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Sex differences in blood pressure responses of Pekin ducks to sound stimuli and conspecific vocalizations

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ABSTRACT

We tested Pekin ducks with playbacks of 5 different vocalizations plus a no noise and white noise stimulus as our controls ($N = 15$ ducks/sex/treatment). The "AM long" call is a common vocalization made by both sexes. "Honk" is also produced by both sexes and is thought to be an alarm or distress call. "Pips" and "harmonics" are common vocalizations made only by hens. The "egg laying squiggle" is also only made by hens. Trials consisted of an initial recording in a quiet condition with 5 consecutive measurements of heart rate, blood pressure and respiratory rate. A specific vocalization was then played on repeat while 8 more measurements of blood pressure, heart rate and respiratory rate were taken. Finally, 5 measurements of heart rate and blood pressure were taken post playback along with a final recording of respiratory rate. Data from all blood pressure measurements (systolic, diastolic and mean arterial pressure [MAP]), heart rate (HR) and respiratory rate (RR) were subject to a principal component analysis (Proc Princomp in SAS 9.4). The significant principal components (Prin 1 loaded strongly on blood pressure and Prin 2 loaded strongly on HR and RR) were then analyzed by ANOVA with repeated measures (Proc Mixed, SAS 9.4, subject=duck ID). Our results showed there was a significant main effect of playback type on the blood pressure of ducks during the playbacks ($P = 0.0276$). Ducks experienced an increase in blood pressure when played back the honk vocalization, as well as the white noise control. Additionally, there was a significant interaction between sex and treatment on the after-stimulus blood pressure ($P = 0.0008$): after the harmonic vocalization was played, the drakes still experienced an increase in blood pressure, but the hens experienced a decrease. The drakes, but not the hens, experienced a decrease in blood pressure after the AM long vocalization was played. Our data show that there are sex differences when it comes to vocalization playbacks in Pekin ducks, but overall, the honk vocalization and white noise control significantly increased ducks' blood pressure. This study represents a critical steppingstone toward understanding how Pekin duck vocalizations affect conspecific physiology.

Introduction

Various stressors affect the welfare and production of poultry species, causing the industry to lose millions in revenue. Stress can decrease welfare and production, and stressful events may happen in barns, for all livestock species, including Pekin ducks. There are stressors that develop unbeknownst to producers such as disease, external stressors like heat or cold stress, and even routine management practices like egg collection, vaccinations, or daily placement of bedding during duck management (Cherry and Morris, 2008; Chen et al., 2021). Moreover, stressor identification is primarily reactive rather than proactive, leading to a reduction in health and productivity of the flock. Now, however, there may be a way to catch these increased stress levels by understanding the context under which bird vocalizations are given, rather than just waiting for a detectable decrease in welfare that will inevitably lead to production losses.

Wild birds are known to have many different types of vocalizations that include, but are not limited to, begging signals, contact calls, migratory flight calls, food calls, and alarm calls (Briefer, 2012, 2018; Manteuffel et al., 2004; Ballentine and Hyman, 2021). Alarm calls are

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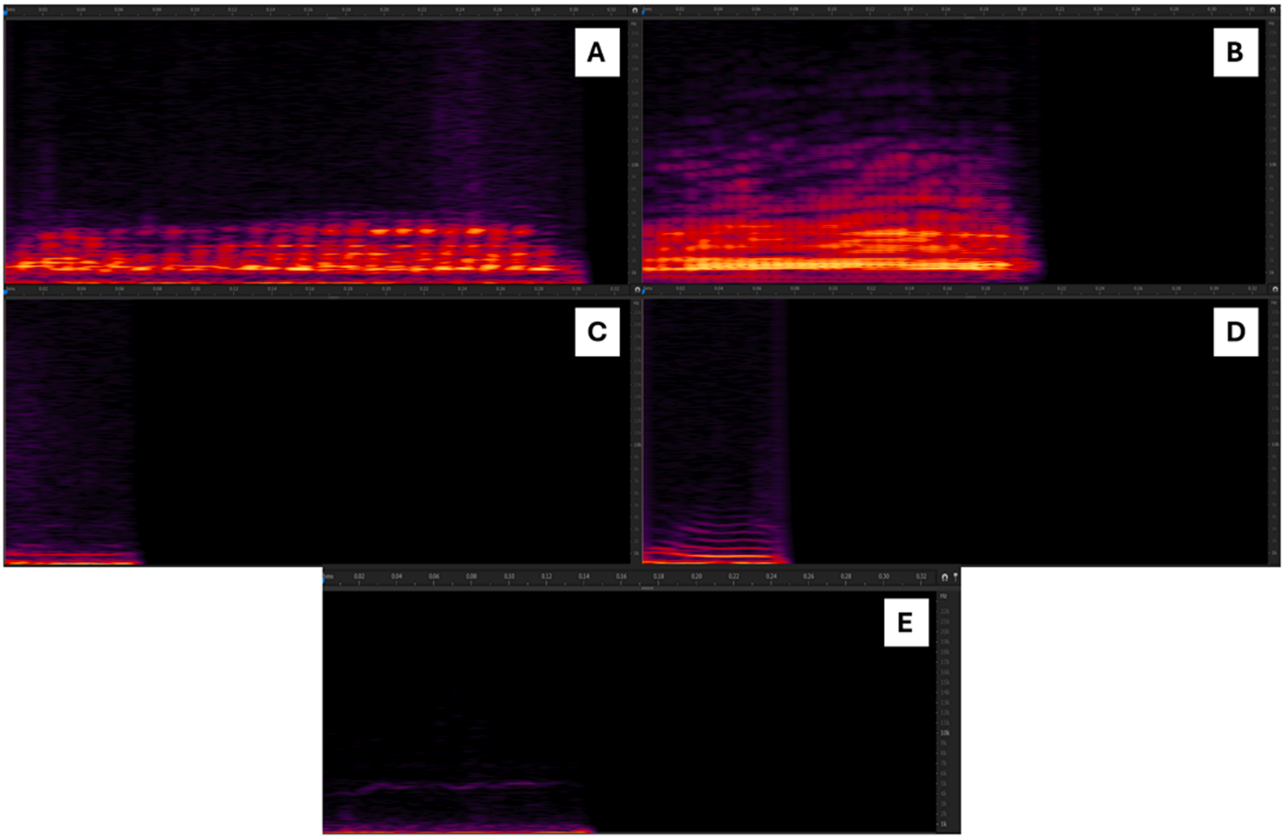

Fig. 1. Spectrograms of the vocalizations used as playbacks. (A) AM Long; (B) Honk; (C) Pip; (D); Harmonic; (E) Egg Laying Call. The X axis is Time (seconds), and the Y axis is Frequency (Hz). All spectrograms have been standardized to start at 0:00:00 and end at 0:00:328 s.

key anti-predator strategies, and different types of alarm calls are characterized by the context in which they are given and the level or type of predatory threats that exist, but also to some degree by the way that they sound (Ficken, 1990; Ficken and Popp, 1996; Caro, 2005; Ballentine and Hyman, 2021). For example, New Holland honeyeaters (*Phylidonyris novaehollandiae*) produce more elements per call as well as lower pitched calls as more dangerous threats are perceived (McLachlan and Magrath, 2020; see Morton 1977). Contact calls allow birds to coordinate movements of a group and to recognize preferred social partners. They function as a means of keeping touch between conspecific individuals and encode various kinds of social information (Kondo and Watanabe, 2009). In short, vocalizations can provide a great deal of information about a bird's status and welfare.

A relevant aspect of vocal repertoires relates to how specific vocalizations affect conspecific physiology. For example, Thompson et al. (1968) found that when starlings are played back distress calls, their heart rates increase from 300 bpm to 700 bpm, but when they are played back feeding calls, their heart rates increase from 300 bpm to only 340 bpm. The difference in how these two types of calls differentially affect their physiology suggests that these calls encode different types of information. Another study showed that when starlings are played back one of three calls (a synthesized starling distress call, a synthesized sound designed to repel birds, or a synthesized starling alarm sound) the birds heart rate significantly increased during the stress call and alarm call, but not during the synthesized sound designed to repel birds (Thompson et al., 1979). When starlings were played back white noise, a pure tone, or a distress call during the summer and the winter, both the white noise and the distress calls elicited greater responses in the summer, indicating greater susceptibility to auditory stimuli in the summer (Johnson et al., 1985). However, these important aspects of the vocal repertoire of poultry are generally unknown. If we could learn how the different vocalizations impact conspecific physiology, we can begin to understand what types of information these vocalizations transmit.

We recently described the nearly complete repertoire of Pekin duck vocalizations and showed that there are at least 16 distinct types of vocalizations that are dependent upon the social and environmental states of the signalers (Schober et al., 2024). For this current study, we hypothesized that specific Pekin duck vocalizations should be able to alter the physiology of conspecifics based on the information differentially encoded in the calls. We used 5 of the most common Pekin duck vocalizations obtained from our previous study (Schober et al., 2024), as well as white noise and no sound as controls. If we could better understand how Pekin duck vocalizations affect conspecific physiology, we can then determine which Pekin duck vocalizations are alarm calls, distress calls or calming calls. If we understand which duck vocalizations are in these categories, we can then begin to develop an automated system to monitor vocalizations in commercial barns to monitor flock welfare in real time.

Materials and methods

We tested Pekin ducks with playbacks of 5 different vocalizations, with a no noise and white noise stimulus as our controls ($N = 15$ ducks/sex/treatment). The experiment was designed to measure the response of 30 ducks (15 females, 15 males) to each of 7 treatments (5 vocal stimuli and 2 controls). Each bird was to be given the 7 stimuli in random order to avoid the confound of time. However, several birds were lost before they were fully tested. We therefore used replacement birds (of the same sex as the lost bird) to fill out the missing stimuli from the 7-treatment design for each of the lost birds. This resulted in a data set of the response to 7 treatments for 15 birds of each sex. The list of ducks used and what treatments they were exposed to are listed in Supplemental Table 4. All but one of the vocalizations used were previously described in Schober et al. (2024; Fig. 1). The five vocalizations were as follows: (1) The "AM long" vocalization is made by both hens and drakes and is classified as having more than 8 pulses. (2) The "honk" vocalization is made by both hens and drakes and is thought to be one of their alarm or distress calls. Pekin ducks produce this sound when picked up and it is characterized by a noisy, loud sound with a higher frequency than the AM long vocalization. (3) The "pip" vocalization is a common vocalization made by only hens and is characterized by a bowed shape that starts and ends at the same frequency. (4) The "harmonic stack" vocalization is also made only by hens and is characterized as a constant harmonic stack of frequencies that can be integrated into other vocalizations. (5) We added a fifth vocalization (the "egg call") that was not described in our original paper. This vocalization is soft, tonal, and high-frequency (8-9 kHz) and is given only by females while they are laying an egg (Schober et al., 2024b).

A petMAP g3 veterinary BP monitor (petMAP, Tampa, FL) was used to measure the ducks' heart rate and blood pressure. Ducks were taken singly into a closed off room (47 dB). One person held the duck for each playback while another person recorded vitals. Ducks were all habituated to being caught and held prior to the onset of this study. The trials initially consisted of 5 consecutive measurements of heart rate and blood pressure followed by a measurement of respiratory rate under the no-noise stimulus. Then, one (of 7) stimulus was played on repeat (60 dB) while 8 more measurements of blood pressure and heart rate were taken concurrently with a recording of respiratory rate. Finally, the stimulus playbacks were stopped, and 5 measurements of heart rate and blood pressure were taken under the no-noise stimulus followed by a final recording of respiratory rate. The trials were repeated until there was a total of 15 replicates for each of the 7 treatments for each sex.

Three estimates of blood pressure were measured: diastolic and systolic pressure from which mean arterial pressure (MAP) was calculated. MAP is an index of the blood flow to the organs in the body. It is the average blood pressure in an individual during a single cardiac cycle. We calculated MAP with the formula: MAP = (2/3)*diastolic blood pressure + (1/3)*systolic blood pressure. When using the petMAP G3 veterinary monitor, we saw that the blood pressure measurements were less variable than the heart rate measurements. Ducks have an average resting heart rate of about 178 BPM (Jones and Holeton, 1972). The monitor had a measurement range of 40-240 BPM. As such, the heart rate could have been over the peak measurement range for some stimuli, and potentially inaccurate when heart rates approached that peak range. These patterns are consistent with our results showing that blood pressure was a better predictor of the physiological response to different call types compared to heart rate (see below).

Statistical analyses

Data from all blood pressure measurements (systolic, diastolic and MAP, heart rate (HR) and respiratory rate (RR) were subject to a principal component analysis (Proc Princomp in SAS 9.4). Each principal component (Prin 1 and Prin 2) was separately treated as the dependent variable in a repeated measures ANOVA (Proc Mixed, SAS 9.4, subject=duck ID). Note that the repeated-measures statistical design used to test for stimulus effects on our physiological measurements (Proc Mixed in SAS 9.4) is robust to unbalanced data sets (SAS Institute Inc. 2023). $P \leq 0.05$ was considered significant. The independent variables included stimulus type (5 vocalization types plus white noise and no noise controls), sex, and the pre-stimulus systolic blood pressure for Prin 1 and the pre-stimulus heart rate for Prin 2. These latter variables test for the potential that the blood pressure principal component (for example; Prin 1) was correlated with the systolic blood pressure of the duck before the stimulus was broadcast independent of any stimulus effect.

We tested for normality of residuals using a quantile plot. We had two clear outliers (4.81 standard deviations above the mean for a female during the no-sound control and 5.09 standard deviation above the mean for a female during the AM long stimulus) for the ANOVA run on the data set including only Prin 1 responses during the playback and one clear outlier (2.96 standard deviations above the mean for a female during the harm vocalization) for the Prin 2 data set run during the sound stimuli. Similarly, we had one outlier for the data set including only Prin 1 responses after the stimulus presentation (−4.51 standard deviations below the mean for a female after the no-sound control). These were removed in the final model resulting in a normal distribution of model residuals. The after-stimulus Prin 2 data were overall not normally distributed. These residuals were normalized using a square-root (Prin2 + 2.1) transform and the subsequent removal of a single outlier (−1.237 standard deviations below the mean for a male after the honk vocalization).

Finally, we tested for a simple sex effect on pre-stimulus estimates of blood pressure, heart rate and respiration rate. This analysis included a principal component analysis of the pre-stimulus measures recorded before each stimulus presentation. We then ran a repeated measures ANOVA with Prin1 as a function of duck sex. These data were normally distributed.

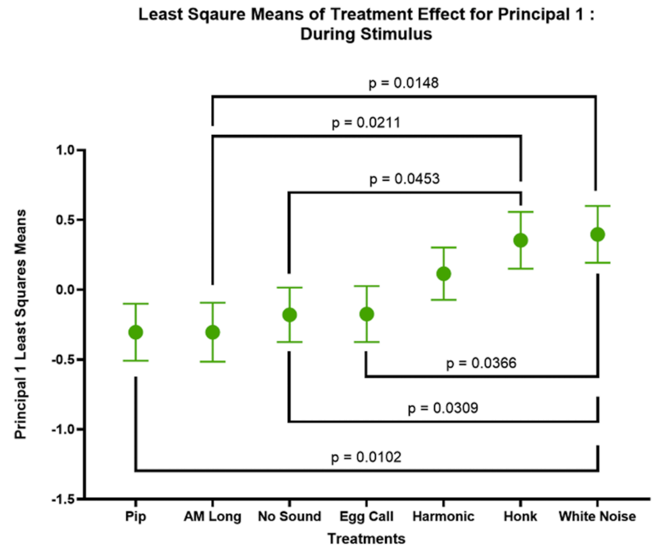

Fig. 2. Least squares means ± standard errors for the treatment effect of call playback type on principal component 1 (blood pressure measurements) during the stimulus. Both sexes are combined in this analysis. The honk had the greatest effect to increase blood pressure suggesting an alarm call. White noise also significantly increased blood pressure suggesting an adverse environment.

Results

Sex effects

The principal component analysis of physiological measures before stimulus presentation resulted in one significant principal component (PRIN 1: $\lambda = 2.83$; eigenvectors: systolic = 0.550, diastolic = 0.554, MAP = 0.589; proportion variance explained = 0.565). A repeated measures ANOVA indicated that there was no significant sex difference in the magnitude of Prin 1 ($F_{1,93}$= 3.32, $P = 0.0716$).

Stimulus treatment effects

The principal component analysis of our 5 physiological measures showed that there were 2 significant dimensions. The first principal component loaded strongly and positively on the blood pressure measurements (PRIN 1: $\lambda = 2.59$; eigenvectors: systolic = 0.5306, diastolic = 0.5715, MAP = 0.618; proportion variance explained = 0.517). The second principal component loaded positively on blood pressure and heart rate (PRIN 2: $\lambda = 1.01$; eigenvectors: HR = 0.840, RR = 0.541; proportion variance explained = 0.202).

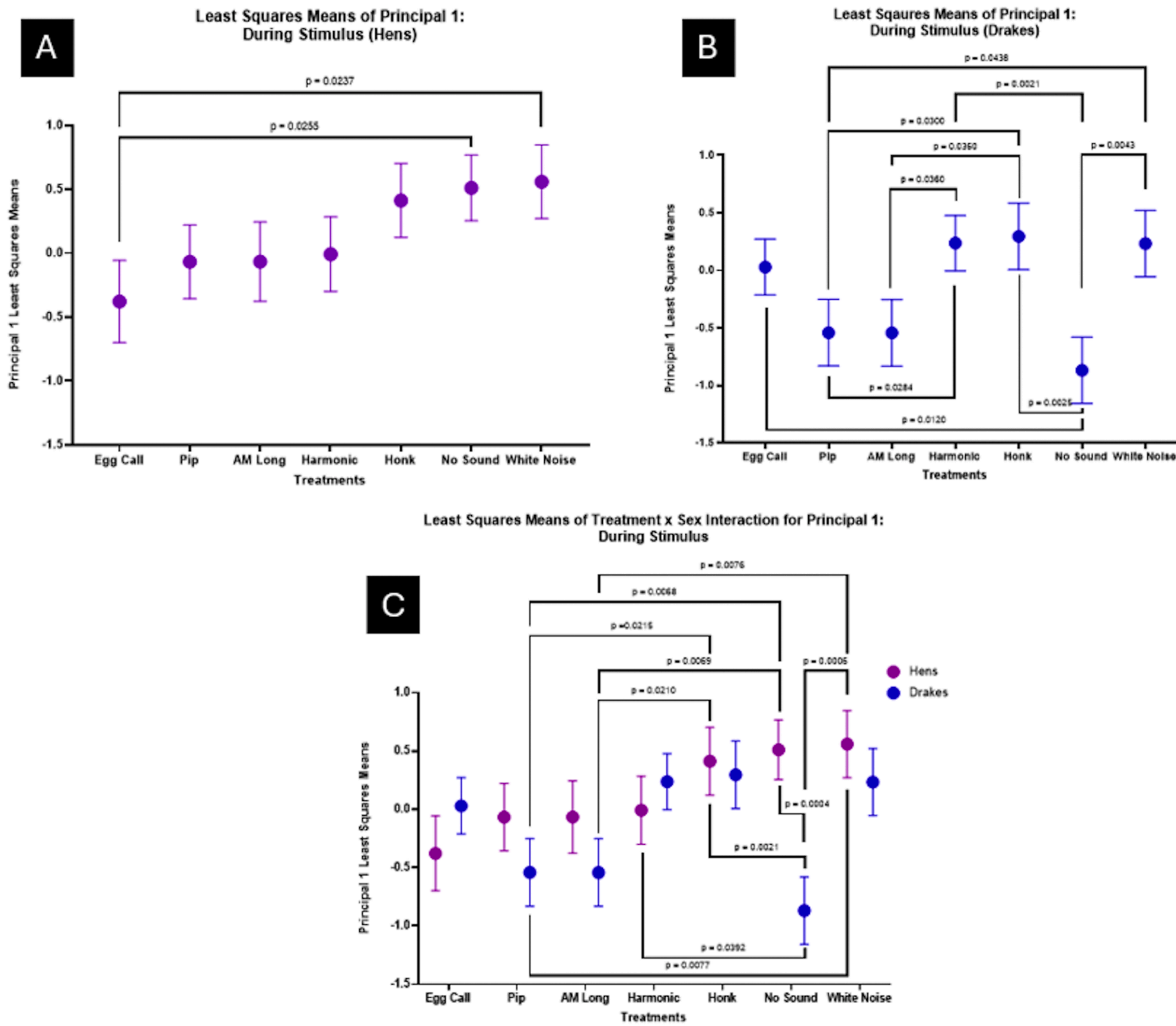

Fig. 3. (A) Least squares means ± standard errors of principal component 1 (blood pressure measurements) for hens during the stimulus. (B) Least squares means ± standard errors of principal component 1 for drakes during the stimulus. (C) Least squares means ± standard errors of treatment x sex interaction effect on principal component 1 during the stimulus. A sex difference was observed in that hens had a greater reaction to vocalizations than did drakes.

Principal 1 (mean arterial pressure): during-stimulus effects

There was a significant main treatment effect on the blood pressure of ducks during the playbacks ($F_{6,177}$= 2.43, $P = 0.0276$) resulting from several patterns of stimulus-induced changes in blood pressure. Ducks during the no-sound control had significantly lower blood pressure when compared to the white noise control ($t_{169} = -2.18$, $P = 0.0309$). Data and all paired contrast analyses are illustrated in Supplemental Table 1 and Fig. 2. We conclude that the honk vocalization and the white noise control increased the ducks' blood pressure compared to blood pressure patterns in response to the other stimuli, controlling for a sex effect and the pre-stimulus systolic blood pressure. However, these conclusions are confounded by a significant sex by treatment effect.

There was no significant main effect of sex on blood pressure ($F_{1,56}$ = 2.42, $P = 0.125$). However, there was a significant interaction between playback treatment and sex ($F_{6,167} = 2.45$, $P = 0.0265$). Data are illustrated in Supplemental Table 2 and Fig. 3. Overall, the honk vocalization significantly increased blood pressure for both hens and drakes, and the egg vocalization significantly lowered hens' blood pressure when compared to the two controls.

Principal 1 (systolic, diastolic, and mean arterial pressure) after stimulus effects

There was no significant effect of playback treatments on blood pressure ($F_{6,173} = 0.91$, P 0.458). However, there was a significant interaction between sex and treatment on the after-stimulus blood pressure ($F_{6,173} = 4.03$, $P = 0.0008$). Data are illustrated in Supplemental Table 3 and Fig. 4. Overall, hens after the harmonic vocalization had lower blood pressure compared to hens after the white noise, AM Long vocalization, and no sound. After the AM long vocalization, drakes had lower blood pressure when compared to drakes after the white noise, honk, and harmonic vocalization.

Principal 2 (heart rate and respiratory rate) during-stimulus effects

Principal 2 was most strongly related to heart rate and respiratory rate. Heart rate and respiratory rate during the playbacks were not significantly influenced by treatment ($F_{6,181} = 1.54$, $P = 0.167$), sex ($F_{1,51} = 0.01$, $P = 0.921$). Fig. 5 illustrates these results.

Principal 2 (heart rate and respiratory rate) after stimulus effects

Principal 2 was correlated with the pre-stimulus heart rate ($F_{1,212}$ =

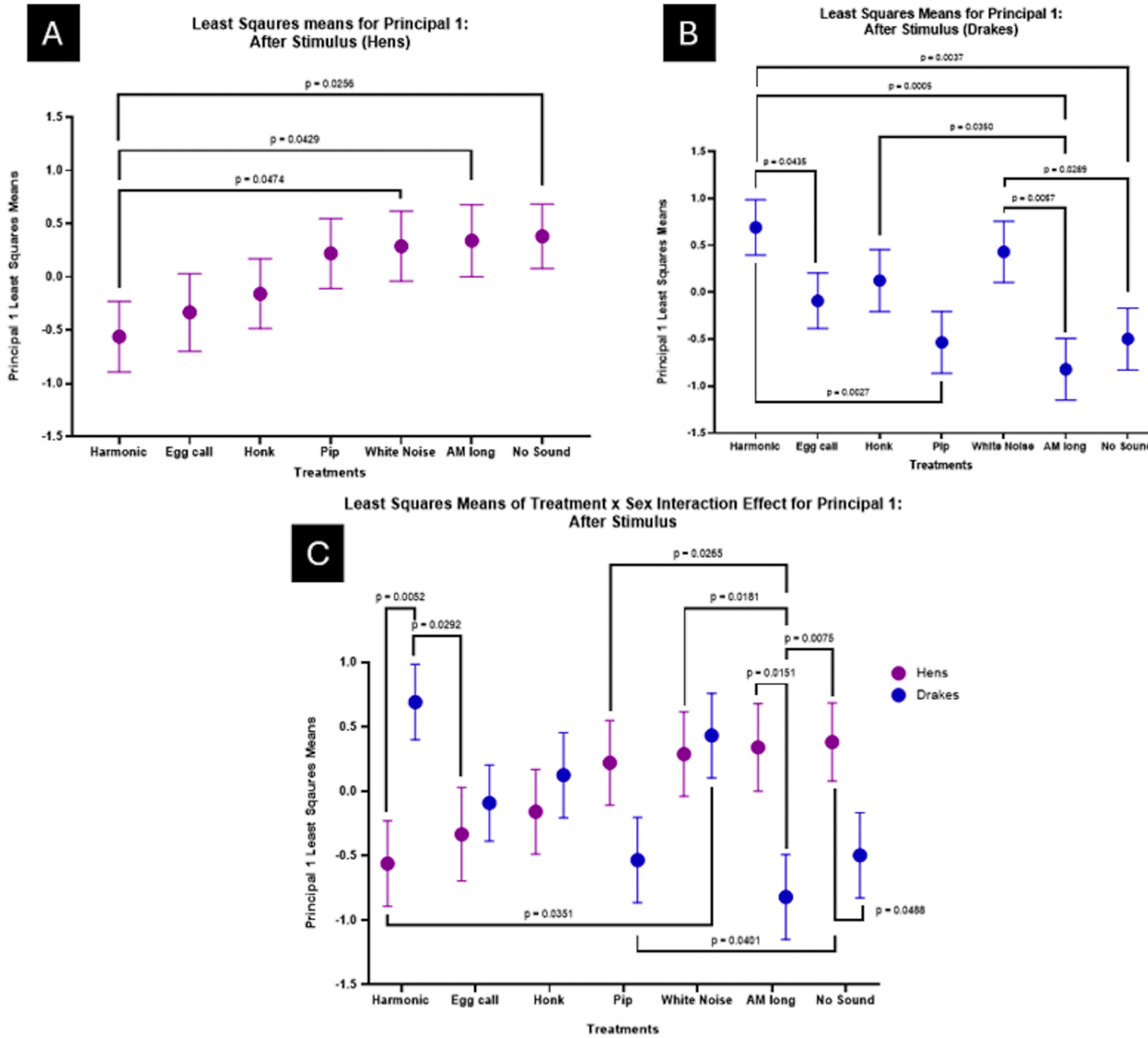

Fig. 4. (A) Least squares means ± standard errors of principal component 1 (blood pressure measurements) for hens after the stimulus. (B) Least squares means ± standard errors of principal component 1 for drakes after the stimulus. (C) Least squares means ± standard errors of treatment x sex interaction effect on principal component 1 after the stimulus. A sex difference was observed in that hens had a greater reaction to vocalizations than did drakes even after the stimuli had stopped.

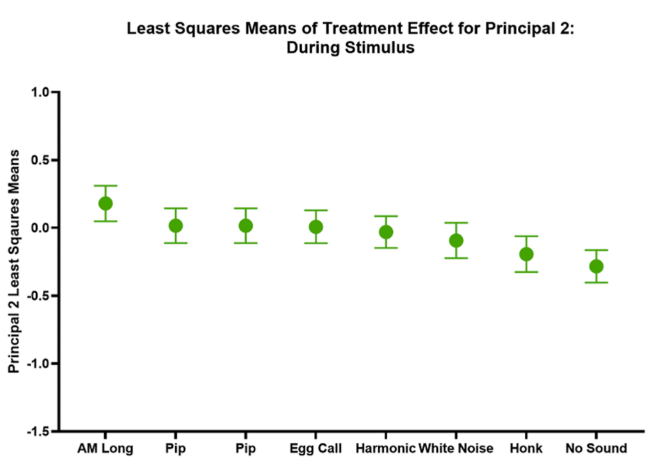

Fig. 5. Least squares means ± standard errors for the treatment effect of call playback type on principal component 2 (heart rate and respiratory rate measurements) during the stimulus. No significant responses were observed.

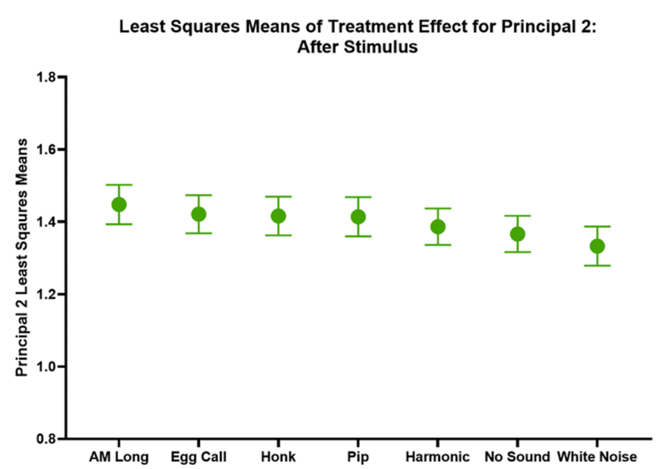

Fig. 6. Least squares means ± standard errors for the main effect of call playback type on principal component 2 (heart rate and respiratory rate measurements) after the stimulus. No significant responses were observed.

93.9, $P = <0.0001$). However, neither sex ($F_{1,48} = 1.41$, $P = 0.124$) nor treatment ($F_{6,171} = 0.58$, $P = 0.240$) affected the level of Principal 2 (Fig. 6).

Discussion

Vocalizations in wild birds have been studied for decades (Mathews, 1904; Saunders, 1941; Miller and Gottlieb, 1978), and their effects on conspecifics have been studied for nearly as long (Thompson et al., 1968, 1979). However, researchers are just scratching the surface of vocalizations in poultry (Evans et al., 1999; Marx et al., 2001; Lee et al., 2015), and there is no research being conducted on the effects of Pekin duck vocalizations on conspecific physiology. Therefore, the purpose of our study was to determine the effects of conspecific vocalizations on Pekin duck physiology, as a step to creating a system that can detect and interpret stress vocalizations in a commercial flock. To achieve this, we played back 5 vocalizations and 2 controls and measured the ducks' heart rate, blood pressure, and respiratory rate before, during and after the stimulus.

We found that the honk vocalization and a white noise control significantly increased ducks' blood pressure (Principal 1) when compared to the no-sound control. Our results also showed significant sex differences. We saw that the drakes' blood pressure was significantly higher than the hens during the harmonic vocalization and the hens' blood pressure was significantly higher than the drakes during the AM long call and the no-sound control. Hens' blood pressure was significantly lower after the harmonic vocalization compared to after the no-sound control. The drakes' blood pressure was significantly higher after the harmonic call, white noise control, and honk vocalization compared to after the no-sound control. Previous studies from our lab have shown that Pekin ducks have strong sex differences when they are exposed to stressors, such as heat or transportation stress, pharmacological manipulation of the stress response, and endogenous glucocorticoid levels (Bergman et al., 2024; Oluwagbenga et al., 2022, 2023, 2024; Schober et al., 2024b; Tetel et al., 2022, 2022, 2022b; Tonissen et al., 2022).

Given all of these observations plus the fact that ducks also show a sex difference in their vocal repertoire (Schober et al., 2024), it is not surprising that we also see a difference in their physiological responses to some of these vocalizations that may be related to fear or predator calls. These studies suggest that hormonal differences between sexes are responsible as is well established in mammals (see Becker et al., [2005] and McCarthy et al., [2017] for a review of sex differences in the brain)—however, this possibility is beyond the scope of this current project. There does appear to be a disconnect between heart rate and blood pressure responses in our study. Although this could be related to sensitivity issues of our HR monitor, it is also not surprising physiologically. The sympathetic nervous system is certainly capable of mass activation; however, it is also capable of fine-tuned responses. Although neural pathways associated with fear and stress responses in ducks are the focus of other studies, it is important to note that we are providing auditory stimuli that directly activate brain pathways. It is likely that the sympathetic responses we observed are the result of many downstream factors from the initial central neural circuits activated (see Roelofs and Dayan, [2022] for a review of the complex interactions between central fear and autonomic circuitry). Regardless of specific neural circuits involved, our data do show that Pekin ducks exhibit sex differences in regard to changes in blood pressure in response to hearing a given vocalization, in particular to distress vocalizations.

Our data showing that the honk vocalization significantly increases ducks' blood pressure suggests that this vocalization is a type of distress call. Schober et al. (2024) showed that the honk vocalization was given most when a person was sitting in the anechoic chamber with the ducks. This observation suggests that the honk vocalization is a distress call. Balentine and Hyman (2021) describe distress calls as a loud, harsh, broad-frequency sound that could be described as a "scream." Distress calls are given when an animal has been attacked or captured by a predator. In Schober et al. (2024), the person sitting in the chamber with the ducks was likely considered a threat. Thompson et al. (1968) also showed that when starlings were played back a conspecific distress call, their heart rate increased 130 % above their baseline heart rate. Given our belief that the honk vocalization is a distress call, this vocalization could be used to determine if flocks of Pekin ducks are stressed in a commercial setting. Future studies, however, are needed to determine if there are different types of honk calls depending upon the degree of the perceived threat.

It is also important that we showed that white noise can elicit an increase in blood pressure, as in a commercial barn there are a lot of different noise stimuli that producers may not be aware could cause distress. White noises that are common in barns may include fans, feed augers, or the hum of radiators. Although a solution to this possibility is not readily available, it is important for equipment designers to understand that these types of sounds may actually be distressing to poultry. Our data also showed that the ducks during the pip, AM long, and egg vocalization had significantly lower blood pressure than during the white noise control. Since there will also be some sort of white noise in a barn, it may be interesting to see if playing these vocalizations as a form of auditory enrichment can reduce stress and improve their affective state.

Our study offers a first step to identifying stress calls in Pekin ducks. Our results showed that the honk vocalization had the most significant physiological response across ducks. This suggests that the honk call it is a stress vocalization. Future studies should address the confounding factor of handling the ducks during the playbacks by using implantable dataloggers. Future studies should also determine how social groups affect physiology during playbacks. Once these have been identified, the results could be used to detect early stages of stress in production ducks in order to address threats to welfare before they impact duck production. Knowing what calls our ducks make when stressed, producers could electronically monitor their flocks' vocalizations and receive alerts as to when an increase in honk vocalizations (or other stress calls) is detected. The stressor could then be detected and addressed before any economic losses or a decrease in welfare can occur.

Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104735.

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