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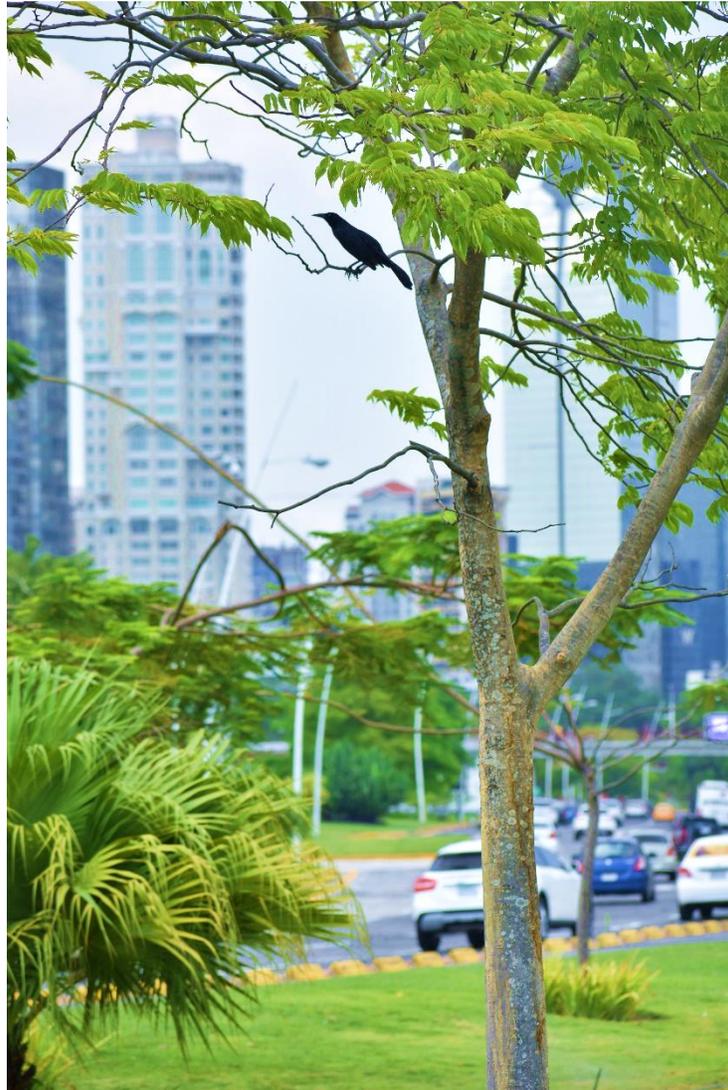Benjamin P. Gregory

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ABSTRACT

Urbanization is one of the fastest-growing threats to the biodiversity of the planet, but not all animals are extirpated by the drastic environmental changes of urbanization, and many are even able to thrive in urban landscapes. Behavioral flexibility is often cited as one reason that successful urban animals can so rapidly adapt to a foreign environment with novel stressors. A well-studied example of urban behavioral flexibility is urban-living birds singing with higher-frequency songs in areas with high levels of traffic noise. The present study investigated this trend in *Quiscalus mexicanus*, an icterid bird with a huge vocal range, highly sexually selective mating system, and behavioral flexibility, that has become abundant in the rapidly-expanding urban areas of Central America. The peak frequency and syllable count of two different call types were compared between a high-traffic area along the Cinta Costera highway in Panama City, and a low traffic area in the parks of the small, canal-zone town of Gamboa, Panama. One call type showed a significantly higher peak frequency in Panama City, while the other did not, likely because it had a much higher default pitch to begin with. Syllable counts also differed significantly between the sites for both call types but were not consistent in direction of change. These results add a new species and geographical region to the growing literature of pitch up-shifting in urban living birds and provide one explanation for the urban success of *Quiscalus mexicanus*.

ACKNOWLEDGEMENTS

INTRODUCTION

Urban Behavioral Flexibility

Urbanization poses one of the greatest threats to global biodiversity. Not only are habitats fragmented, polluted, and outright destroyed; but animal contact with humans increases, predator communities change, and increased levels of noise and light pollution are introduced, all factors that are highly disruptive to ecosystems that were present pre-urbanization (Sol et al., 2013). However, although many animals are pushed past their ability to adapt and are driven out of urbanized regions, there are animals that take advantage of the new anthropogenic biome and find success in cities. A growing body of evidence suggests that many of these successful animals adjust to the novel stressors and conditions of an urban lifestyle by altering their behaviors through behavioral flexibility (Shochat et al., 2013). Behavioral flexibility is defined as an individual's capacity to change its behaviors along with changes in the environment by inhibiting old behaviors that in the new environment might be deleterious, and by innovating new behaviors or using old ones in new contexts (Logan 2016). Urban behavioral flexibility has important implications for the future of life on this planet. It allows a population to adapt rapidly to the novel conditions of cities and gain generational stability, permitting the propagation of genes and thus future evolution and longer-term adaptation to the fastest growing habitat (Dukas, 2013; Snell-Rood, 2013; Dingemanse & Wolf, 2013).

Birds seem to be particularly successful at urban living, likely because of the high concentration and low seasonality of food, and the reduced pressure of predation (Shochat et al., 2010). Consequently, birds are one of the most thoroughly examined groups regarding their urban behavioral changes, and a vast array of changes have been identified across many species spanning all stages of life history (Sol et al., 2013).

Food variety and distribution is drastically different in urban environments compared to natural habitats, so it follows that food preference and foraging behaviors show plasticity among urban-living birds. For example, Sol et al. (2011) found that common mynas (*Acridotheres tristis*), an introduced passerine bird that is common in Australian urban areas, show a significant difference in their treatments of novel food resources depending on their environment. Specifically, mynas captured from urban areas were less hesitant to try an unfamiliar food source than mynas captured from suburban areas, and were quicker to innovate in a foraging problem-solving task than their suburban counterparts.

Predation is also typically relaxed in urban settings, leading to changes in predator avoidance and escape behaviors in birds. Møller and Ibáñez-Álamo (2012) found that, from a sample of over one thousand individual birds from 15 different species, urban individuals tended to wriggle less when held, display less aggressiveness, and emit more alarm screams than rural individuals. These differences also related strongly to time since introduction to an urban environment, implying that the behavioral change was gradual, perhaps driven by processes of learning. Similarly, contact with humans is much more frequent in urban environments, and urban birds have demonstrated much lower aversion to humans (Rodríguez-Prieto et al., 2008), and even the ability to learn and recognize individual human faces and alter their foraging habits based on that information (Belguermi et al., 2011).

Another novel environmental characteristic of an urban habitat is the presence of artificial light. Lebbin et al. (2007) found that a population of insectivorous birds that typically forage during the day were nocturnally foraging on the large swarms of insects attracted to floodlights. Other studies have also demonstrated that bird species drastically shift their breeding behaviors

due to artificial light pollution, causing urban females to lay their eggs earlier than rural females, and influencing male mating success (Kempenaers et al., 2010).

However, of all avian behaviors, communication is likely the most crucial to reproductive success (Sol et al., 2013). Male birds use vocalizations to attract mates, claim and defend a territory, and compete for resources and social status with other males (Tolentino et al., 2018). Because of its variety of important functions, vocalization behavior is subject to strong sexual and natural selection in most bird species (Proppe et al., 2011). Therefore, the high levels of disruptive anthropogenic noise in cities originating from traffic, construction, and various recreational activities may be a challenge to this system of communication, as calls could be masked. It would follow that bird species with a broader vocal range would fare better in noisy urban environments, and males of those species that adjust their calls to be better heard would have a selective advantage over those males that do not (Mendes et al., 2011). Indeed, a growing number of studies have demonstrated that this assumption holds up in urban bird populations in a wide range of species across many continents (Proppe et al., 2011; Mendes et al., 2011; Slabbekoorn, 2012).

Halfwerk and Slabbekoorn (2009) exposed suburban-living great tits (*Parus major*) in the Netherlands to low-frequency traffic sound and found that the birds switched their vocalizations to higher-pitched songs, demonstrating a short-term mechanism for song masking avoidance. In Spain, Mendes et al. (2011) showed a graded and behaviorally plastic response to traffic noise from the center of Salamanca out into suburban areas with common blackbirds (*Turdus merula*). The closer to the center of the city the birds were recorded, the higher the dominant pitch of the birds' calls were, in correlation with the increasing traffic noise. In another study with common blackbirds, this time in Vienna, Nemeth and Brumm (2009) found that blackbirds living in the forest surrounding the city sung at a lower pitch and with longer intervals between their songs than conspecifics living in the city center. These studies have been performed in North America as well as Europe. Proppe et al., (2011) recorded the songs of black-capped chickadees (*Poecile atricapilus*) living along roadsides in Edmonton, Canada, and directly compared the instantaneous traffic noise with the peak frequency of the *bee* note in the male *fee-bee* song. The chickadees sung higher frequency and shorter duration songs when traffic noise was louder, and lower frequency songs when the traffic was less intense.

Finally, in a rare study from a tropical region, Tolentino et al. (2018) analyzed the vocalizations of nine bird species in forest fragments with varying distances to urban areas in Brazil. Bolstering the growing trend, eight of the nine bird species were found to shift the pitch of the vocalizations higher when living closer to urban areas. The trend is clear that the low-frequency sound of traffic and other urban ambient disturbances correlate strongly with a behavioral shift in bird vocalization towards higher pitches (Tolentino et al., 2018). However, very few studies of this kind have been performed in tropical, highly biodiverse regions of the world (Sol et al., 2013).

Study species: *Quiscalus mexicanus*
General Description

The great-tailed grackle (*Quiscalus mexicanus*) is a new world blackbird in the family Icteridae (Beedy and Pandolfino, 2013). Great-tailed grackles are native to coastal ecosystems such as mangroves and estuaries (Athanas and Greenfield, 2016; Beedy and Pandolfino, 2013) but in the past few centuries have become incredibly pervasive across human-modified and urban environments in Central America (Christensen, 2000; Wehtje, 2003). The species is highly sexually

dimorphic, males averaging 45cm in length, 160-265g in weight, and fully black in plumage, with an iridescent purple or bluish sheen on their heads, backs, and underparts (Jaramillo and Burke, 1999). Corresponding with the common name, males also have a very long, keel-shaped tail that is thought to have arisen due to high pressure from sexual selection (Johnson et al., 1999). Females are smaller, with an average length of 38cm and an average weight of 85-130g. Females are also less visually outstanding, with dull brown feathers on their backs and undersides, shorter tails, and little to no iridescence in their plumage (Jaramillo and Burke, 1999).

Overview of Expansion and Urban Success

Great-tailed grackles are highly-successful urban invaders. They are found at much higher densities in cities than anywhere else, taking advantage of the reduced nest-predation and higher food density of urban environments (Christensen, 2000). The species originated in the coastal regions of Central America and northern South America, but their range is presently much larger than that, expanding all the way across Central America and Mexico, and continually moving northwards into the United States and even Canada (Christensen, 2000; Wehtje, 2003). The history of this range expansion starts several centuries ago. Sometime in the late fifteenth century, the emperor of the Aztecs, Auitzotl, commanded that a group of grackles be brought inland to Tenochtitlan from the gulf coast, likely for ornamental use of the males' attractive iridescent feathers (Haemig, 2012; Wehtje, 2003). There in Tenochtitlan, the grackles were fed and kept until they quickly spread and thrived in the central basin of Mexico, where they continued to find habitat in human-modified landscapes through the colonial age of the sixteenth century and into the present (Wehtje, 2003). Since 1880, the range of the great-tailed grackle in North America has increased an astronomical 5530%, primarily due to the use of human-modified land such as public parks, golf courses, and parking lots as foraging and breeding habitat (Wehtje, 2003).

Further south though, evidence shows that great-tailed grackles have always lived closely with humans. Smithsonian Archaeologist Richard Cooke, who studies Cerro Juan Díaz, a site in modern day Panama that was inhabited from 200 BCE to 1400 CE, reports that his team has found abundant *Quiscalus mexicanus* bones in their excavations, many of which were found with garbage dumps that had food leftovers (Cooke, 1998). Like Mexico, the colonial era and the urbanization and land alteration that came with it facilitated the expansion of the great-tailed grackle across Central America where they are now omnipresent in urban settings. It is evident that *Quiscalus mexicanus* finds the greatest success in being commensal with humans, but the reasons for this are not quite as well understood. Even in the ever-expanding size and development of twentieth and twenty-first century cities, grackles continue to find outstanding success.

Behavioral Flexibility

Like many species that have invaded urban environments, *Quiscalus mexicanus* has demonstrated a high degree of behavioral flexibility in controlled lab experiments with captured individuals (Logan 2016a, 2016b, 2016c). Logan (2016a) performed an experiment in which eight *Quiscalus mexicanus* individuals captured from California were trained on a color association task. The grackles learned very quickly to associate a gold-colored tube with food over a silver-colored tube. The test came when the researcher switched the experimental setup so that the food was always in the silver tube rather than the gold tube. The grackles reversed their preference

very quickly in an average of 91 trials, which is on par with many other bird species tested with similar methodologies (Logan 2016a).

Next, Logan (2016a) tested the grackles with the Aesop's Fable test, a commonly-used problem-solving test involving a narrow transparent tube half-filled with water with a small piece of food floating on top. The test animal needed to figure out that dropping small items into the tube would raise the level of the water and thus bring the food upwards to make it accessible. The grackles did not spontaneously learn to drop items in the water, showing a failure in innovation, but once trained, were successful at the task. Logan then added a further challenge for the grackles and allowed them to choose between larger and smaller objects, the larger of which would displace more water and therefore act as a more efficient means of retrieving the food. Four of the six grackles in this part of the study showed a preference for the larger objects and were thus more efficient problem solvers, while the other two did not seem to develop a preference. Finally, Logan then tested flexibility in the Aesop's Fable problem by attaching magnets to the inside of the tube that would snag the larger objects before they reached the water. Therefore, the grackles that had learned to prefer the large stones would have to reverse their preferences and use only the small stones to be able to reach the food at all. Again, the grackles demonstrated behavioral flexibility and reversed their preferences (Logan 2016a).

Later that same year, Logan (2016b) performed more problem-solving tasks with captured *Quiscalus mexicanus* individuals. These new tests were geared more towards determining if the grackles had the ability to innovate new solutions to problems, meaning the grackles were presented with a problem in accessing food that required the creation of a new behavior, usually involving the manipulation of a tool. Logan demonstrated that the grackles were not particularly adept at innovation, as they were unable to invent solutions to the problems presented, even after observing a human solve the task. The problems were identical to problems that have been successfully solved by other birds deemed to be behaviorally flexible like New Caledonian Crows (Logan 2016b). These results show that grackles can be behaviorally flexible, as demonstrated by the first set of experiments, but not necessarily innovative, suggesting that these two terms are not interchangeable and refer to different abilities.

Finally, Logan (2016c), also performed a series of tests on *Quiscalus mexicanus* individuals with the goal to determine if behavioral flexibility correlates with other traits assumed to be connected to urban invasion success such as neophobia, exploration, risk aversion, persistence, or motor diversity. Through the same colored tubes test as the first set of experiments, Logan correlated these traits with behavioral flexibility, but did not find correlations for any of them, indicating the behavioral flexibility is a further source of individual variation rather than a result of variation from the other traits. However, the sample size of this study was rather small, and might have been insufficient to find correlations (Logan 2016c).

The results of Logan's experiments on *Quiscalus mexicanus* unequivocally show the behavioral flexibility of the species. When a preference or a behavior is learned, they are quickly able to reverse or alter that behavior when the environment is altered. However, great-tailed grackles do not seem to be as innovative as some of the other behaviorally flexible birds studied in similar contexts, but far more research needs to be done to make a concrete conclusion. And finally, behavioral flexibility may not be connected to other traits that are traditionally considered to be

adaptive for urban invaders, but again, the research on this species is scant and new (Logan, 2016a; 2016b; 2016c).

Mating System, Sexual Selection

        Quiscalus mexicanus breed in large colonies of up to over one hundred nests in close proximity to each other. Each nest is built by a female within the small territory of a territorial male (Beedy and Pandolfino, 2013). Great-tailed grackles have a highly polygynous mating system, and it is likely that their extreme sexual dimorphism is partially a result of this sexually competitive environment (Bjorklund, 1991). More specifically, Johnson et al., (2000) described the mating system of *Quiscalus mexicanus* as non-faithful frank polygyny, a situation in which a large proportion of the males in the population have more than one mate, some of the males only have a single mate, and the majority of the males have no mates at all. Those males that have more than one mate are the territorial males, who are larger and able to acquire and defend a favorable territory for nesting. Territorial males are far more reproductively successful than the other males, siring an average of 8.3 nestlings each in Johnson's study, whereas the other males sired less than one on average (Johnson et al., 2000). Johnson et al. point to the size of the territorial males as the primary cause for their success, but it is also likely that their calls, both in ability to attract females and repel potentially competitive males, were instrumental in their success. Kok (1971) and Townsend (1927) explain that the courtship and displays that males use to compete between one another for females are complex and highly competitive. Males face off in mock fights in which they pose intimidatingly at one another, cocking their tails and craning their necks backwards. On occasion they fly up at each other and strike, competing for physical superiority (Townsend, 1927). Clearly. male great-tailed grackles are subject to very intense sexual selection, and their reproductive success is highly contingent on their courtship, intrasexual competition, and vocalization prowess.

Vocal Range

        Quiscalus mexicanus are infamous for their huge variety of calls and wide vocal range. Their sounds are described as bizarre, torturous, not so pleasing, and even like the sounds of an alien spaceship (Beedy and Pandolfino, 2013; Athanas and Greenfield, 2016; Kok, 1971). Despite this, however, very few studies have been done on the vocalizations of great-tailed grackles. Kok (1971) performed an intensive survey of the vocal behaviors of the great-tailed grackle, and determined that, though sixteen distinct patterns of sound were described for males, the distinction between one call type or another is not always obvious, and mid-call mixing and transitioning occurs often. Kok (1971) also suggests that this huge range of vocalization is likely the evolutionary result of the intense courtship and sexual selection behaviors described in the previous section.

Present Study

        It is possible that because of their abundance in cities, highly polygynous and competitive mating system, and huge vocal range, urban male great-tailed grackles may follow the well-observed trend of increasing the pitch of their mating calls to compete with anthropogenic noise. The present study aimed to investigate this possibility by comparing the peak frequency and syllable count of urban-living great-tailed grackle calls between a high-traffic area in downtown Panama City along the Cinta Costera highway, and a low-traffic small town surrounded by Soberanía National Park in Gamboa, Panama.

The main study site for Panama City was along the Cinta Costera roadway, a recently constructed six-lane avenue that runs along the coastline of the city between Paitilla and El Chorillo that features green spaces and a scenic walkway (GlobeNewswire, 2015). The trees and green space in the region provide ample living opportunity and space for great-tailed grackles, and the proximity of loud and constant traffic supplies the anthropogenic noise.

Gamboa, historically the home of the Panama Canal dredging division, is about 25 kilometers inland from Panama City and the Cinta Costera, and sits on the confluence of the Río Chagres and Lago Gatún (Niedzialek, 2007). Gamboa is surrounded by Soberanía National Park, a 220 km^2 protected area with high bird and mammal diversity (Robinson, et al., 2004). Downtown Gamboa features large green areas with ample trees that hosts a considerable *Quiscalus mexicanus* population.

The prediction of this study is that the vocalizations of male grackles in Panama City will be considerably higher-pitched on average compared to the vocalizations of male grackles in Gamboa due to the huge discrepancy in vehicle traffic noise. The large and growing collection of studies showing this phenomenon in other species, in combination with the demonstrated behavioral flexibility of *Quiscalus mexicanus*, and the evident intensity of sexual selection in *Quiscalus mexicanus* populations makes this prediction very confident.

This research is particularly important because the vast majority of research done on the behavioral plasticity of urban living animals has been performed in temperate regions of the world with relatively lower biodiversity, and in much older European and North American cities with well-established urban bird populations (Sol et al., 2013). By investigating a potential behavioral alteration in a tropical and more biodiverse region of the world, in more recently developed urban areas, this study adds an important new dimension to the current understanding of urban behavioral adaptation.

RESEARCH QUESTION

Does vocalization pitch and syllable count of male great-tailed grackles (*Quiscalus mexicanus*) differ between a population living along the Cinta Costera in Panama City, and a population in downtown Gamboa, Panama?

METHODS AND MATERIALS
Localities and Sampling Sites

The Cinta Costera highway and downtown Gamboa were the two main localities of the study. The Cinta Costera was chosen as the high traffic site because it consists of a strip of green park space with abundant trees running alongside a very busy and very loud highway (See Fig. 1A). Downtown Gamboa was chosen as the low traffic site because of its similar green, tree-inhabited park space accompanied by much less vehicle traffic due to the low population and rural location of the town (See Fig. 1B). The two localities are separated by 25 kilometers of national parks, protected forests, and small rural settlements (See Fig. 1C). Five sampling sites were chosen at each locality. Sampling sites were located by observing grackle behavior and watching for females bringing building material up into a tree, or males performing their display on a high branch (Bjorklund, 1991). Sampling sites did not always necessarily fall near a known nesting location, as abundance of vocalizing male grackles was prioritized for selection. Two days of exploratory observational study were done in each locality to narrow down the best five sites that

were found to consistently host vocalizing male grackles. When a site was decided, its GPS coordinates were saved.
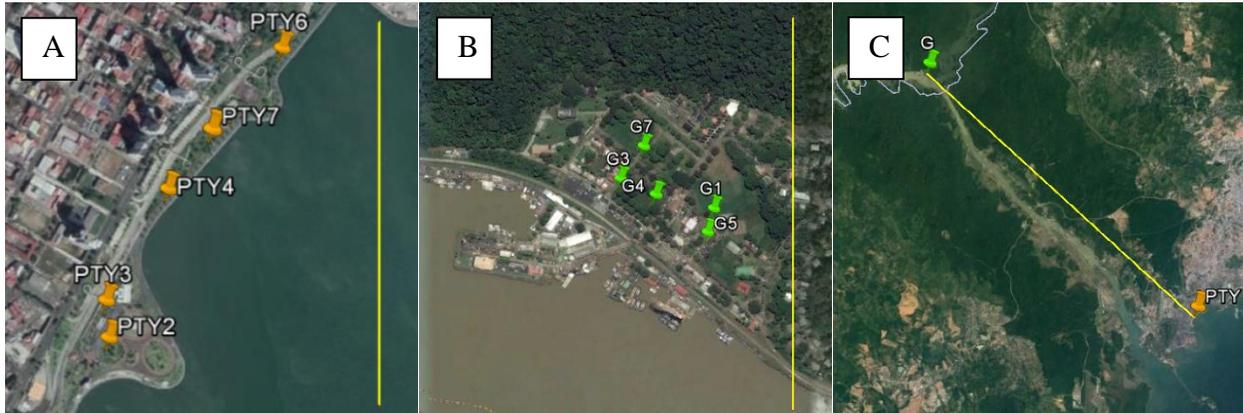

Figure 1: Satellite images showing the locations of the localities and sampling sites. A) is the Cinta Costera in Panama City with its five sampling sites. B) is downtown Gamboa with its five sampling sites. C) is south-central Panama. The yellow lines in A and B represent one kilometer. The yellow line in C represents the 25 kilometers that separate the Panama City (PTY) and Gamboa (G) localities.

Sampling

All sampling occurred between 7:00AM and 10:30AM to maximize data collection when birds are typically most active (Mendes et al., 2011; Proppe et al., 2011). Daily samples were done at each sampling site in Panama City for seven days from April 12th, 2019 through April 18th, 2019; and then in Gamboa for seven days from April 21st, 2019 through April 27th, 2019. The order of sampling between the five sampling sites was randomized each day. A fifteen-minute audio recording was done at each site. At the initiation of the recording, the time, date, temperature, humidity, and weather conditions were recorded. Temperature, humidity, and weather data came from Google Weather services. Audio recordings were done with a Samsung Galaxy S7 smartphone using an app called Hi-Q MP3 Recorder (Version 2.4.1) to capture sound in the WAV sound format at 705kbps. The smartphone was held 1.3 meters off the ground with a tripod and the microphone was angled directly upwards to capture audio from every direction.

Data Processing

For each recording, Cornell Lab of Ornithology's RavenPro (Version 1.5.0 - Build 43) acoustic analysis software was used to calculate values (Mendes et al., 2011; Proppe et al., 2011). Hann window spectrograms were used. Two different types of calls were analyzed and were chosen because they were the most commonly heard in the field in the exploratory observation days. These two calls were dubbed sweep (S) and beep (B) calls. For each recording every male *Quiscalus mexicanus* B and S call above a volume threshold (55 decibels in Panama City, and 50 decibels in Gamboa) was marked and labeled. They were recognized visually by carefully scanning the spectrogram (See Fig. 2), and each one was also confirmed auditorily. Once all applicable calls were labeled, three (maximum) of each were randomly chosen for analysis (Mendes et al., 2011; Proppe et al., 2011). For each chosen call, peak frequency, number of syllables (discrete call units), and peak volume was recorded. In addition, traffic noise data was generated by calculating average volume and peak frequency of all sound between 700 and 1300

Hz for 60 seconds centered on the given call (Sandberg 2003, Proppe et al., 2011). That way, each call had local traffic sound data associated with it.
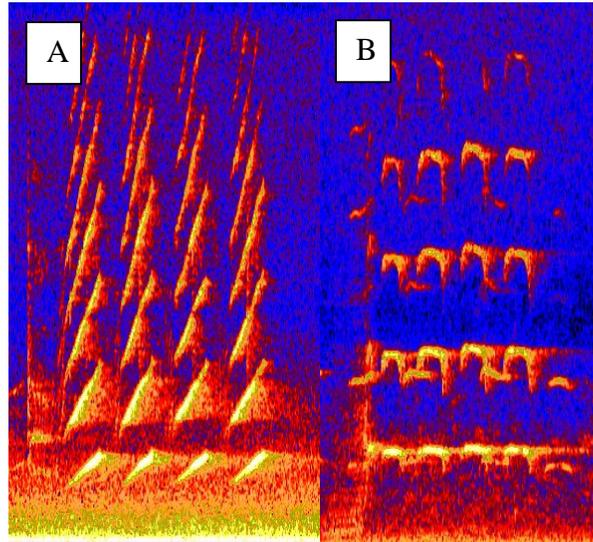

Figure 2. A typical example of the spectrographic representation of each type of call examined. A) is a sweep (S) call. B) is a beep (B) call. Both calls in this example would be considered to have four syllables.

Statistical analysis

An entire day of Cinta Costera sampling was excluded from the statistical analysis because the nearer side of the highway was closed, drastically reducing the volume of the traffic and potentially confounding that day's data. In addition, two days into the Cinta Costera sampling, one of the sites was abandoned and substituted for another because it was yielding very little data, so the data from that initial site were also excluded from analysis.

All statistical analyses were performed in IBM's SPSS Statistics (Version 25). Independent samples two-tailed t-tests were performed for peak frequency and syllable count for each call type between the Cinta Costera and Gamboa locations with alpha levels of 0.05. A t-test was also performed on the traffic amplitude data for both sites, using both call types, also with an alpha level of 0.05, to assess whether the traffic noise differed in intensity between the two sites. T-tests were also performed on temperature, relative humidity, and sample time data between both sites. Further, a Pearson Correlation Coefficient was calculated for both B and S calls, comparing peak frequency and the average traffic amplitude. Finally, another Pearson Correlation Coefficient was calculated for peak frequency and peak volume of the calls, both B and S.

RESULTS

The total sample size for S calls was N=124 (81 Cinta Costera, 43 Gamboa), and for B calls was N=90 (60 Cinta Costera, 30 Gamboa).

There was a significant difference in the peak frequency of the sweep (S) calls in the Cinta Costera (M=3394.1 Hz, SD=525.6 Hz) and Gamboa (M=3019.8 Hz, SD=457.1 Hz) sites; t(122)=3.943, p<0.001 (Fig. 3). There was also a significant difference in the syllable count of the sweep (S) calls in the Cinta Costera (M=3.1, SD=2.1) and Gamboa (M=2.1, SD=1.5) sites; t(122)=2.769, p=0.007.

Figure 3. Box plots representing the comparison of peak frequency of S calls (left) and syllable count of S calls (right) between the Cinta Costera and Gamboa localities.
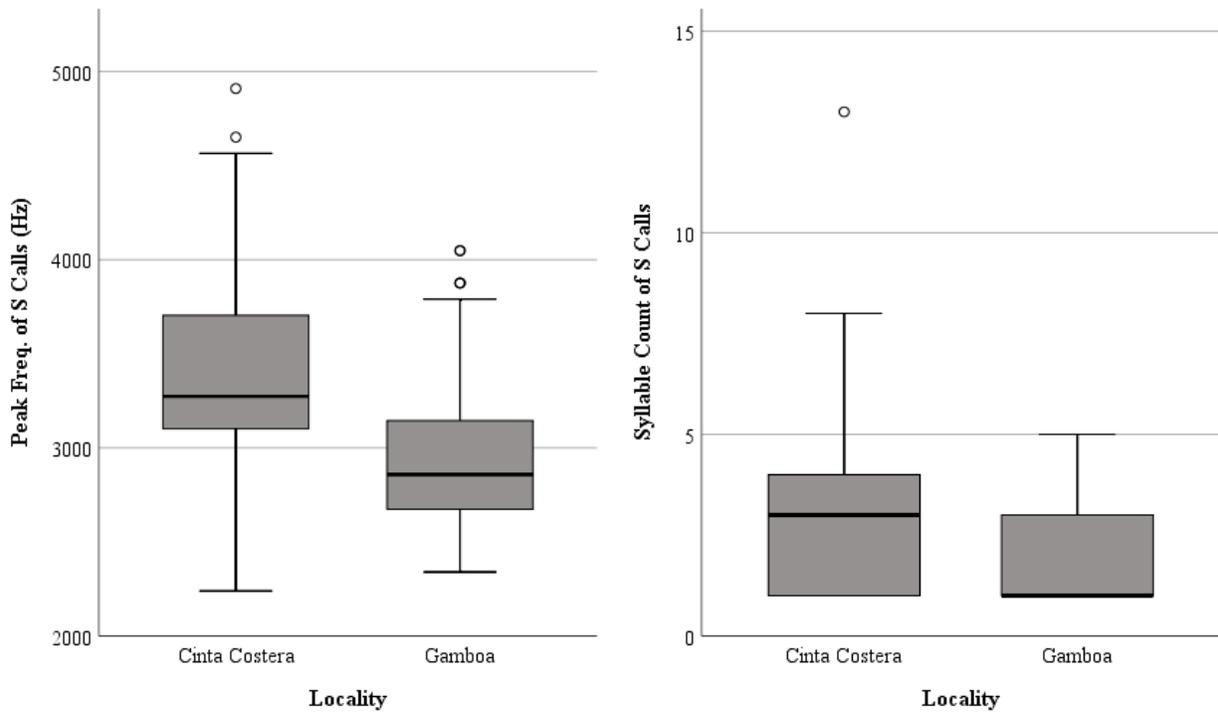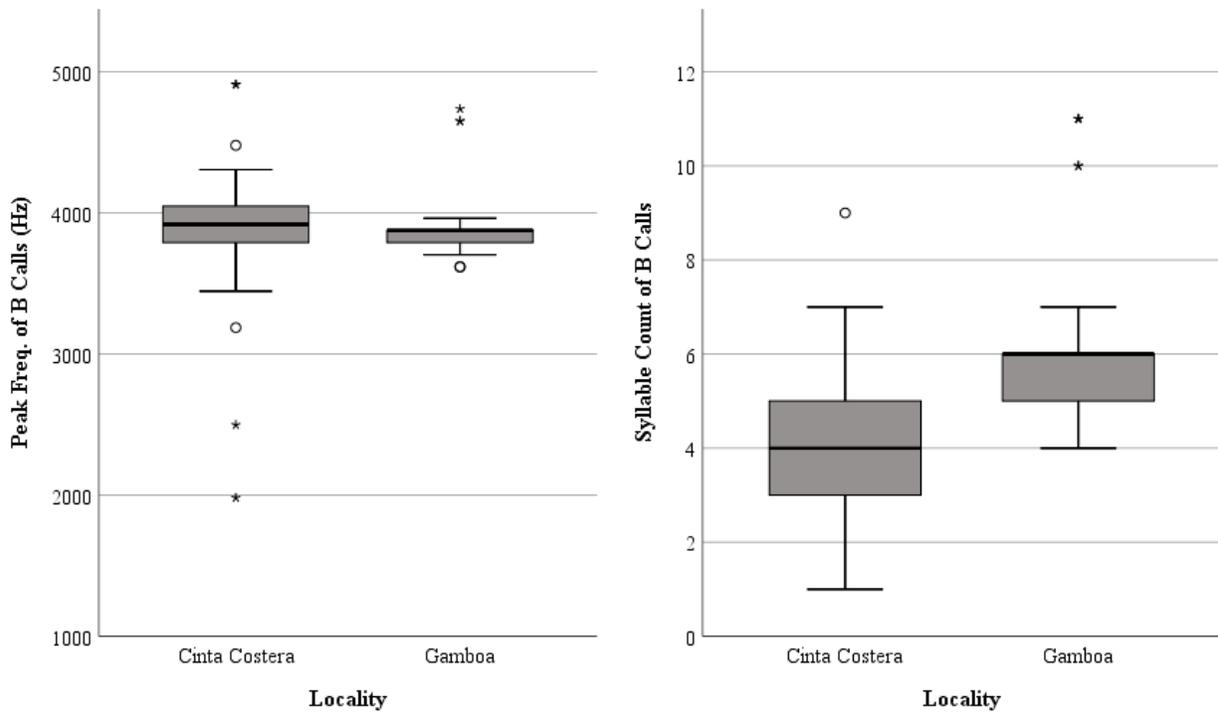
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Figure 4. Box plots representing the comparison of peak frequency of B calls (left) and syllable count of B calls (right) between the Cinta Costera and Gamboa localities.

There was not, however, a significant difference in the peak frequency of the beep (B) calls in the Cinta Costera (M=3903.2 Hz, SD=414.3 Hz) and Gamboa (M=3899.4 Hz, SD=280.3 Hz) sites; $t(88)=0.046$, $p=0.964$. There was a significant difference in the syllable count of the beep calls (B) in the Cinta Costera (M=3.9, SD=1.4) and Gamboa (M=6.2, SD=2.2) sites; $t(41)=-5.293$, $p<0.001$ (Fig. 4)

There was a significant difference in the traffic volume in the Cinta Costera (M=66.9 dB, SD=2.8 dB) and Gamboa (M=47.1 dB, SD=5.5 dB) sites; $t(91.7)=28.792$, $p<0.001$. There was also a significant difference in the sampling temperature in the Cinta Costera (M=29° C, SD=1° C) and Gamboa (M=27° C, SD=1° C) sites; $t(212)=11.490$, $p<0.001$. In addition, there was a significant difference in the sampling relative humidity in the Cinta Costera (M=68%, SD=5%) and Gamboa (M=76%. SD=5%) sites; $t(212)=-11.626$, $p<0.001$. Finally, there was a significant difference in the time of sampling between the Cinta Costera (M=8:37:18AM, SD=0:37:17) and Gamboa (M=8:02:17AM, SD=0:12:30) sites; $t(203.9)=8.390$, $p<0.001$.

There was a weak, positive correlation between peak frequency of S calls and traffic volume, $r=0.263$, N=124, $p=0.003$ (Fig. 5). There was not, however, any correlation between peak frequency of B calls and traffic volume, $r=0.056$, N=90, $p=0.600$. There was also no correlation between peak frequency and peak volume for S calls, $r=0.059$. N=124, $p=0.513$; or B calls, $r=-0.044$, N=90, $p=0.681$.
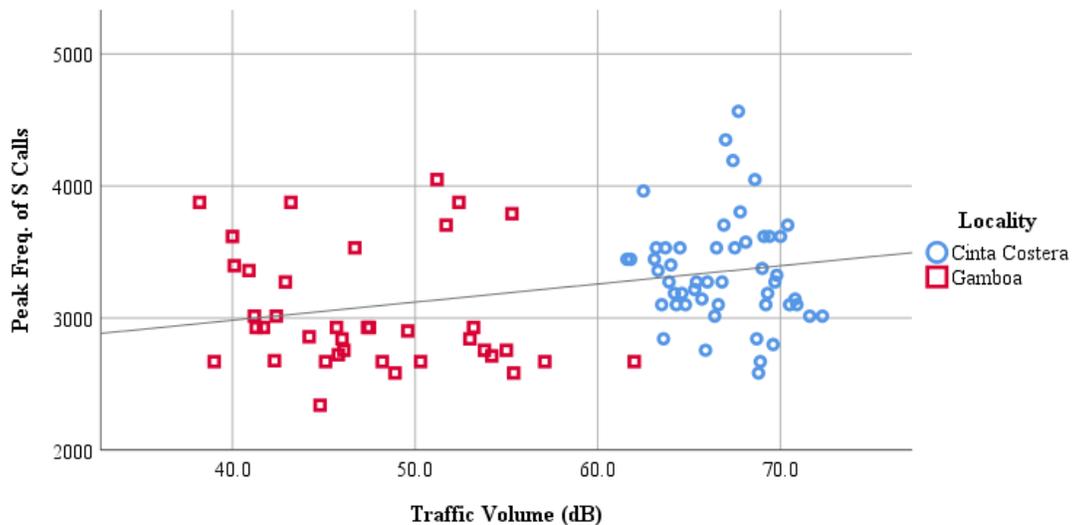

Figure 5. Every S call displayed on a scatterplot comparing its peak frequency and its associated traffic volume, with a best-fit linear trendline.

DISCUSSION
General Discussion

The population of *Quiscalus mexicanus* living along the Cinta Costera highway in Panama City produced higher-frequency sweep (S) calls than the population living in downtown Gamboa. This result coincides with the predictions of the study and with the trends identified in the literature, further bolstering the growing understanding that urban-living birds very frequently shift the pitch of their calls upwards to avoid masking by traffic noise (Tolentino et al., 2018; Halfwerk & Slabbekoorn, 2009; Mendes et al., 2011; Luther & Derryberry, 2011). However, it has recently come into contention whether frequency is the feature being prioritized for alteration. Nemeth et al. (2013) found that the common blackbird (*Turdus merula*) prioritized

higher-frequency call elements not because they were higher-frequency, but because they were physiologically more able to be produced at higher amplitudes. So, rather than shifting the frequency of the call up to escape the low-frequency sound of traffic, it could be that the birds are responding to the noise by singing louder, and higher-pitched songs are easier to sing loudly. The methodology of the present study did not allow conclusions to be drawn in this regard, because distance from microphone to subject was not controlled. Call volume in this study is more representative of the distance between the microphone and the subject, and any variation in the amplitude of the call produced at the source is impossible to ascertain.

  The syllable count for the S calls was lower in Gamboa than in Panama City. Past studies have found mixed results on how the duration of songs varies between traffic-intensity conditions, with some matching the present study and revealing shorter songs in rural environments (Ríos-Chelén, 2012), and others revealing shorter songs in urban environments (Proppe et al., 2011; Salbbekoorn & Boer-Visser, 2006). It is likely that this feature of communication is more variable according to the individual species in question, as it has been shown that species do in fact respond differently to variation in traffic noise (Tolentino, 2018; Hu & Cardoso, 2010). In addition, the present study did not directly measure the duration of the song, but the number of syllables, or discrete song units, which may not necessarily correspond exactly with the duration of the song. A song with fewer, longer syllables may end up being longer in duration than a song with many, very rapid syllables. A future investigation or revision of the data could account for this fact and directly measure the song duration in addition to syllable count.

  For beep (B) calls, there was no difference in frequency between the two sites, and in fact, the average frequencies were remarkably close. This suggests that, perhaps, the nature of B calls does not allow as much variation. It is possible that the message communicated by B calls would be lost if the frequency of the notes was to change to drastically in one direction or the other. More likely, though, is that the B calls are already high enough in frequency in their "default" form that they need not worry about being obscured by traffic. The average frequency for B calls, around 3900 Hz (Figure 4), was much higher than that for S calls, around 3100 Hz (Figure 3), so perhaps the S calls are on the lower range of the *Quiscalus mexicanus* call repertoire and therefore required the up-shift when other, naturally higher calls may not. Hu and Cardoso (2010) found that species that typically sing higher frequencies do not need to adjust their pitch as much in traffic, species that typically sing lower frequencies cannot adjust their pitch enough and resort to other means to avoid masking, and species with midrange frequencies do adjust their pitch. Because of the immense vocal range of *Quiscalus mexicanus* (Kok, 1971), it is possible that their vast repertoire of song types may fall into different categories, some low enough to necessitate up-shifting, and others high enough that traffic noise is not an inhibitor to successful communication.

  Syllable count for B calls was higher in Gamboa than Panama City, the inverse of the case for S calls. Again, previous studies have found mixed results for traffic's effect on duration, and syllable count is likely not a perfect proxy for duration.

  There was a very large difference in the traffic noise between the two sites as was expected. This was most likely the cause of the difference in S call frequency, due to the correspondence with past studies. However, it should be noted that temperature, humidity, and time of sample all also differed significantly between the two sites, so it cannot be definitively stated that these environmental conditions could not have also influenced the nature of the calls. Finally, due to the distance between the two sites, sampling days were not alternate, and were separated by two days. The difference in timing means that the sampling of each locality could have fallen

during different phases of the breeding season. However, the breeding season of *Quiscalus mexicanus* is defined as March through July (Johnson et al., 2000), and this study took place in mid-April, so it seems unlikely that the discrepancy in timing had very much of an effect on the behavior of the subjects.

Potential Errors

The three calls of each type were entirely randomly selected from each 15-minute recording. Therefore, it is possible that two or more of the analyzed calls from a given recording could have been very close to each other temporally, even in direct sequence after a brief pause. Proppe et al. (2011) ensured that the songs chosen from their recordings were spread out roughly equally to avoid the possibility of frequency being affected by social factors like song matching between males. The present study did not account for that possibility. The potential consequence of this oversight is the clustering of data around certain frequencies as males match each other, but as the sampling was entirely random, there were likely few instances in which this occurred.

Another potential error is the inconsistency in analysis between the two sites. For the Cinta Costera site, the minimum amplitude for a song that would be considered for analysis was 55 decibels, while it was 50 decibels in Gamboa. The reason for this discrepancy is the much lower population of *Quiscalus mexicanus* in Gamboa, and the necessity to widen the analysis criteria to gather a larger sample size of data. In addition, the severe difference in traffic noise between the two sites made it more difficult to analyze quieter songs in the Panama City locality, as they were often masked. However, there was no correlation between the peak volume of the calls and their peak frequency, so it is safe to say that the slightly different call amplitude criteria between the two sites most likely did not confound the data. Because the distance of the subjects from the microphone was variable, the only reason that there might be a correlation and thus a confounding factor would be the possibility that the higher frequencies of the calls would dissipate in the distance between the subject and the microphone, causing the peak frequency data to skew slightly lower in the Gamboa locality. But the distances involved in this study were not great enough for that phenomenon to pose a reasonable threat to the integrity of the data, and that is backed up by the lack of correlation between peak frequency and peak volume.

CONCLUSION

Quiscalus mexicanus demonstrated what a growing selection of other urban-living birds have: that living in regions with high levels of traffic noise correlates with higher-pitched calls. S-calls, whose default frequency is lower, were higher in a higher-traffic region, while B-calls, whose default frequency is higher, were not. The number of syllables was different between the two sites, but in opposite directions for the two call types, suggesting that the relationship between syllables and traffic noise is more complicated and may depend on call type.

Future research should continue to focus on the effects of traffic noise on the communication behavior of *Quiscalus mexicanus*. Rather than just measuring syllable count, future studies should also take note of the duration of each call as other studies in the literature have done to examine if the duration varies between sites. Additionally, future research should add one or more sites with intermediate levels of traffic noise to create a gradient and search for a potential graded effect of traffic on call pitch. Finally, a more controlled future study could involve the capture of grackles from sites of different traffic intensity so that the volume and pitch of calls can be compared more accurately in a lab setting.

Investigating the means by which animals find success in anthropogenic biomes is vital to understanding the biotic impacts, both present and future, of the sixth mass extinction. Species that can use behavioral plasticity to quickly adapt to new stressors and maintain stable populations in the environmental turbulence of the Anthropocene will be more likely to survive and subsequently go on to shape the evolutionary future of life on Earth.

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