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A study on the role of social information sharing leading to range expansion in songbirds with large vocal repertoires: Enhancing our understanding of Great-Tailed Grackle (*Quiscalus mexicanus*) breeding and non-breeding vocalizations

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ABSTRACT

The acoustic adaptation hypothesis posits that animal sounds are influenced by the habitat properties that shape acoustic constraints (Ey and Fischer 2009, Morton 2015, Sueur and Farina 2015). Alarm calls are expected to signal important habitat and receiver-dependent information (Ripmeester et al. 2010, Sheldon et al. 2020), and we want to test whether *Q. mexicanus* alarm calls differ between populations and ecological contexts across the US as expected under the acoustic adaptation hypothesis (three US subspecies: *Q. m. nelsoni*, *Q. m. monsoni*, and *Q. m. prospidicola*; Figure 1). The alarm call vocalization in *Q. mexicanus* is known to vary in tone, range and pitch (Kok 1971). Alarm calls signal low intensity excitement (Kok 1971) and research in other species has shown that differences in the acoustic qualities of alarm calls reflect the urgency of threats tailored to the receiving audience (Carlson et al. 2020, Sheldon et al. 2020, McLachlan and Magrath 2020). However, due to the ecological importance of alarm calls in minimizing risk to group members, natural selection could promote stabilizing selection on alarm calls, resulting in homogenous alarm call structure across subspecies regardless of habitat and receiver. For this reason, we will also test whether *Q. mexicanus* songs differ between populations and ecological contexts across the US as natural selection likely promotes disruptive selection on song structure to facilitate subspecies recognition during mating season (Cruz-Yepez et al. 2020, Simpson et al. 2021). In this project we will enhance our understanding of the vocal repertoire of *Q. mexicanus*, by 1) recording and describing alarm calls and songs, 2) testing a null hypothesis that differing vocalizations will correlate with subspecies-specific soundscapes, and 3) test an alternative hypothesis that vocal signal characteristics correlate with range expansion. We will improve the description of vocalizations by recording vocalizations from each subspecies and

analyzing the tone, range and pitch of vocalizations using spectrograms generated with Raven Lite 2.0 (Cornell Lab of Ornithology). Recording of alarm calls will take place during the non-breeding season, and of songs during the breeding season. We will only record alarm calls during the non-breeding period to avoid differences associated with reproduction. For our first objective, a phylogenetic principal component analysis (PPCA) will be conducted to identify correlations among measures of vocalization structure across subspecies while accounting for phylogenetic history. For our second objective, a phylogenetic generalized least squares analysis (PGLS) will be conducted to determine if subspecies vocalization characteristics are explained by social and habitat contexts within a phylogenetic context. To test whether vocalizations have functionally diverged and to help explain differences in range expansion, we will conduct a reciprocal playback experiment measuring responsiveness to recordings from within each subspecies compared to those from other subspecies. We will use the results of the PPCA and playback experiment to test whether vocal signal characteristics (both signal and response) are significant regional drivers of predicted distributions for *Q. mexicanus* in the US using an ensemble distribution model. If vocal signal skill is learned from context-dependent experiences unique to each subspecies (i.e., in line with the acoustic adaptation hypothesis), then individuals should share vocal characteristics with and respond to the signals of their own subspecies but not to signals of other subspecies. Tone, range, and pitch of vocalizations as well as low responsiveness will be a significant explanatory variable in all regional models (i.e., differences in vocal signals will distinguish subspecies distributions). However, if differences in regional models are due to variation in responsiveness according to subspecies, then skill in vocal communication could contribute to differences in range expansion among subspecies. Generalized linear models will be used to analyze the data of the playback experiment. All quality control processing and statistical analyses will be performed in the R environment (Araya-Salas and Smith-Vidaurre 2017, R Core Team 2020, Araya-Salas 2021). Data collection will stop once the minimum sample size is reached (n=88 per population, per season, calculated using a power analysis to detect small differences in signal strength). Quantifying differences in signal strength of vocalizations between *Q. mexicanus* subspecies will contribute to our understanding of vocal plasticity in heterogeneous landscapes. We will additionally test a series of alternative hypotheses using an ensemble distribution modelling approach to determine whether the distributions of each subspecies in the US are correlated with habitat variables (i.e., wetlands that do not freeze in winter, proximity of wetlands to disturbed habitat) and co-occurrence data with other blackbird species (i.e., occurrence data will be sourced from the citizen science platform, eBird).

Figure 1. Distributions of the three *Q. mexicanus* Great-tailed Grackle subspecies in the US. This map depicts the relative abundance of individuals from eBird data (map from Johnson and Peer 2020) and we drew subspecies distributions using range descriptions by Clements et al. (2019). Occurrence records denoted with purple reflects year-round site occupancy, red reflects site occupancy during the breeding season only, and blue reflects wintering congregations. Stars reflect the proposed research areas.

1. Research Questions

Could social vocal communication explain differences in the northern reach of year-round range expansion in three great-tailed grackle subspecies: *Q. m. monsoni*, *Q. m. nelsoni*, and *Q. m. prosopidicola*?

Background - Bird species with large vocal repertoires and geographic ranges are thought to have high plasticity in acoustic qualities in order to match tone, range, and pitch to the heterogeneous soundscape (Ríos-Chelén et al. 2012). This is true in oscine birds that learn their songs (Kroodsma 1982, 2004) such as the banded wren (Molles 2006), the kōkako (Bradley et al. 2013), and our study taxa the great-tailed grackle (Quiscalus mexicanus Kok 1971). While Q. mexicanus is an urban-adapted species, selection pressures in urban environments may compromise previously effective status signals (Swaddle et al. 2015), used to obtain or defend resources that benefit survival and reproductive success. Birds using vocal signals in urban noise have been shown to use lower performing vocalizations (Phillips et al. 2020), and less complex vocalizations (Phillips and Katti 2020, Phillips et al. 2020). Following the simulation work of Hudson and Creanza (2021) on the evolution of birdsong as a functional trait in oscine birds, we aim to test whether selection on signal skill

(measured as the descriptive qualities of alarm calls and songs, and the responsiveness to vocalizations in a playback experiment) may play a role in range expansion. Although oscine birds learn their vocalizations, there is evidence of a genetic component of vocalizations (Snyder and Creanza 2019), as well as morphology (beak size and shape; Podos 2001) and physiology (affecting trill rates and auditory sensitivity; Podos 1996, Prather et al. 2012). Thus, our analyses of song variables will be conducted in a phylogenetic context to account for evolutionary history between subspecies.

Study taxa - *Q. mexicanus* is a highly vocal resident species with a large vocal repertoire (Kok 1971, Johnson and Peer 2020) and subspecies have recently expanded their ranges to occupy a wide geographic range northward in the US with unequal success (Christensen 2000, Wehtje 2003, DaCosta et al. 2008). We aim to test whether social vocal communication could explain differences in the northern extent of year-round invasion fronts across *Q. mexicanus* subspecies. *Q. m. nelsoni* has the northernmost year-round range and is the earliest divergent subspecies within those being studied (Figure 2; DaCosta et al. 2008). *Q. prosopidicola* has year-round populations further south, but which are at higher densities and sister taxa *Q. m. monsoni* has seasonally ephemeral populations that retreat close to the border with Mexico during the non-breeding season (Figure 1; Clements et al. 2019, Johnson and Peer 2020). While *Q. m. nelsoni* and *Q. m. monsoni* may come into secondary contact in the southwestern US during the breeding season (DaCosta et al. 2008, Johnson and Peer 2020), there is no evidence of hybridization between these two subspecies which are not each other's closest relatives (DaCosta et al. 2008; Figure 2). There is a possibility that secondary contact could select for increased differences in song between these two subspecies to promote reproductive isolation.

Figure 2. A simplified phylogeny of *Q. mexicanus* subspecies in this study. This figure was created by matching the maximum-likelihood topology of a grackle phylogeny built using mitochondrial with sample locations from DaCosta et al. (2008). Note: We do not provide a timescale for this phylogeny as we plan to create a new phylogeny and do not wish to present false estimates (see Other Variables, below).

2. Hypotheses

Hypothesis 0 - The acoustic adaptation hypothesis governs animal sounds. Differences in the vocalizations of *Q. mexicanus* subspecies are habitat and context-dependent and unique to each subspecies. The northern reach of the year-round range is explained by habitat availability (measured using ensemble modeling).

Prediction 1 (vocalizations = match habitat): The vocalizations of each subspecies will be different from one another (measured using PPCA), and subspecies will not recognize one another (measured using GLM).

Prediction 2a (habitat = wetlands): Because *Q. mexicanus* rely on wetland habitat to meet their life history needs (Johnson and Peer 2020), differences in the extent of range expansion in *Q. mexicanus* subspecies in the US are explained by the availability of year-round habitat beyond the current range. The extent of the ranges

of each subspecies will be delineated by the presence of year-round wetland habitat that does not freeze during winter within the area they are known to breed in. The range of *Q. m. nelsoni* should have more year-round unfrozen water in wetland habitat compared to the northern breeding areas of the other two subspecies.

Prediction 2b (habitat = disturbed): Because disturbed habitats are more likely to be invaded than undisturbed habitats (Marvier et al. 2004), differences in the extent of range expansion in *Q. mexicanus* subspecies in the USA are explained by the amount of disturbed habitat near wetlands. Note: This is not a mutually exclusive hypothesis from Prediction 2a. The extent of ranges of each subspecies will be delineated by the availability of disturbed habitats adjacent breeding wetlands. The range of *Q. m. nelsoni* is expected to include more disturbed habitat adjacent breeding wetlands compared to the northern breeding areas of the other two subspecies.

Alternative 1 - Different rates of range expansion in *Q. mexicanus* subspecies in the US are explained by competitive release through the lack of co-occurrence with other blackbird species because successful invasions can occur when a population gains a competitive advantage in a new area.

Prediction 3 (vocalizations = no differences): The vocalizations of each subspecies will be no different from one another than they could be due to chance (measured using PPCA), and subspecies may or may not recognize one another (measured using GLM).

Prediction 4 (habitat = fewer competitors from other species): *Q. m. nelsoni*, the northernmost subspecies, is expected to co-occur with fewer blackbird species compared to the range of the other two subspecies.

Alternative 2 - Differences in the extent of range expansion in *Q. mexicanus* subspecies in the US are explained by the strength of social vocal communication which is expected to convey important information for meeting life history needs (e.g., the urgency and type of danger, the location and possibly nutrition of food sources, or the location of nocturnal roosts).

Prediction 5a (vocalizations = expansion~vocalization complexity): *Q. m. nelsoni*, the northernmost subspecies, is expected to have more complex alarm calls compared to the other two subspecies. This will reflect that more detailed information is being communicated.

Prediction 5b (vocalizations = expansion~enhanced social communication): *Q. m. nelsoni* individuals are expected to respond more to playback of their own subspecies as well as other subspecies, reflecting more flexibility in their vocal communication compared to the other subspecies. *Q. m. monsoni* and *Q. m. prosopidicola* are expected to respond less to playback of their own subspecies and not at all to the alarm calls of the other subspecies. Responsiveness to playback will be measured as the distance moved after the onset of the playback experiment (either toward or away from the speaker), the number of vocalizations made by

the target individual, and the number and duration of any non-vocal displays made in response to the playback (e.g., head-up displays) during the observational period following playback.

Prediction 6 (habitat = regional models include vocalization and responses as significant explanatory variables): Any patterns in the drivers of regional distributions that distinguish *Q. mexicanus* subspecies will include differences in vocalizations or responsiveness.

Note: All hypotheses will be tested separately for non-breeding and breeding vocalizations.

Sampling Plan (Sections 3-8)

3. Existing Data

This preregistration was written prior to the collection of any data.

4. Explanation of existing data

This preregistration was written prior to the collection of any data.

5. Data collection procedures

Training

Both authors had previous experience with observing the species and recording birds for a minimum of one year to determine a list of best practice methods (see below). This training period also included practice with two different sets of recording equipment (Marantz versus Mix Pre-3).

Below we list training steps that facilitate robust acoustic data collection:

- 1) Be familiar with the how gain level affects recording quality
- 2) Be familiar with what distance is appropriate for the study species to not affect their behaviour (both for setting up the speaker for playback and recording)
 - a) Be familiar with how the study species shows signs of behavior change (e.g., flying away, erect posture, stopping one behaviour to be more vigilant because of your presence etc.)
- 3) Be able to identify and distinguish different types of vocalizations from the study species (to ensure that both team members are recording the same vocalization types)
- 4) Understand how to position yourself with respect to the bird and sources of interfering background noise that could improve the quality of recordings
- 5) Understand how background noise can affect recording quality

Below we list a set of 'best practices' that we follow:

- Keeping the bird being tested in view at all times
- Recording microhabitat, demographic, other variables (see below)

- Record and conduct experiments only in suitable weather conditions (e.g., not when stormy or raining) and away from noisy vehicles or machinery to limit acoustic interference
- Use .wav files for playback experiments and not compressed sound files
- Always carry multiple sets of replacement batteries for both the recording device and the microphone
- Format the SD card in the audio recorder between recording days to not run out of memory

Audio-recordings

Alarm calls will be recorded in the wild during the winter months (December - February) and summer months (May - August) of 2020, 2021, and 2022. Because individuals are not marked, we will first record the individual and then expose it to the playback experiment. In this way, our two vocalization data sets will not be independent and will thus give us more power to assess the strength of responses to playback in comparison to how different the individual's vocalization was compared to the vocalization it is exposed to in the playback experiment. More practically, this will enable us to avoid accidentally resampling individuals and to only visit sites once for the duration of the project.

Grackles will be recorded 5-10 times using Rode NTG-2 shotgun microphones (frequency responses = 2-8 Hz) and saved to a Mix-Pre 3 audio recorder. Recordings will be uploaded to an external hard drive and backed up using the DropBox cloud. Grackles will be recorded from a distance of approximately 5 meters to reduce the potential of interference from researchers. Alarm calls will be identified using spectrograms generated with Raven Lite 2.0 (Cornell Lab of Ornithology). An alarm call is defined as a low, single-syllable "chut" with a wide frequency range used by grackles throughout the year in low intensity excitement (Kok 1971).

Microhabitat Variables

Microhabitat structure and the soundscape can affect bird vocalizations. To account for this, we will note the habitat type (using terrestrial ecosystem classifications of Sayre et al. 2009), the substrate the individual made the vocalization from, and measure decibels of background noise at the time of recordings using the free app [Decibel X](#).

Demographic Variables

Because our results could be affected by the social context in which vocalizations are produced or responded to, during all recordings and the playback experiment we will record the group size, as well as the age and sex of all individuals in the group. Although it is possible that grackle density beyond the group could play a role in vocal variability and responsiveness during our study, assessing local *Q. mexicanus* population density is beyond the scope of the proposed research.

Other Variables

We will also account for additional variables that could affect our results including: the time of day, date, weather (temperature, wind speed and direction, cloud cover, precipitation), latitude, longitude and elevation (meters a.s.l.), and the estimated distance from the individual we are recording/testing. As differences in bill morphology and body size can shape vocalization evolution (Demery et al. 2021), we also account for

differences in body size between subspecies by using the average mass and bill morphometrics (measured from museum species at Louisiana State University). We will also account for phylogenetic relatedness using a phylogeny built either from mitochondrial genes for each subspecies in GenBank (<https://www.ncbi.nlm.nih.gov/gene>), or through the OpenWings project (<https://www.openwings.org/>) if genetic data is available for these subspecies when it is time for us to analyze our data.

Cleaning Sound Files for Playback Experiment

Quality control processing will be performed in the R environment (R Core Team 2020) using RavenR (Araya-Salas 2021) and warbleR (Araya-Salas and Smith-Vidaurre 2017). Calls that overlap with other acoustic signals will be removed from further analyses in favor of retaining high-quality recordings for the playback experiment.

Conducting the Playback Experiment

Stimulus vocalizations from recordings with a high signal-to-noise ratio will be chosen from the data set of measured vocalizations from each subspecies. Recordings will be high-pass filtered at 0.85 kHz and normalized to an equal peak amplitude in Signal version 3.1.1. Exemplar vocalizations will be subdivided into those that originated from each subspecies, creating 3 different categories of playback alarm call stimuli representing “*Q. m. nelsoni* - male”, “*Q. m. monsoni* - male”, and “*Q. m. prosopidicola* - male”, and 3 song stimuli representing “*Q. m. nelsoni* - male”, “*Q. m. monsoni* - male”, and “*Q. m. prosopidicola* - male”. We made sure that there were no other systematic differences between the playback categories. These groups will allow us to distinguish between the effect of the acoustic qualities and the effect of the stimulus origin.

The playback experiment will be conducted with a different focal individual that is at least 5 km away from any other tested individual. A trial will start by placing the loudspeaker at a distance of about 10 m from the focal individual with the observer being another 5-10 m further away. The calls will be played by an iPhone using bluetooth to connect to the UE Roll speaker. The behavior of the focal animal will be scored for 1 minute during a pre-playback phase. Subsequently, the playback stimulus will be presented followed by 3 minutes of silence. The behavior of the responding bird will be scored for 3 additional minutes. The assignment of which stimulus the receiver will be subjected to (from 6 stimulus options described above) will be randomly selected using the random number generator at <https://www.random.org>.

Large scale habitat data

We will use remotely sensed land cover data from the National Land Cover Database (NLCD) and the Global Human Modification of Terrestrial Systems (Kennedy et al. 2020) to assess Predictions 2a and 2b.

Co-occurrence data

Point location data will be gathered from the citizen science resource, eBird (www.eBird.org). To minimize the effect of known biases in eBird data (e.g., different sampling effort across species and geographic areas; Johnston et al. 2020), we will use the same number of eBird records from only complete checklists to model all *Q. mexicanus* subspecies and co-occurring blackbird species that will be randomly selected (following MacPherson et al. 2018). This hypothesis will be tested using point data for all species gathered from this

source (i.e., we will not do counts of other blackbird species in the field). In addition, we will remove all eBird records of *Q. mexicanus* subspecies from our background points layer in our ensemble model.

6. Sample size

Planned Sample:

We plan to sample 88 individuals from each of the three subspecies in both the breeding and non-breeding season (total sample size $n=528$).

7. Sample size rationale

At least 35 individuals from each region should be sampled if large differences between the vocalizations of each subspecies have relevant impacts to communication, and at least 88 individuals from each region should be sampled if medium differences between the vocalizations of each subspecies have relevant impacts to communication. We believe it will be unrealistic for us to sample enough individuals if small differences between the vocalizations have relevant impacts to communication ($n=542$ per subspecies).

Ability to detect actual effects:

We used the free software tool [G*Power](#) (v 3.1.9.7) to conduct a power analysis based on confidence intervals to estimate the sample size (i.e., the number of independently sampled individuals) that would be needed to detect a difference (if there is one) for t tests. This software tool uses pre-set drop down menus and we chose the options that were closest to one of our planned analysis methods, the Kruskal Wallis one-way ANOVA, because there were no options for ANOVA or PCA. The power analysis is only an approximation of the effect size we might detect additionally because it is unclear what kinds of effect sizes we should expect for subspecific comparisons of vocalization data.

8. Stopping rule

Data collection stopping rule:

We will record vocalizations during the non-breeding and breeding seasons and then we will compare the recordings from each season to account for potential differences associated with eliciting a mate. We stopped collecting data once we reached the estimated sample size required to detect a medium effect between populations ($n=88$ per population; total $n = 264$).

Variables (Sections 9-11)

9. Manipulated variables

As this is an observational study, we will not be manipulating any variables.

10. Measured variables

Measured variables for this study are broken up into two categories: a) outcome measures, and b) predictors or covariates. All spatial data sets (occurrence, co-occurrence, and geospatial environmental) will be set to the largest pixel size of any data set which is 30 m resolution.

Category a) Outcome measures:

Pitch of alarm calls - how low or high the vocalizations are (Hertz)

Tone of alarm calls - determined by the pitch, quality, and strength of the calls

Range of alarm calls - the span from the lowest to the highest note the voice produced (listed as: lowest value - highest value)

Strength of intrapopulation vocal communication - do they respond to the calls (i.e., cocking head, vocalizing in return, coming close to the speaker)

Area Under the Curve (AUC) Values - These values will be used to compare regions within the ensemble distribution model framework. Higher AUC values reflect better fit distributions models that will be used to measure correlations between subspecies occurrence and habitat.

Percent contribution - The proportional contribution of each explanatory variable in distribution models are assigned according to changes in model gain when each layer is modified.

Permutation importance - This is a second assessment of importance calculated by randomly permuting the values within each explanatory variable. Permuted environmental data allow comparisons between AUC models and permutation analyses distinguish the influence of environmental variables from one another, allowing for the inclusion of relevant variables regardless of correlation between them.

Root mean square error (RMSE) Values - These values will be used as a second performance measure comparing regions within the ensemble distribution model framework. Lower RMSE values reflect better fit models.

Kappa Values - These values will be used in addition to AUC to compare regions within the ensemble distribution model framework. Higher Kappa values reflect better fit distribution models (Monserud and Leemans 1992).

True Skills Statistic (TSS) Values - These values will be used in addition to AUC and Kappa to compare regions within the ensemble distribution model framework. Higher TSS values reflect better fit distribution models (Allouche et al. 2006).

Category b) Predictors and Covariates:

Individual sex - Sexes are distinguished by plumage color such that females have dull brown and males have glossy black plumage.

Individual age - determined by eye color: yellow for adults, brown/amber for juveniles

Group size - the number of individuals in the flock with the test individual

Age and sex of individuals in group - groups may be made of adults, or juveniles of either sex (male or female) and these will be listed

Background noise - measured using decibel X iphone app (kHz)

Weather - measured using Beaufort's wind scale, and estimate of % cloud cover, the ambient temperature

Microhabitat - This is the specific habitat where each test bird is found and is at a finer resolution than the terrestrial ecosystem classification. Examples will include: gravel parking lot, paved parking lot, gas station parking lot, prairie, desert etc.

Macrohabitat - This is the terrestrial ecosystem classification for each location based on Sayre et al. (2009).

Land cover - This will be used to assess the role of specific land cover types in occurrence for each subspecies using the NLCD. We will identify wetland habitat using this dataset and add a 5 km radius buffer to include pixels that could be within the typical territory size of an individual.

Urbanization - A global dataset of development and development potential (Global Human Modification of Terrestrial Systems) will be used to categorize pixels to level of urbanization and also to calculate a distance to disturbance from wetlands classified using the NLCD dataset.

Co-occurrence - We will gather wintertime occurrence data on 5 other species of blackbirds to assess levels of co-occurrence with great-tailed grackle subspecies: tri-colored blackbird (*Agelaius tricolor*), red-winged Blackbird (*Agelaius phoeniceus*), brown-headed cowbird (*Molothrus ater*), common grackle (*Quiscalus quiscula*) and boat-tailed grackle (*Quiscalus major*).

11. Indices

To test our null hypothesis, we will conduct a phylogenetic principal component analysis to identify correlations among measures of call structure across the three subspecies. A nested ANOVA will be conducted to determine differences in call characteristics. Generalized linear models will be used to analyze the data of the playback experiment.

Design Plan (Sections 12 - 15)

12. Study type

Our test of Hypothesis 0 is an experiment because we are randomly assigning treatments to study subjects we encounter. Our tests of the alternative hypotheses are observational as we are not randomly assigning treatments to test these alternatives.

13. Blinding

For blinding, the personnel who analyze the data collected from each study population will not be aware of the treatment applied to any given individual (i.e., the second author will analyze the data collected by the first author on the *Q. m. nelsoni* and *Q. m. monsoni* subspecies, and the first author will analyze the data collected by the second author on the *Q. m. prosopidicola* subspecies).

14. Study design

This study compares the strength of intrapopulation communication with respect to alarm calls in three subspecies of *Q. mexicanus* using an independent measures experiment. The study also tests alternative hypotheses that explain the range expansion in *Q. mexicanus* subspecies based on habitat requirements and

competitive release. We do not anticipate any need for counterbalancing of test individuals because there is no evidence to suggest that individuals should differ in their alarm calls or responsiveness to alarm calls. It's important to note that we will have no control over the ambient noise levels where wild individuals are encountered.

15. Randomization

In the playback experiment, the assignment of which stimulus the receiver will be subjected to (from 6 stimulus options described Data Collection Procedures) will be randomly selected using the random number generator at <https://www.random.org>.

Analysis Plan (Sections 16 - 22)

16. Statistical models

Analysis Plan:

Describing Vocalizations

We will first classify different elements of the alarm calls using visual inspection of spectrograms within the Raven acoustical software platform. Using the warbleR package we will reduce our samples to only those with high signal-to-noise ratios following the package author guidelines. We will record the following parameters: tone, range, pitch, total duration, and total number of elements. Then, a Kruskal-Wallis test will be used to evaluate alarm call similarity within populations using the R statistical software platform (see Valderrama et al. 2013). As the identification of specific alarm call elements will be new, we may add additional variables that describe these vocalizations once more literature has been reviewed and we gain experience using the analytical software packages.

Note: All following analyses will be conducted within the R statistical software platform.

Comparing the Vocalizations of Each Subspecies

A Phylogenetic Principal Components Analysis (PPCA) will be conducted to identify correlations among measures of song structure between each subspecies using warbleR ([Araya-Salas & Smith-Vidaurre, 2017](#)) and phytools (Revell 2012). A phylogenetic general least squares analysis will be conducted to determine if there are significant differences in alarm call characteristics in different social contexts using phytools ([Revell, 2012](#)).

Analyzing Data from the Playback Experiment

We will conduct a mixed effect Cox model to identify differences in the strength of responses to playback for each subspecies and treatment (i.e., exposure to subspecies or control; following Keen et al. 2020). Separate models will be conducted for each parameter of signal quality described above (i.e., tone, range, pitch, total duration, and total number of elements). We will also include non-vocal displays in this analysis (e.g., silence, head-ups, moving closer to or further away from the speaker) in case subspecies differ in their modes of response to stimuli (e.g., Jedlikowski et al. 2021). We will include stimulus (control versus treatment) as fixed

effects and individual bird identity, individual bird sex, group size and composition, microhabitat, weather, and subspecies as random effects.

Ensemble Distribution Models

A spatially explicit ensemble model will be used to average regional models pixel by pixel to account for variation in differences in habitat availability, the competitive landscape, and spatially explicit vocalization data from our study individuals across the northern extent of *Q. mexicanus* subspecies ranges (following Curry et al. 2018). As in Curry et al. (2018) we will use random forest regression trees to create distribution models using the R package randomForest (Breiman 2001, Liaw and Wiener 2002). The regional models will be set to reflect each subspecies range, and this approach will allow us to identify unique differences affecting predicted distributions among US *Q. mexicanus* subspecies. We will select background points (pseudo absence points) from the range of each subspecies. To minimize the effect of known biases in eBird data ([e.g., different sampling effort across species and geographic areas; Johnston et al., 2020](#)), we will use the same number of eBird records from only complete checklists to model all *Q. mexicanus* subspecies that will be randomly selected ([following MacPherson et al., 2018](#)). In addition, we will remove all eBird records of *Q. mexicanus* subspecies from our background points layer in our ensemble model. We will use the Louisiana State University's High Performance Computing cluster to conduct this computationally expensive analysis.

17. Transformations

We do not anticipate transforming, centering, or recoding any of the data.

18. Follow-up analyses

We do not plan to conduct any follow-up analyses.

19. Inference criteria

We will use p-values ($\alpha=0.05$) to make inferences for predictions 1 and 3. We will use AUC values and root mean square error (RMSE) to determine model fit, in addition to exploring Kappa and True Skill Statistic as potential alternatives. We will use percent contribution and permutation importance to distinguish the importance of each variable in ensemble distribution models.

20. Data exclusion

We do not plan to exclude any data from analyses. Any outliers will be included in the results unless we find reason that the experiment was not conducted properly for that individual.

21. Missing data

As we are only conducting a single experiment that lasts ~8 minutes, we do not anticipate having any missing data from individuals. However, if we do not reach minimum sample sizes in a single year, we will collect additional data in a second year to reach minimum sample sizes (estimated at 35 individuals per population).

22. Exploratory analysis (optional)

We plan to explore our recording data to more thoroughly describe the alarm call across the three *Q. mexicanus* subspecies being studied. These descriptions will entail simple statements describing the pitch, range, tone, elemental composition, and total duration of alarm calls using ANOVA to statistically compare these parameters between each subspecies.

Script (Section 23)

23. Analysis scripts (optional)

Not applicable.

Other (Section 26)

24. Other (optional)

Not applicable.

Data accessibility

Data will be available online: www.github.org/mmacphe/Quiscalus_vocalizations.

Partitioning the results

We may decide to partition the results into more than one manuscript once analyses are completed.

Author Contributions

Samantha Bowser: concept = hypothesis development; writing = literature review, main author, technical methodology knowledge; funding = grant applications.

Maggie MacPherson: writing = literature review, minimal writing, developing statistical analyses; editing; funding = editing grant applications.

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Conflict of interest disclosure

The authors declare that they have no conflicts of interest. The authors of this preregistration declare that they have no financial conflict of interest with the content of this article. In the last year, both authors have worked under Corina Logan and the second author also collaborated with Dieter Lukas who are both Recommenders at PCI Ecology and Corina Logan is on the Managing Board at PCI Ecology.

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