided a context that helped more students describe the multiple selection pressures in a system as students could more clearly visualize how different selection pressures led to different outcomes. This may be because outcomes were maladaptive for survival (Ritchie, 2007; Chenoweth et al., 2015). When multiple selection forces selected for different types of traits, it may help students better recognize selection can be based off of things other than survival, as contradicting prior knowledge can help guide students in their evolutionary reasoning (Alters & Nelson, 2002; Anggoro, 2019).

Differences emerged in how students described fitness and inheritance, components related to reproduction. The emphasis on survival in real-world use of fitness and instruction of evolution may lead students to emphasize survival in their reasoning (Bishop & Anderson, 1990). The results indicated that providing examples where there are multiple selection pressures selecting for different traits guided students recognize the role of reproductive potential in fitness and the role of inhertiance in evolution (Figure 3.1). The presence of sexual selection that selected for a different trait variant than

survival-based selection may have guided students to include the importance of the evolutionary components related to reproduction. Inheritance is greatly affected by reproductive potential as rates of inheritance are affected by how often an organism successfully reproduces. The close connection between reproductive potential and inheritance may explain why student responses included inheritance more often in their widowbird responses where reproductive potential had a more apparent influence on evolution since ornamental tails for mating are common in the population (Gregory, 2009).

Inheritance plays an integral role in the evolutionary story as it explains the individual steps of the seemingly gradual changes observed in a population over many generations (Gregory, 2009). When students described inheritance, they reasoned about the instances where genes were being passed on, rather than gradually changing in a population. They also addressed that favorable traits were passed on more often, instead of favorable traits being picked up by individuals within a lifetime. The inclusion of inheritance and reproductive potential led to more responses including the level of detail necessary to connect the evolutionary components (Nehm et al., 2012; Salter & Momsen, 2018). It is possible that students' increased use of inheritance in their responses help them more fully describe the mechanisms of change over time.

In the scenario where sexual selection and artificial selection opposed one another, students were once again able to describe how multiple selection pressures could select for different types of traits. They described how male fitness could be reduced if they sang at a lower frequency near low-frequency traffic noise and how rural counterparts would be seen as more fit if they sang at lower frequencies compared to

urban males. They also described how traits learned in a lifetime could not be inherited because the trait must be associated with genetic information that is passed on through reproduction. Once again, sexual selection was more clearly observed by students and improving their description of the role of reproduction.

Context influences how students reason about evolution in several categories such as whether there is a gain or a loss of a trait (Nehm & Ha, 2010). It seems that changing the context of selection pressure may help students recognize that forces other than survival can drive evolutionary change by providing a discrepant event that guides students to think outside of survival. Contradicting students' prior reasoning may guide students to apply evolutionary components and selection forces that they would typically not apply (Alters & Nelson, 2002). For example, if a trait is maladaptive for survival, but benefits reproductive potential, a student may better recognize the role of reproductive potential as a part of fitness. In scenarios where there was sexual dimorphism and sexual selection opposed selection based on survival (i.e., widowbirds), more students described mate choice and identified reproductive potential as a part of fitness, regardless of original definition of fitness. This suggests that students did know that reproductive potential was important to fitness, but they didn't recognize its importance in scenarios where the role of mate choice was not apparent. Instructors cannot assume that students recognize selection forces simply because they are there, but providing contexts where different selection pressures oppose may guide students to recognize them.

Teaching Implications

Using more examples with selection forces selecting for different types of traits in teaching may benefit evolution instruction and assessment, and may help students better

recognize the role of multiple selection forces in evolutionary change. Instructors must consider selection forces in teaching and assessment examples as the different selection forces may impact how students are thinking about evolution, as context can affect evolutionary reasoning (Nehm & Ha, 2011). Providing scenarios with multiple selection forces may help instructors better determine which students are committed to survival-based reasoning, as some students persist in applying only survival even a selection pressure favors a trait variant that is maladaptive for survival (Bishop & Anderson, 1990; Price & Perez, 2016).

Sexual selection should be emphasized more when students are learning about natural selection, as it can serve as a good example of how multiple selection forces can act upon a trait. In the textbook used by the students in this study, sexual selection is found within the natural selection section, and it includes that sexual selection can seem maladaptive when it is actually beneficial to fitness (Urry et al., 2014). However, if the goal is for students to consider all possible evolutionary forces when reasoning about evolution, instruction may need to go further and include more details about the different impacts of different selection forces, including sexual selection. The textbook describes directional selection, disruptive selection, and stabilizing selection, different ways in which selection pressures can interact to select for different or similar traits. However, it attributes all selection interactions to survival-based selection situations, such as environmental change leading to population isolation. It would be beneficial for the textbook to include how sexual selection may also contribute to directional selection or oppose other types of selection, creating a disruptive selection situation like the widowbird example in the interview of this study. Leaving out selection force

interactions may be a missed opportunity for students to better understand the role of multiple selection forces.

Providing examples with different selection pressures selecting for different variants of a trait can guide students to apply more evolutionary forces to future scenarios. Guiding students to consider more options than just natural selection may help students not be so committed to survival-based evolutionary reasoning. Providing examples with diverse selection may lead students to apply more diverse components, and the ability to apply more components is indicative of better holistic evolutionary reasoning (Nehm & Ha, 2011). Scenarios with multiple selection pressures are a potential tool to improving evolutionary instruction by providing students with more diverse contexts that reflect the complexity of evolutionary change.

Study Limitations

In this study there were a small number of interviews (n = 12). For future studies, I would recommend interviewing more students to determine if changing selection pressure interactions in scenarios leads to larger scale patterns in student reasoning. Instruments used for larger populations of students, such as the ACORNS and CINS, could also include questions that touch on selection pressure interactions (Anderson et al., 2002; Nehm & Reilly, 2007). This study focused on introductory biology students. Interviewing more advanced students, perhaps after taking an evolution course, may provide more information about the development of student reasoning as they progress through a biology program. There has been a learning progression study of students in the years prior to university, but we do not know how student reasoning of selection develops as they progress through university programs (Scheuch et al., 2019).

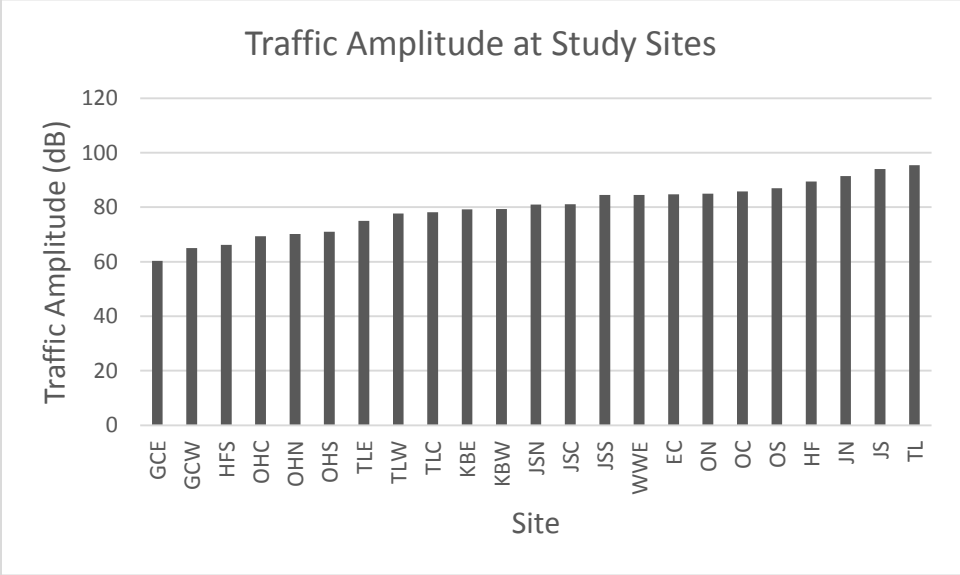
The order of the interview stayed the same for this study, but there may be an effect of the order on student reasoning. The order of the interview could be randomized to control for this effect. When using a larger student population, the order of the interview could also be randomized to for some and not for others to determine if the progression of contexts affected student reasoning. If there is a learning progression across the scenarios, the scenarios may provide an opportunity to explore student learning progressions as they reason about different selection force interactions.

APPENDICES

APPENDIX A: STUDY SITE LOCATIONS

	Name	Coordinates	Distance from Road (m)
2017	Glacier Creek East	41°20'9.66"N 96° 8'22.43"W	50
	Glacier Creek West	41°20'8.50"N 96° 8'47.26"W	50
	Holy Family Shrine	41° 4'42.41"N 96°16'43.65"W	62
	Oak Hills North	41°12'8.34"N 96° 5'44.25"W	63
	Oak Hills South	41°11'27.92"N 96° 6'5.88"W	56
	Oak Hills Central	41°11'52.95"N 96° 5'46.21"W	50
	Twin Lakes East	40°49'20.68"N 96°56'26.65"W	58
	Twin Lakes West	40°49'18.92"N 96°58'1.77"W	53
	Twin Lakes Central	40°49'27.40"N 96°56'44.84"W	281
	Kirkpatrick Basin West	40°49'23.57"N 97°39'54.44"W	205
	Kirkpatrick Basin East	40°49'18.82"N 97°39'41.18"W	59
	Jack Sinn North	41° 2'43.61"N 96°38'19.10"W	106
	Jack Sinn Central	41° 1'50.22"N 96°38'29.85"W	107
	Jack Sinn South	41° 1'37.18"N 96°38'24.42"W	50
	Werner Wetlands	40°53'54.99"N 96°35'16.92"W	137
	East Campus	40°49'51.93"N 96°39'16.02"W	53
	Whitehead	40°52'43.8"N 96°40'48.3"W	153
	Riverside	41°15'18.45"N 96°15'40.67"W	50
2018	East Campus	40°49'51.93"N 96°39'16.02"W	51
	Holy Family Shrine	41° 4'30.53"N 96°16'37.85"W	370
	Jack Sinn NW	41° 2'43.80"N 96°37'51.83"W	189
	Jack Sinn NE	41° 2'43.48"N 96°38'15.54"W	760
	Jack Sinn S	41° 1'50.10"N 96°38'29.23"W	94
	Oak Hills C	41°11'52.04"N 96° 5'57.04"W	300
	Oak Hills N	41°12'9.23"N 96° 5'50.01"W	197
	Oak Hills S	41°11'29.02"N 96° 6'7.92"W	115
	Twin Lakes	40°49'27.74"N 96°56'40.24"W	277
	Riverside	41°15'18.45"N 96°15'40.67"W	50

APPENDIX B: TRAFFIC AMPLITUDE AT STUDY SITES



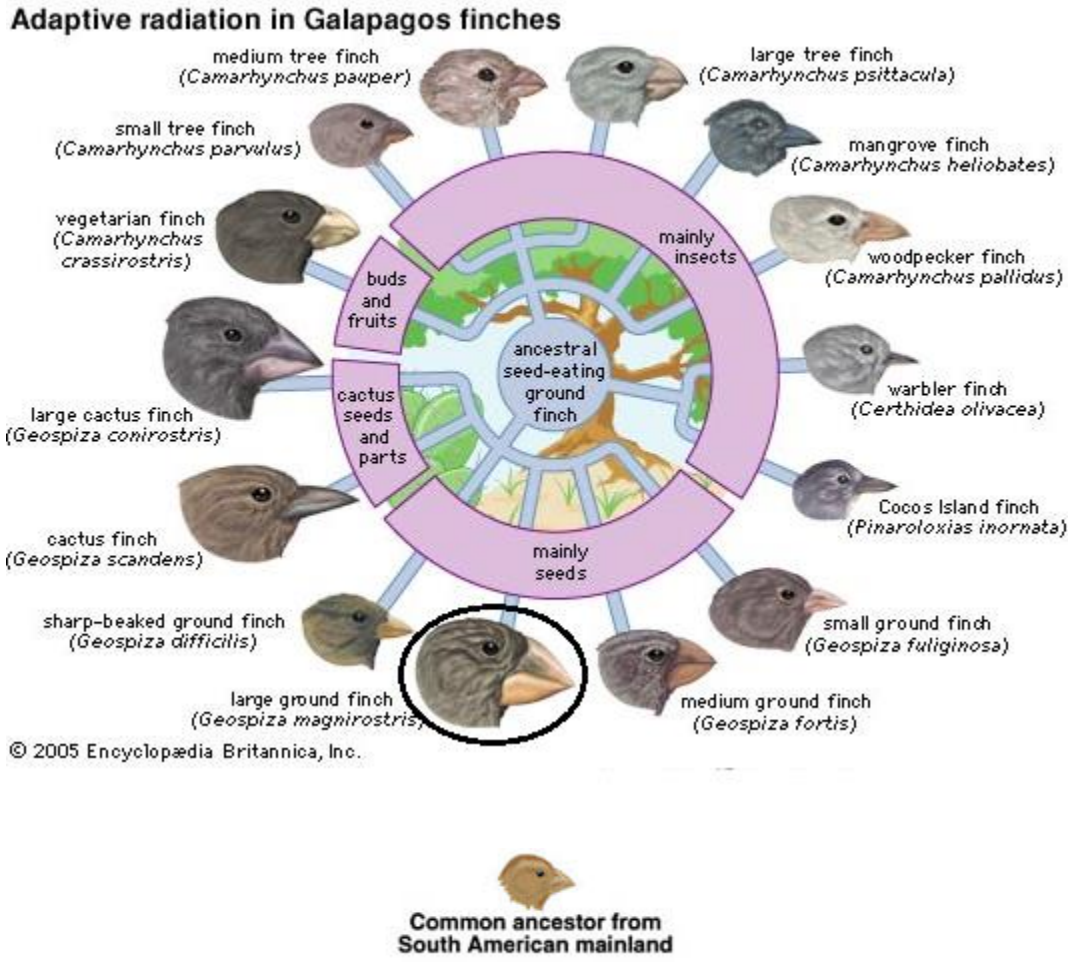
APPENDIX C: SPECIES PREDICTED PROBABILITY

Traffic Noise and Species Detection Probability									
	Species	60	65	70	75	80	85	90	p-value
Masked	AMRO	0.50	0.56	0.63	0.69	0.74	0.79	0.83	0.153
	COGR	0.12	0.17	0.23	0.30	0.38	0.47	0.56	0.066
	NOCA	0.26	0.29	0.32	0.36	0.39	0.42	0.46	0.388
	BLJA	0.20	0.19	0.18	0.17	0.16	0.15	0.14	0.730
	MODO	0.35	0.31	0.27	0.23	0.20	0.17	0.14	0.318
Unmasked	COYE	0.69	0.55	0.43	0.33	0.23	0.16	0.12	0.016*
	YEWA	0.50	0.40	0.30	0.23	0.16	0.11	0.09	0.052
	RWBB	0.66	0.56	0.44	0.33	0.24	0.17	0.11	0.023*
	DICK	0.34	0.33	0.32	0.31	0.30	0.29	0.28	0.783
	EAME	0.46	0.35	0.29	0.22	0.16	0.12	0.10	0.060

APPENDIX D: STUDENT INTERVIEW INFORMATION

Darwin's Galapagos Finches

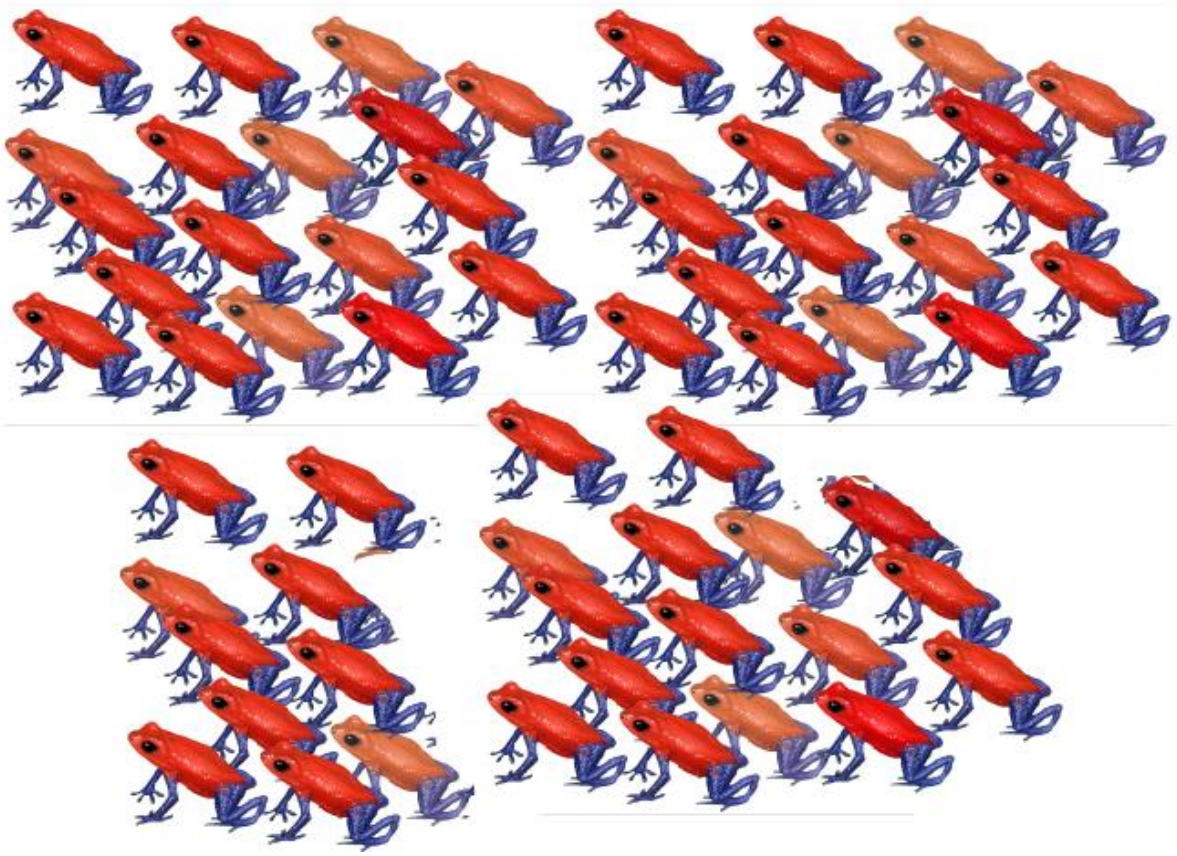
In the pretest, you were given an example of evolution acting on Galapagos finches. The 14 different species of finch all descended from one single species after being geographically isolated on separate islands. The islands can have different food sources. A major distinguishing feature of the different species is beak size and shape.



Poison Dart Frogs

Female **Male**

Male Poison Dart Frogs



Long-Tailed Widowbird

Female

Image Source: Eric Landsberg

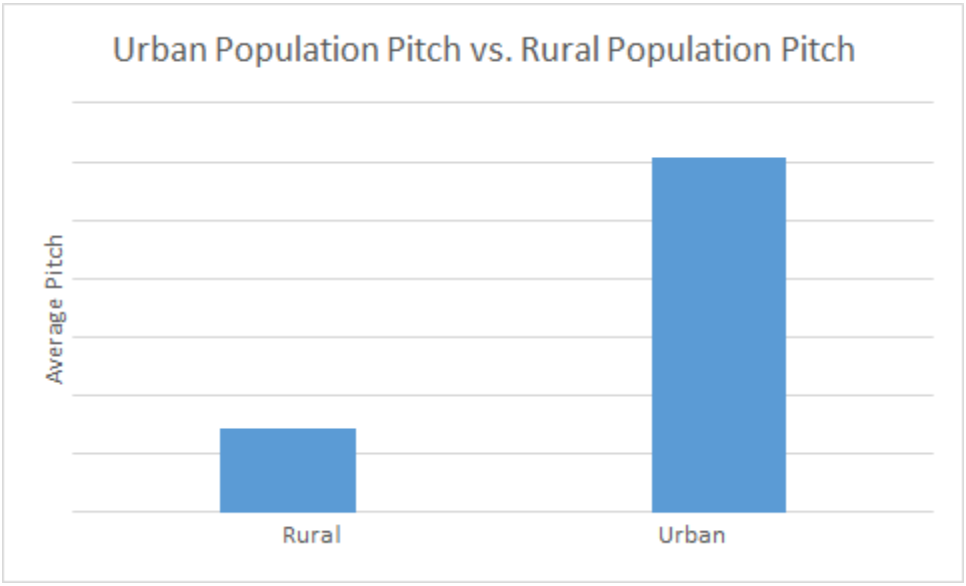
Male

Image Source: Chris JeK

Long-Tailed Widowbird

Black-capped Chickadees

There is evidence of Black-capped Chickadees increasing the pitch of their songs in response to loud traffic noise. Females prefer mates with lower-pitched songs.



APPENDIX E: INTERVIEWER SCRIPT

Interviewer: "DATE, TIME, STUDENT #"

Interviewer: "I am interested in how students reason about evolution. Today I will be asking you a series of questions about evolution. Some questions will be about evolutionary concepts in general, while others will be related to specific ecological contexts. As you answer these questions, please walk me through your reasoning by thinking out loud to the best of your ability. If you need me to restate or reword a question at any time, please feel free to ask.

1. In your own words, define fitness.
2. In your own words, define the process of adaptation.
3. In your own words define natural selection.
4. In as much detail as possible, please describe the process of evolution."

1. <u>Darwin's Galapagos Finches</u>

[Student shown finch scenario from APPENDIX D]

Interviewer: "In the pretest, you were given an example of evolution acting on Galapagos finches. The 14 species of finch all descended from one single species after being geographically isolated on separate islands. The islands can have different food sources. A major distinguishing feature of the different species is beak size and shape.

1. Does having a larger beak affect fitness?
2. Describe how the Large Ground Finch evolved a larger beak size than that of the common ancestor."

<u>Poison Dart Frogs</u>

Interviewer: "Next, I will provide some images of Poison Dart Frogs. First here is an image of a male and female [Student shown poison dart frog male and female image from APPENDIX D].

1. Are there observable differences between the male and female frogs? Why?
2. Do you think a predator would avoid males or females more?
3. How does color affect fitness?"

Interviewer: "Here is a population of males [Student shown frog population image from APPENDIX D].

4. What do you observe about the male population?
5. Describe how evolution has acted on this population to cause it to appear as it does currently."

Interviewer: "Now I will provide you with three figures to fill in [Provided graphs from APPENDIX D]. The x-axis is skin color redness for all three and the y-axes are survival, reproductive potential, and fitness.

6. Create a line graph based on your interpretation of the relationship between the factors on the two axes."

Long-tailed Widowbirds

Interviewer: "Now we will move on to a scenario with Long-tailed Widowbirds. First, here are pictures of a male and a female Long-tailed Widowbird [Student shown widowbird male and female image from APPENDIX D].

1. Are there observable differences between the male and female widowbirds? Why?
2. Do you think a predator would have an easier time capturing a male or a female? Why?
3. How does tail length affect fitness?

Interviewer: "Now here is a population of male Long-tailed Widowbirds [student shown image of widowbird male population from APPENDIX D]

4. What do you observe about the male population?
5. Describe how evolution has acted on this population to cause it to appear as it does currently"

Interviewer: "Now I will provide you with three more figures to fill in [Provided graphs from APPENDIX D]. The x-axis is tail length for all three and the y-axes are survival, reproductive potential, and fitness.

6. Create a line graph based on your interpretation of the relationship between the factors on the two axes."

4. Black-capped Chickadees

Interviewer: "[Student shown Chickadee image and figure from APPENDIX D] There is evidence of Black-capped Chickadees increasing the pitch of their songs in order to be heard over loud traffic noise. Females prefer mates with lower-pitched songs.

1. Please interpret this graph for me.
2. Why do songs have different pitches?
3. Is there variation in the songs of individual males within each specific population? Why/why not?
4. How do different pitches influence fitness?
5. Is a male changing its pitch in response to traffic noise an example of evolution? Why or why not?
6. Singing is a learned behavior in Chickadees. How would offspring be affected by this change in behavior?
   -Are learned behaviors inherited? Why or why not?
7. Can singing evolve in chickadees? Why or why not?

APPENDIX F: QUALITATIVE CODE BOOK

	Coding Instructions	Code	Code Descrption	Example Quotes
Selection	Code for the inclusion of selection occurring based on survival (NS) and/or selection occurring based on attracting mates (SS). Some responses may include no selection force (N) or both types of selection forces (B).	NS	Natural Selection: The student describesthe process of natural selection, or selection based on survival or a change in the environment. Including the term "natural selection" without describing the process is not suffient.	*"At the population level, there is a certain amount of variation and then over time as* ***natural selection*** *occurs and is acting on that variation"* *"The finches that had smaller beaks wouldn't survive and be able to reproduce while* ***the finches that had larger beaks would be able to survive and eat the seeds****. Then that trait would probably eventually just become common throughout the whole population to get to the large ground finch with a bigger beak."*
		SS	Sexual Selection: The student describes an evolutionary change based on mate preference or reproductive success	*"They could help them live longer, but* ***if they're reproducing then it must be attracting mates*** *or it's just that the ones who aren't as aren't are more likely to be killed"*
		N	The response does not include any selection force.	
		B	The student describes selection based on survival and attracting mates.	*"Maybe the medium tail is more favorable because* ***if it's too long, a predator will get them and if it's too short a female won't mate with them****. So having a tail length in the middle is the best."* *"The middle of the road trait is allowing them to* ***not get captured*** *as much as the long tailed ones and* ***able to attract a mate*** *therefore they are reproducing more."*
Variation	Code for the inclusion of a source of variation and whether they describe variation in the population. There will be two codes for each response, one for source of variation followed by one for variation in the population. Example: V, 0 if the student described variation in the population but did not include a source of variation.	M	The response includes a source of variation, and the source is a mutation.	*"I think there'd have to be like a* ***mutation*** *for like a larger beak size."*
		S	The response includes a source of variation that is not a mutation.	*"Even the population that was present with this common ancestor,* ***at least a couple of them had to have had larger beaks.*** *"*
		V	The student describes variation in the population.	*"There was a different* ***variation of the red*** *in these frogs"*
		0	The response does not include variation in a population or a source of variation (0,0 if none for both).	
Fitness	Code for the use of survival and reproductive potential in student responses when they are directly asked about fitness. Responses that include how both survival and reproduction affect fitness should be labelled "SR." If only survival or reproduction is used alone, label "S" or "R."	S	The student describes fitness as the ability to survive or bases fitness on survival alone.	*" it's central a who* ***survives*** *the best."*
		R	The student describes fitness as reproductive potential alone.	*"Fitness is the ability of an individual to* ***have offspring successfully****."*
		SR	The student includes both survival and reproductive potential in their description of fitness.	*"Fitness is the ability to* ***reproduce and survive*** *"* *"The long tail is a negative and a positive because not only does it make you easier to spot, which makes you* ***more likely to be caught*** *by a predator, but it a* ***lso helps you find a mate and pass on your traits****, which would make you a more fit species or more fit organism."*
Inheritance	Code for whether responses include inheritance. Responses marked "I" may include the term inheritance directly or the student may describe the passing on of genes or traits to the next generation. If there is no mention of inheritance, label "0."	I	The student clearly describes the passing on of genes or traits; includes the terms "inheritance" or "passed on."	*"Red frogs available to reproduce and* ***pass on*** *their bright red to their offspring."* *"Traits that are helping them survive are being* ***inherited*** *and reproduced way more than traits that aren't working so great."*
			The student describes reproduction and connects it to change over time, and it can be inferred that the student is describing inheritnace, but the student does not directly include inheritance.	*"I see more red than the orange. I guess that the r* ***ed frogs were more successful in having offspring, so that caused the population to have a change in the alleles*** *so that more of the frogs nowadays are red than they were in the past."*
		0	The response does not include inheritance.	
Change over time	Code for whether responses include a description of a change in the population over time. Responses may describe change over many generations (MG) or one generation (OG).	MG	The student describes a change in the population and includes that it occurs over multiple generations.	*"The individuals with more relative fitness are going to be able to, to have more offspring, making their traits* ***more present in the next generations, and that will lead to the change of alleles in a population.*** *"* *"Evolution would come not immediately, obviously it will have to happen over* ***multiple generations*** *in order for something to happen"*
		OG	The student describes a change in the population in a single generation.	*"The population probably* ***changed due to chance or mutation****. One ended up with the different sized tail and if it was more beneficial for them to have that sized tail, they would probably reproduce more."* *"So the finches that had smaller beaks wouldn't survive and be able to reproduce while the finches that had larger beaks would be able to survive and eat the seeds. And then that trait would probably* ***eventually just become common throughout the whole population*** *to get to the large ground finch with a bigger beak."*
		0	No mention of a change in the population over time.	

APPENDIX G: DATA FROM INTERVIEW QUESTIONS

Interview Question	**Data from question**
In as much detail as possible, please describe the process of evolution by natural selection	
In your own words, define fitness.	Baseline fitness definition
In your own words, define the process of adaptation	Baseline adaptation definition
Describe how the Large Ground Finch evolved a larger break size than that of the common ancestor. Follow up: Does genetics play a role in the change in beak shape	Baseline natural selection definition
IF NEEDED: All of the seed-eating species can be found on Santa Cruz Island (see map). Please explain how speciation occurred in a single island?	Baseline evolution definition
The male on the bottom has a higher fitness than the male on the top. Why might this be?	
If the ancestral species of dart frogs was duller, how did bright color evolve in poison dart frogs? Follow-up: Does genetics play a role in this scenario?	Fitness reasoning with familiar scenario
Do you think a predator would have an easier time catching a male or female? Why?	Evolution reasoning with familiar scenario
How might reduction of tail feather length affect male fitness?	
The average widowbird male has a tail length similar to that of bird 2. Describe how evolution led to the intermediate length instead of the tail length of birds 1 and 3?	Fitness reasoning when natural and sexual selection are concurrent
Please fill in the graphs based on your interpretation of the relationship between the factors on the two axes.	Evolutionary reasoning when natural and sexual selection are concurrent
How could this affect an individual male (Chickadee)?	Student description of relationship between components of fitness and fitness with phenotype when sexual selection reinforces natural selection
How could this affect the overall population (Chickadee)?	
What do we need to know to determine if this scenario has an effect on the evolution of Black-capped Chickadees?	Fitness reasoning when natural and sexual selection are opposing, and students are told of female preference

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