universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Behavioural and Endocrinological Response of Ring Dove Females (*Streptopelia risoria*) to Unimodal and Multimodal Courtship Playback“

verfasst von / submitted by

Daniela Biegler, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2021 / Vienna, 2021

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 878
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Verhaltens-, Neuro- und Kognitionsbiologie UG2002
Betreut von / Supervisor:	Univ.-Prof. Dott. Leonida Fusani, MPhil PhD

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Acknowledgments

I want to thank everyone who worked with me on this project and made this experiment and thesis possible. I first want to thank Cliodhna Quigley and Leonida Fusani for their guidance and supervision. Your feedback was invaluable.

Thank you also to Silvia Colombo and Donata von Bistram, who helped me acquire the stimulus material needed for running this experiment. A second, third, and fourth huge thank you goes to Silvia Colombo, also, for helping me run the experiment, scoring part of the videos analysed for this master's thesis, and staying involved in the project as our resident quiver expert.

A further thank you to Darja Čepon, who was also responsible for part of the behavioural scoring, and Adele Tuozzi for scoring all recorded audio for female vocalisations, both from the experiment proper and the GoPro recordings. I hope you're not sick of the doves' cooing ☺

Last but not least, I want to thank the staff at the Animal Care Facility and the Konrad Lorenz Institute of Ethology, namely Alexandra Bohmann, Barbara Timmler, and Nathalie Skupa, for caring for the doves and the many other animals housed at these facilities.

I enjoyed working with all of you!

Abstract

Bird courtship often features a combination of audio and visual channels, but it is unclear if signal components carry unique or redundant information, and the response to the entire signal may differ to the combined response to single signal components. We investigated multimodal signalling in ring dove courtship by exposing 21 females to high-quality recordings of male courtship over 7 days. Females were divided into three conditions: (1) multimodal audio-visual, (2) unimodal video, and (3) unimodal audio. In the unimodal conditions, audio (foliage) and video (vacuum sound) channels were occluded using alternate familiar stimuli. For this thesis, half the available recordings were scored for behaviours of interest, and behavioural occurrences were compared between stimulus (playback of courtship) and control (playback of empty cage) intervals as well as between conditions. Chasing, a behaviour associated with sexual stimulation, and preening were significantly higher in stimulus intervals. Preening rate was significantly lower in the unimodal audio condition, but no other behaviour differed between conditions, indicating that visual and audio channels carry redundant information in ring dove courtship. However, future analysis of the full dataset might reveal further effects. Additionally to behavioural reactions, female physiological response was assessed through blood oestradiol levels, but we did not find a significant increase, nor was the percentage change different between conditions. Overall, playback seems to have been successful in mediating male courtship to females, and future analysis of this dataset will further investigate multimodal signalling in ring doves.

1 Introduction

Animals communicate by transmitting information in the form of signals, using various sensory channels during this process. Signals and cues emitted by animals may be detectable by humans, but may also fall outside human perception (e.g. echolocation used by many bat species) or use sensory channels that are not accessible by humans at all (e.g. electric communication used by some fish).

Signals can consist of only a single component, but many animals, including humans, also use signals that consist of multiple components. For example, humans combine speech with facial expressions and gestures to enrich conversational content. If the components of such signals use the same sensory channel (or modality), they are referred to as multicomponent signals (Johnstone, 1995; Rowe, 1999). If the signal components hail from different sensory channels (e.g. combine visual and auditory information), the resulting signals are classified as multisensory or multimodal (Higham & Hebets, 2013).

Signal components of multicomponent or multimodal signals may carry the same information (redundant signal hypothesis), but each signal component may also contain unique information (multiple message hypothesis) (Johnstone, 1996; Møller & Pomiankowski, 1993). These different types of components seem to serve different functions in animal communication: Redundant components have been theorised to ensure that the information reaches the receiver, especially if other factors in the environment may occlude part of the signal (e.g. vocalising in a noisy environment), whereas non-redundant signal components may maximise the information content that can be included in a signal (Partan & Marler, 2005).

Some studies have suggested that the response to the entire component signal is not the same as the combined response to its single components, resulting in an enhancement effect (Partan & Marler, 1999, 2005). For example, in wolf spiders (*Schizocosa ocreata*), females approach males performing multimodal courtship more often and present more receptivity displays in response to multimodal compared to unimodal courtship signals (Uetz et al., 2009), and in pigeons (*Columba livia*), multimodal playback of male courtship elicits a stronger response in females than playback of visual or audio components alone (Partan et al., 2005).

Multimodal signals have been described in a variety of contexts, but are especially abundant in courtship, which denotes the sequence of behaviours that enables animals to find partners in order to copulate and reproduce (reviewed in Candolin, 2003; Mitoyen et al., 2019). Combinations of many modalities may occur in multimodal courtship displays. For example, male wolf spiders (genus *Schizocosa*) use a blend of visual and vibratory signals to court females (Scheffer et al., 1996), and peacock spiders (*Maratus volans*) apply vibratory and motion signals in addition to complex body ornaments (Girard et al., 2011). In vertebrates, courtship is often studied in frogs, who show acoustic, but also visual, seismic, and chemical communication (reviewed in Starnberger et al., 2014), and in birds, as a result of some species' striking plumage and highly elaborate courtship dances, which are very conspicuous to humans as well (e.g. manakins (Pipridae), reviewed in Fusani et al., 2014).

To investigate the information content of signal components in multimodal signals, cue isolation experiments have been used in past studies. These experiments involve confronting animals either with the whole component signal or only parts of it, and measuring their behavioural (as done in e.g. wolf spiders, reviewed in (Uetz & Roberts, 2002) or physiological responses (as done in e.g. ring doves, see Friedman, 1977; Lambe & Erickson, 1973). However, in recent years, playback experiments have emerged as the more convenient method to study the information content of signal components (methodology most recently reviewed in Rosenthal, 2019). A main advantage of playback is that it allows for highly standardised exposure to the signal and manipulation of single or multiple signal components. Test subjects may therefore be exposed to all or only part of the signal under controlled conditions, and still experience the same exposure to the signal, overall.

A difficulty one encounters in the playback methodology is that the equipment used is largely designed for the range of human perception, but an animal's perception may differ from what humans can discern, so special equipment may be needed (Fleishman et al., 1998; Rosenthal, 2019). Advances in technology have made it easier to use playback even when the species being investigated differs quite dramatically in its perception to that of humans, but limitations still exist, such as the lack of depth cues and the inability of cameras to capture and

monitors to display some features of visual signals, e.g. ultraviolet and polarised light (Fleishman et al., 1998; Fleishman & Endler, 2000; Rosenthal, 2019).

When studying birds, specifically, audio equipment is not a problem in most cases, as the frequencies humans can perceive exceed the majority of birds' perceivable frequency range (Beason, 2004). However, the refresh rate of many monitors is lower than the flicker-fusion threshold of many bird species, leading to birds perceiving single frames one after the other instead of a fluid, moving image (Fleishman & Endler, 2000; Rosenthal, 2019). Additionally, some bird species are capable of perceiving ultraviolet or polarised light, therefore colours depicted by monitors may look unnatural to the birds viewing the playback (Fleishman & Endler, 2000; Rosenthal, 2019). Still, visual playback has been employed successfully in a number of bird species, including fowl (*Gallus gallus*, Smith & Evans, 2008), rooks (*Corvus frugilegus*, Bird & Emery, 2008), and zebra finches (*Taeniopygia gutata*, Galoch & Bischof, 2007).

Playback experiments in pigeons (*Columba livia*) have found that video motion quality is crucial in eliciting a courtship response from individuals of both sexes, and that courtship response is highest when the frame rate (or image presentation rate, IPR) was set to 60 frames/second (Ware et al., 2015). Partan et al. (2005) used playback to investigate female behavioural response to courtship and were able to show that females reacted more strongly in response to multimodal audio-visual playback compared to unimodal video or audio playback, indicating an enhancement effect. However, this study used cue isolation, and one could argue that cue occlusion is the more ecologically valid setup to investigate multimodal signalling, as single signal channels may be blocked by some sort of barricade, but do not occur in isolation without a reason in nature.

Ring doves (*Streptopelia risoria*) are an excellent species to study in laboratory settings because they can be kept easily in aviaries and will show courtship and other behaviours of interest even in very unnatural experimental setups (Cheng, 1979). They belong to the family Columbidae and are thought to be a domesticated variant of the African collared dove, *Streptopelia roseogrisea* (Baptista et al., 1997). Ring dove behaviour is well-documented (Craig, 1909; Miller & Miller, 1958), and previous work conducted on ring doves includes an extensive canon of literature exploring how reproductive behaviour affects hormonal secretion and anatomical development (reviewed in Cheng, 1979, 2003, 2008).

Ring dove courtship and mating behaviour follows a predictable cycle on a regular time schedule (described in Lehrman, 1964): The male begins by “chasing” (or “charging”, Craig, 1909) the female (Lovari & Hutchison, 1975) and courting her using his relatively simple coo-bow display, which consists of a cooing vocalisation and a bowing movement (Miller & Miller, 1958). During the early stages of courtship, females will often start preening in response to the male’s coo-bow display (Cheng, 1979). If nesting material and an appropriate nesting location are available, the male will then start the nest soliciting display. This display features the same vocalisation as the coo-bow display, but the coos are sometimes described as softer and less hurried (Miller & Miller, 1958). Additionally, nest cooing is accompanied by a distinct crouching body posture and flipping of the wings at a frequency of about two flips per second (Craig, 1909; Miller & Miller, 1958). After a few days of exposure, the female will answer the male’s nest coos with her own and start building the nest with the material the male brings to her. Nest calling will decrease in the male throughout the nest-building process, but will increase in the female until the eggs are laid seven to eleven days after the start of courtship (Miller & Miller, 1958). Both parents will incubate the eggs and feed the young once they hatch.

This behavioural cycle is mediated by physiological changes which also cause anatomical development such as the growth of the oviduct in females (Lehrman, 1964). The hormones responsible for these changes are oestrogen, which induces female sexual responsiveness, and progesterone, which is necessary to bring about nest building and incubation behaviours (Cheng & Silver, 1975; Korenbrot et al., 1974; Liley, 1976). Although it was long assumed that the male’s courtship and nest cooing induce the secretion of these hormones in the female, Cheng (1986) was able to show that if females are exposed to playback of their own vocalisations, follicular development will progress even in the absence of males and even when the female herself has been muted. These initial findings were further substantiated through a series of experiments (reviewed in Cheng, 1992, 2003). However, male courtship and nest calling is still necessary in most cases as it is the main factor initiating female vocalisation, and females paired with males are also stimulated through tactile and visual aspects of courtship (Cheng, 2003).

In addition to the coo-bow and the nest coo, both male and female ring doves also vocalise using perch coos. These coos feature the same vocalisation as the coo-bow and the nest coo, but are given from a perch position (Miller & Miller, 1958) and often occur when a bird is isolated (Davies, 1974). Vocalisations given by females, i.e. perch coos and nest coos, are especially interesting in the context of this study because a similar experiment conducted in pigeons found that females vocalised more often in the unimodal audio condition compared to the unimodal video and multimodal audio-visual condition (Partan et al., 2005).

The multimodality of ring dove courtship has been investigated by Lambe & Erickson (1973) who found that only females who saw the male's shadow in addition to hearing his courtship vocalisations laid eggs. Likewise, Friedman (1977) was able to show that oviduct development is greater in females that can see and hear a courting male than in females that can only hear the male, presumably because females need assurance that males are courting them and not another female before beginning their nest soliciting display.

Previous work conducted in the dove lab at the University of Vienna has focused not only on the physiological changes females undergo in response to courtship, but also on their immediate behavioural responses to it, which had been largely neglected up until this point. Mitoyen et al. (2021) recorded interactions between male and female pairs and of individual birds without an interaction partner. They analysed male and female behaviour and investigated how female response differs between the presence and absence of males, and how the presence of courtship changes behavioural response in females. They found that when males courted, females showed a higher number of steps and tail quivering events, confirming quivering as a sign of sexual interest in doves. However, number of preening events did not depend on the presence of courtship, and female vocalisations were found to occur extremely rarely.

Mitoyen et al. (submitted) expanded on these findings by exposing females to synchronous or asynchronous multimodal audio-visual playback of male courtship. They demonstrated the efficacy of using playback to study courtship in the ring dove, as females showed higher numbers of behavioural events for steps and preening during stimulus intervals (when male courtship videos were played) compared to control intervals (in which a silent empty testing compartment was shown). Tail quivering events were also higher during stimulus intervals, further

confirming this behaviour as a sign of sexual stimulation in doves. Surprisingly, they also encountered chasing in females, even though it had hitherto only been described in males (Craig, 1909; Lovari & Hutchison, 1975). Since chasing was also higher in the stimulus compared to control intervals, it can be assumed as a sign of sexual interest for the female as well.

In this study, we first obtained high-quality recordings of male ring dove courtship and then showed these recordings to female ring doves. Playback was structured to contain both stimulus intervals, in which the females were exposed to male courtship, and control intervals, in which females were shown silent playback of the empty setup. To investigate whether signal components of ring dove courtship carry redundant information, females were split into three groups, and shown either the multimodal courtship or only one aspect of it. A novelty of this study was that instead of showing isolated signal components for the unimodal conditions, we chose to occlude either the video or audio channel using alternate familiar stimuli, in an effort to make the experiment more ecologically valid.

Previous work from the dove lab has shown that playback can be used to elicit a behavioural response in ring doves (Mitoyen et al., submitted), and based on these findings, we predicted that we would find higher behavioural occurrences during stimulus intervals compared to control intervals. Furthermore, since Mitoyen et al. (submitted) observed chasing during their playback study, we expected to see it as well.

Based on previous work by Lambe & Erickson (1973) and Friedman (1977), who found that oviduct development is strongest when ring dove females are exposed to both the male's vocalisations and his image, and on the findings of a similar study conducted on the closely related pigeon (Partan et al., 2005), we expected to see stronger behavioural and endocrinological responses in females assigned to the multimodal condition, and that in particular, vocalisations would be higher in the unimodal audio condition.

2 Materials and Methods

In this experiment, we exposed female ring doves (*Streptopelia risoria*) to courtship recordings of male ring doves once a day for seven consecutive days, and investigated their behavioural and endocrinological responses between stimulus and control intervals as well as between conditions. I contributed to designing and running the experiment, and helped with the recording of the courtship material as well as the habituation of doves to the setup and isolation cages. I selected and cut the courtship clips to be used in the experiment, scored part of the videos obtained from it, and ran the analysis presented in this master's thesis.

2.1 Ethics statement

The experiment was approved by the local ethics committee of the Faculty of Life Sciences, University of Vienna, and by the national committee of the Austrian Federal Ministry of Education, Science and Research (BMWFW permit 66.006/0042-WF/V/3b/2017). All birds were housed following national guidelines. Ring doves are a domesticated species and the birds in our study were used to being handled. All persons directly interacting with the birds were adequately trained and the doves were given time to adjust to new people. Birds were regularly checked by trained animal caretakers and vets. Isolation cages were cleaned daily and equipped with branches for environmental enrichment. In the isolation cages, birds were visually, but not acoustically separated from each other. Both in the aviaries and in the isolation cages, food, water, and grit were given ad libitum. All doves involved in the experiment were habituated to the setup and the isolation cages prior to the start of the experiment. Additionally, for the duration of the experiment, all birds were weighed daily to assess any sudden changes indicating stress. This was never observed for any of the birds involved in the experiment.

2.2 Subjects and Phases

2.2.1 Courtship Recordings

In the first phase of the experiment, six ring dove males were chosen and repeatedly exposed to a female to obtain courtship videos. The two females used for this phase did not partake in the subsequent experiment. Of these six males,

courtship was observed in three of them, but only two courted with high enough frequency to be chosen as stimulus males. Male courtship was recorded at 120 frames/second to ensure courtship videos looked as natural as possible to females. Audio was sampled at 48 kHz together with the output of the camera hardware synchroniser, which allowed audio and video recordings to be synchronised in post-processing. Due to the nature of the recording procedure, the first coo of a courtship bout was not always recorded. If it was recorded, it was not used in the playback to avoid biasing female response, especially because it is unclear if the first coo carries additional information.

For the duration of courtship recording, the males were kept in individual cages (50x38x60 cm).

2.2.2 Playback

After obtaining the courtship recordings, we were able to proceed to the playback phase. A total of 21 females partook in the courtship playback and were exposed to the courtship clips once a day for seven consecutive days. This interval was chosen because behavioural and physiological changes in females follow a regular time schedule (Lehrman, 1964).

Females were transferred to individual isolation cages (cage type 1: 50x38x60 cm (9 cages), cage type 2: 46x32x48 cm (3 cages)) and kept there for six (experimental group 1, see below) or five (experimental group 2) days before testing began. This was done in an effort to return females to a baseline hormonal state in order to adequately assess changes in blood hormone levels before and after the experiment.

Isolation cages were kept in a temperature-controlled room (20 °C) at a set 14/10 hour light/dark cycle. Before testing began, females had been separated from males for at least three weeks. For the duration of the experiment, females were filmed simultaneously in their isolation cages using GoPro cameras (hero4 model) for 30 min each day before testing sessions started and after they ended. The footage was scored for vocalisation behaviours, but this scoring was not analysed as part of this master's thesis.

For logistical reasons, testing had to be split into two groups: Experimental group 1 consisted of nine females, who were exposed to courtship playback from Aug 25 to Aug 31, 2020. Experimental group 2 consisted of twelve females, who

were exposed to courtship playback from Sept 8 to Sept 14, 2020. All birds were ringed with unique ID numbers to allow for easy distinction between individuals.

2.3 Playback Design

For the playback stimuli, 10 courtship clips (8-14 seconds, each containing 4-6 coo-bows) of each male were chosen. Due to the different courting styles of the males, the duration of both the clips (summed duration ~104.2 seconds for stimulus male 1, ~111.6 seconds for stimulus male 2) and the overall playback (~15.5 minutes for stimulus male 1, ~16 minutes for stimulus male 2) varied slightly, but females were exposed to the same total number of coo-bows. The playback was automated using Psychophysics Toolbox (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997; script written by Cliodhna Quigley, Department of Behavioural and Cognitive Biology, University of Vienna), and structured to consist of both stimulus and control intervals. One stimulus interval consisted of five courtship clips shown consecutively, each separated by a short break. Each short break was randomly chosen to be between 0.5 and 1.5 seconds long (uniform distribution), in an effort to make the playback less predictable for the females. Each stimulus interval was followed by a control interval, fixed to be 40 seconds long. Both the short breaks and the control interval featured silent video playback of the empty setup. Each clip was repeated in random order for a total of five times during each session, under the condition that no courtship clip be shown twice in a row.

2.4 Conditions

Females were split into three conditions, each group consisting of seven birds: 1) unimodal audio-only condition (A), 2) unimodal video-only condition (V), 3) multimodal audio-visual condition (AV). Females were randomly assigned to a condition and stimulus male, and conditions were balanced across stimulus males and experimental groups. For the AV condition, females were exposed to both the video and audio recordings of the male courtship. For the A condition, females were exposed to the audio aspect of the recording, but the video of the male was replaced by a still image of a bundle of ivy, which was recorded in the same set-up using the same recording conditions. For the V condition, the visual aspect of the male courtship remained unobscured, but the audio recording was

replaced by the sound of a vacuum cleaner, played at the same dB level (75-80 dB) as the male courtship. The image of ivy and vacuum sounds were chosen in an effort to mask the courtship and give the females a plausible reason for not being able to hear or see the male, thus making the experiment more ecologically valid. The ivy used was visible from the aviary the females were housed in before being moved to the isolation cages. Likewise, the sound of the vacuum cleaner was familiar to the females, as it was the vacuum used to clean their isolation cages. Additionally, prior to the start of the experiment, all females were habituated to the playback of the ivy and the empty setup as well as the sound of the vacuum.

2.5 Setup and Equipment

The setup (see **Figure 1**) used for the experiment was built by Clémentine Mitoyen for her PhD project on ring dove courtship. It consisted of two adjacent aluminium-frame boxes covered with walls of either black fabric or mist netting. The female was placed in one box, while the other housed the equipment necessary to show the courtship playback. The two compartments were separated by a layer of mist netting so that the female was unable to touch the equipment. The visual component of the courtship was shown on a 24-inch gaming monitor (ASUS VG248QE, refresh rate 120 Hz), located approximately 22 cm from the border between compartments. The distance and size of the displayed video (28.5x18.8 cm) were chosen so that the male would appear as natural in size as possible. Additionally, the video was cut so that the male filled up much of the available frame, while the rest of the monitor and its bezels were covered in non-reflective black cardboard. The auditory component of the courtship was played using a speaker (Fostex FE108ez) placed behind the monitor. Visual and auditory response of the females were recorded using a microphone (Sennheiser ME66 K6, directional head with battery powered K66 module) and three cameras (Basler acA1920-155uc). One camera recorded the female from the side, the second from above, and a third camera was directed at the monitor on which the playback was shown. Since the cameras were synced, this third camera allowed us to later differentiate between stimulus and control intervals for all recorded material. All cameras recorded at 60 frames per second. For this master's thesis, be-

havioural scoring was done only on the side view camera, and only for half of the recorded sessions.

Figure 1: Setup in the dove lab as seen from above. Only shows side view camera.

Video (Motif Video Recording System, loopbio gmbh, Vienna, Austria) and audio recording (Audacity, ©1999-2021 Audacity Team) were controlled by a standard gaming computer (graphics card: AMD Radeon Pro WX 4100, 4GB GDDR5) running Ubuntu. The playback was controlled by an Octave script run on the same computer using Psychophysics Toolbox (Brainard, 1997; Kleiner et al., 2007; Pelli, 1997; script written by Cliodhna Quigley, Department of Behavioural and Cognitive Biology, University of Vienna).

The room in which the experiment was conducted was equipped with acoustic foam on the walls and fabric curtains around the experimental setup to ensure optimal acoustic recording quality. The box in which the female was placed was illuminated using a soft box diffuse LED light (Neewer LED max. 45W) behind the camera and LED spotlights (six individual spots) placed above the compartment. This guaranteed optimal visual recordings so that behavioural scoring would be as unambiguous as possible. All lights were powered by DC power supplies to eliminate flicker.

2.6 Analysis

Video coding of female behavioural response was conducted in Loopy (http://loopb.io, loopbio gmbh, Vienna, AUSTRIA), and scoring was performed by three coders. To date, half of the recording sessions have been scored and are reported here. I scored 55 of 77 videos, while my colleagues Darja Čepon and Silvia Colombo scored the remaining 22 videos. To ensure behaviours were coded in the same way, an ethogram was drafted (see **Table 1**), and six videos were chosen to be scored by all coders. Interobserver reliability was assessed by simple visual comparison of scorings, making sure that behaviours were scored within the same time frame and using the same qualifiers (e.g. the same body area for preening behaviour). Once the full dataset is available, interobserver reliability will be assessed using suitable statistical measures. Audio scoring was conducted in its entirety by Adele Tuozzi.

To also investigate the endocrinological response to the courtship playback, blood and faecal samples were taken to measure oestradiol levels. Blood samples were taken by Cliodhna Quigley and Giovanni Spezie, and hormone analysis was performed by Virginie Canoine (Department of Behavioural and Cognitive Biology, University of Vienna). Samples were taken the day before the experiment started and the day after it ended. Post-test samples were available for all females and all samples showed detectable levels of oestradiol (> 5.2 pg/ml). However, in pre-test samples, oestradiol concentration was undetectable in one female, and no pre-test sample could be acquired for another female. The pre-testing value for the former female was set to the detectable level (5.2 pg/ml) for the analysis, while the latter female was excluded from the analysis.

Faecal samples were taken on each day of the experiment as well as the day after the experiment ended. To ensure freshness of faecal samples, cages were cleaned each day after all females had been exposed to playback, and then checked for samples every 1-2 hours until a sample had been obtained from each bird. Due to different defecation habits, we were unable to collect samples of all birds at the same time, but collection time and approximate age of the sample was documented for each collection day. Faecal samples are still pending analysis.

All statistical analyses were conducted in R version 4.0.2 (R Core Team, 2020). All plots were produced using the R package *ggplot2* (Wickham, 2016). Figures made up of multiple plots were arranged and annotated in Inkscape (Inkscape Project. (2020). Retrieved from https://inkscape.org). Additional packages used were *tidyr* (Wickham, 2020) and *dplyr* (Wickham et al., 2020) for data wrangling, as well as *FSA* (Ogle et al., 2021) and *rcompanion* (Mangiafico, 2020) for post hoc testing.

Scoring of all behaviours for all videos was outside the scope of this master's thesis. Therefore, analysis was conducted on select behaviours, and scorings for "step", "chase", and "preen" behaviours were only available for a subset of videos (77 of 147). Additional behaviours that will be considered in future analyses can be found in **Table A1**.

Vocalisation data was available for all videos, but because not all females vocalised, data was treated the same as other behaviours for analysis (see below). Of all vocalisation data scored, nest coos and perch coos (see **Table 1**) were analysed. Each coo was treated as one behavioural event.

Differences between stimulus and control intervals and differences between conditions were analysed separately for all behaviours. Data wrangling was required to get the data in an appropriate shape for analysis. In a first step, raw data was summed separately for each female and experiment day, resulting in total durations per recording session spent on the behaviour for duration behaviours, and total number of behavioural events for event behaviours. For analysis of stimulus vs. control intervals, data was additionally separated by stimulus and control intervals, resulting in 154 values (one value per female and experiment day for stimulus intervals, and one value per female and experiment day for control intervals). For analysis of conditions, all data was summed, regardless of whether the behaviour occurred in a stimulus or a control interval, resulting in 77 values (one value per female per experiment day).

Because total duration of stimulus and control intervals was not equal, and length of stimulus intervals varied slightly between stimulus males (total duration of stimulus intervals per experiment session: ~9.5 min (stimulus male 1) or ~10 min (stimulus male 2); total duration of control intervals per experiment session: ~6 min (for both stimulus males)), I decided to take relative measures for each behaviour of interest. Using the summed values obtained in step one, I calculated

number of events per minute for event behaviours and proportion of time spent on the behaviour for duration behaviours, again separately for females and experiment days.

Additionally, in an effort to avoid biasing the scoring of video data, videos were chosen randomly during the scoring process, hence not all females had the same number of scored videos available. In order to prevent distorting analysis results, I opted to run the analyses on the means of available values. For durations behaviours, I averaged the proportion of time spent on the behaviour over available experiment sessions. For event behaviours, means were calculated based on the number of events per minute in each experiment session. This left me with two values per female (one for stimulus intervals, one for control intervals) for analysis of differences between stimulus and control intervals. For analysis between conditions, I obtained one value per female.

Statistical testing was kept simple for this master's thesis because analysis was performed on a partial dataset. Once the full dataset is available, behaviours and interactions between variables will be investigated using appropriate statistical models. Normal distribution of all data was tested using Shapiro-Wilk normality tests. Only some data was normally distributed, and for better comparability between test results, I chose to use non-parametric tests for all analyses. To assess the efficacy of playback in the ring dove, differences between stimulus and control intervals for all behaviours of interest were tested using paired Wilcoxon signed rank tests. Since stimulus and control intervals were part of the playback for all females, I compared values within subject. To investigate the information content of single signal components in the ring dove courtship display, differences between conditions were assessed for each behaviour of interest using Kruskal-Wallis tests as well as Dunn tests for post hoc analysis if necessary. Females were assigned to only one condition, therefore these values were compared between subjects.

Likewise, blood oestradiol was compared for differences between pre- and post-test levels (paired Wilcoxon test; within-subject design), and percentage change was compared between conditions (Kruskal-Wallis test; between-subject design). The significance level for all tests was set at 0.05.

Table 1: Ethogram of behaviours included in the analysis for this master's thesis. For all scored behaviours, see Table A1.

category	behaviour	definition
body movements	step	The dove lifts one of her feet off the ground and sets it down again immediately. One step counts as one behavioural event.
	chase	The dove moves by setting one foot in front of the other while assuming a distinct body posture. The typical body posture may be more or less clear between birds, but in general, her back is slightly hunched, her head tucked down, and her wing feathers are slightly spread (on one or both wings). She may drag her wings and/or tail on the ground as she walks. Her head may bob back and forth along a horizontal axis. She may move in a circular pattern, but this is not a must.
preening	wing preening	The dove touches her beak to any part of her wings or runs her beak along her wing feathers.
	underside preening	The dove touches her beak to the feathers or skin of the underside of her body
	back preening	The dove touches her beak to the feathers or skin of her back, i.e. the region between her wings and above her tail.
	tail preening	The dove touches her beak to the feathers or skin of her tail, i.e. the region distal of the cloaca.
vocalisations	nest coo	The dove makes a cooing sound while assuming a distinct body posture: She holds her tail high; her head may be lowered so that her tail and head are in one line, but she may also move her head up and around. Both her feet are on the ground, but she may stand only on the tips of her feet. Her wings may twitch. Her neck may inflate and deflate with the coos.
	perch coo	The dove makes a cooing sound while in perch position, i.e. with her tail lower than her head and both feet planted on the ground; her neck may inflate and deflate with the coos.

3 Results

In this study, 21 female ring doves were exposed to recordings of male courtship (stimulus intervals), interspersed with recordings of the empty setup (control intervals). Females were exposed to the playback for 15.5-16 minutes once a day over the course of seven days. Since only the first half of the behavioural scoring is available, I am unable to report differences between experimental sessions. However, I used the preliminary dataset to investigate differences between stimulus and control intervals (within-subject design) to assess the efficacy of playback in this species, and differences between conditions (between-subject design) to answer questions about the information content of the ring dove courtship and whether audio and visual channels carry unique or redundant information.

3.1 Female response in stimulus vs. control intervals

Differences between stimulus and control intervals were analysed separately from differences between conditions. The following text and figures report the results of the analysis for stimulus vs. control intervals.

Steps were observed for all females on all experiment days, both in stimulus and control intervals. Mean steps per minute (**Figure 2**) ranged from 2.598 to 116.263 (group mean = 26.79, SD = 26.64, N = 21) for stimulus intervals, and from 2.878 to 118.713 (group mean = 23.09, SD = 25.83, N = 21) for control intervals. The difference between stimulus and control intervals spanned from -17.497 to +29.856 mean steps per minute (group mean = 3.69, SD = 10.31, N = 21). Positive numbers indicate a higher number of mean steps per minute in the stimulus intervals, negative numbers indicate a higher number of mean steps per minute in control intervals. Wilcoxon signed rank test showed no paired difference between stimulus and control intervals ($V = 164$, $p = 0.0958$).

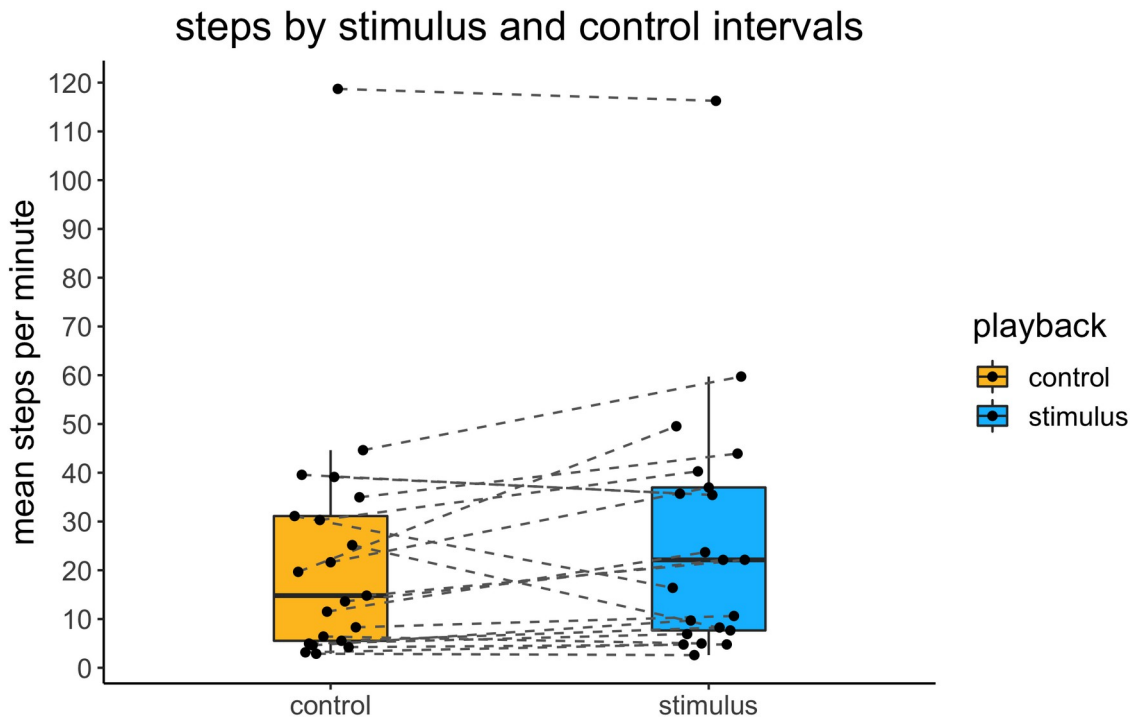

Figure 2: Boxplot and overlayed scatter plot for mean steps per minute. Each datapoint represents the average step rate from 3 or 4 days of the experiment. Connected datapoints represent the same female.

A total of eleven of 21 females showed chasing behaviour at least once. All eleven of these females chased during at least one stimulus interval, while eight females also chased at least once during a control interval. Mean percentage of time spent on chasing (**Figure 3**) spanned from 0 (i.e. the behaviour did not occur in any scoring) to 26.9732% (group mean = 5.09, SD = 8.08, N = 21) for stimulus intervals, and from 0 to 5.52% (group mean = 0.68, SD = 1.44, N = 21) for control intervals. The difference between stimulus and control intervals ranged from 0 to 26.0475% of time spent on chasing (group mean = 4.41, SD = 7.18, N = 21). All females showed higher amounts of chasing during stimulus intervals. Statistical testing revealed a significant difference between stimulus and control intervals (paired Wilcoxon signed rank test, V = 66, p = 0.003857), with chasing being more prevalent in stimulus than control intervals.

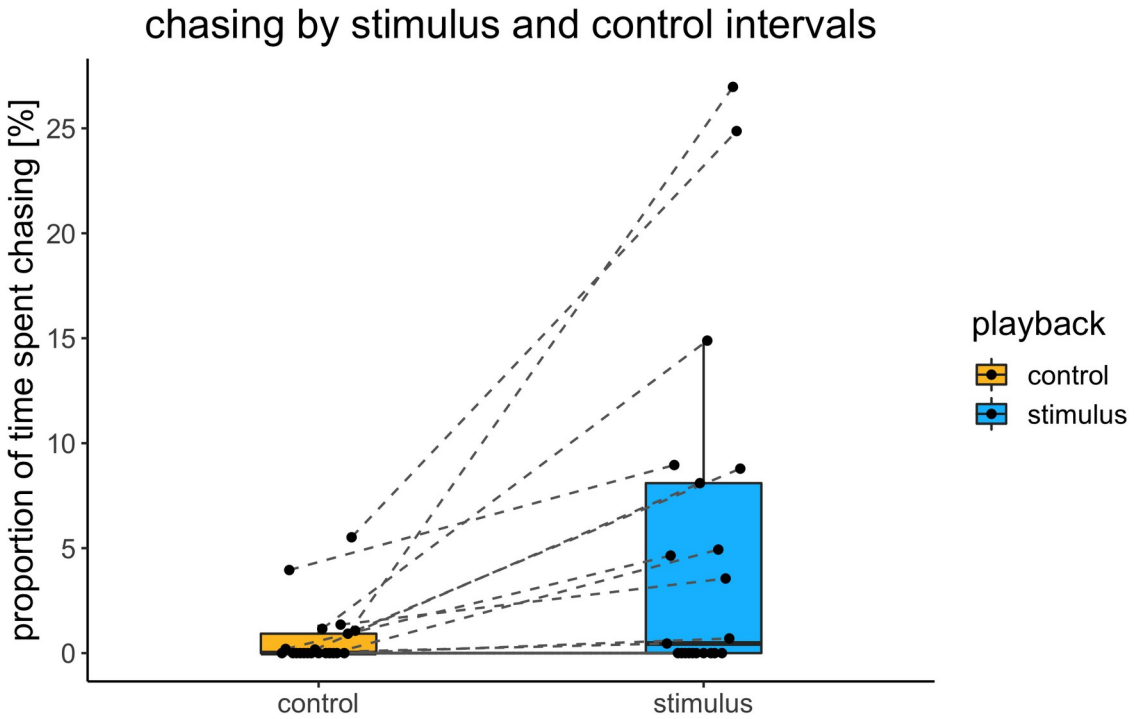

Figure 3: Boxplot and overlayed scatter plot for proportion of time spent on "chase" behaviour. Each datapoint represents the average proportion of time spent on chasing from 3 or 4 days of the experiment. Connected datapoints represent the same female. Some females did not show the behaviour at all (shown as 0 values in the graph).

Preening was observed during at least one experimental session for every female, and tested both as a proportion of time spent on the behaviour and as mean number of preening events per minute. Proportion of time spent on preening (**Figure 4a**) ranged from 0.03425 to 20.94% (group mean = 10.47, SD = 6.75, N = 21) for stimulus intervals, and from 0.01522 to 24.346% (group mean = 7.23, SD = 6.42, N = 21) for control intervals. Differences between stimulus and control intervals spanned from -8.0191 to +15.9429% of time spent preening (group mean = 3.23, SD = 6.49, N = 21). Negative numbers indicate a higher mean proportion of time spent on preening in control intervals, positive numbers indicate a higher mean proportion of time spent on preening in stimulus intervals. There was no difference between stimulus and control intervals for mean proportion of time spent on preening (paired Wilcoxon signed rank test, V = 161, p = 0.1193).

Mean preening events per minute (**Figure 4b**) spanned from 0.07398 to 33.12182 (group mean = 11.73, SD = 9.44, N = 21) for stimulus intervals, and from 0.2192 to 15.5111 (group mean = 5.38, SD = 4.00, N = 21) for control intervals. The difference between stimulus and control intervals ranged from -0.8714 to +28.8467 mean preening events per minute (mean = 6.36, SD = 8.98). Negative numbers indicate a higher preening rate in control intervals, negative numbers

indicate a higher preening rate in stimulus intervals. Preening rate was significantly higher in stimulus compared to control intervals (paired Wilcoxon signed rank test, V = 205, p = 0.001002).

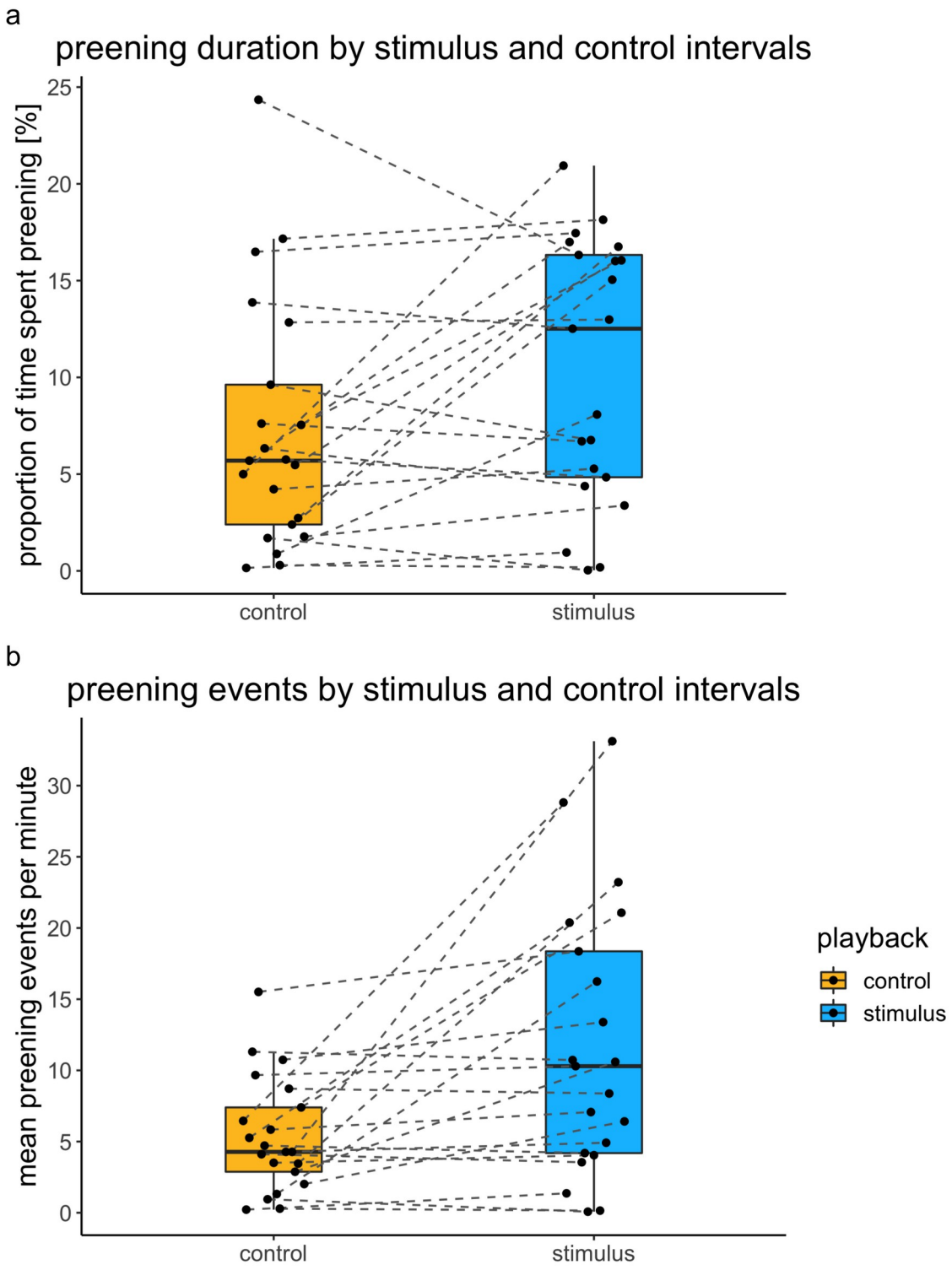

Figure 4: Boxplots and overlayed scatter plots. Connected datapoints represent the same female. (a) Proportion of time spent on preening. Each datapoint represents the average proportion of time spent on preening from 3 or 4 days of the experiment. (b) Mean preening events per minute. Each datapoint represents the average preening rate from 3 or 4 days of the experiment.

Vocalisations were analysed separately for perch coos and nest coos. Seven of 21 females perch cooed during at least one stimulus interval, and nine females showed perch coos during one or more control intervals. Mean perch coos per minute (**Figure 5a**) ranged from 0 to 1.24132 (group mean = 0.17, SD = 0.34, N = 21) for stimulus intervals, and from 0 to 7.5633 (group mean = 0.50, SD = 1.65, N = 21) for control intervals. Differences between stimulus and control intervals ranged from -6.32198 to +0.09848 mean perch coos per minute (group mean = -0.33, SD = 1.38, N = 21). Negative numbers indicate more mean perch coos per minute in control intervals, positive numbers indicate more mean perch coos per minute in stimulus intervals. Statistical testing did not reveal a significant difference between stimulus and control intervals for perch coos (paired Wilcoxon signed rank test, V = 9, p = 0.06655).

Nest coos were observed in six females during stimulus intervals, and five females during control intervals. For stimulus intervals, mean nest coos per minute (**Figure 5b**) ranged from 0 to 2.18212 (group mean = 0.16, SD = 0.49, N = 21). For control intervals, mean nest coos per minute spanned from 0 to 3.4768 (group mean = 0.22, SD = 0.76, N = 21). Differences between stimulus and control intervals ranged from -1.29467 to +0.19605 mean nest coos per minute (group mean = -0.06, SD = 0.29, N = 21). Negative numbers indicate a higher number of mean nest coos per minute in control intervals, positive numbers indicate a higher number of mean nest coos per minute in stimulus intervals. Mean nest coos per minute showed no significant difference between stimulus and control intervals (paired Wilcoxon signed rank test, V = 8, p = 0.675).

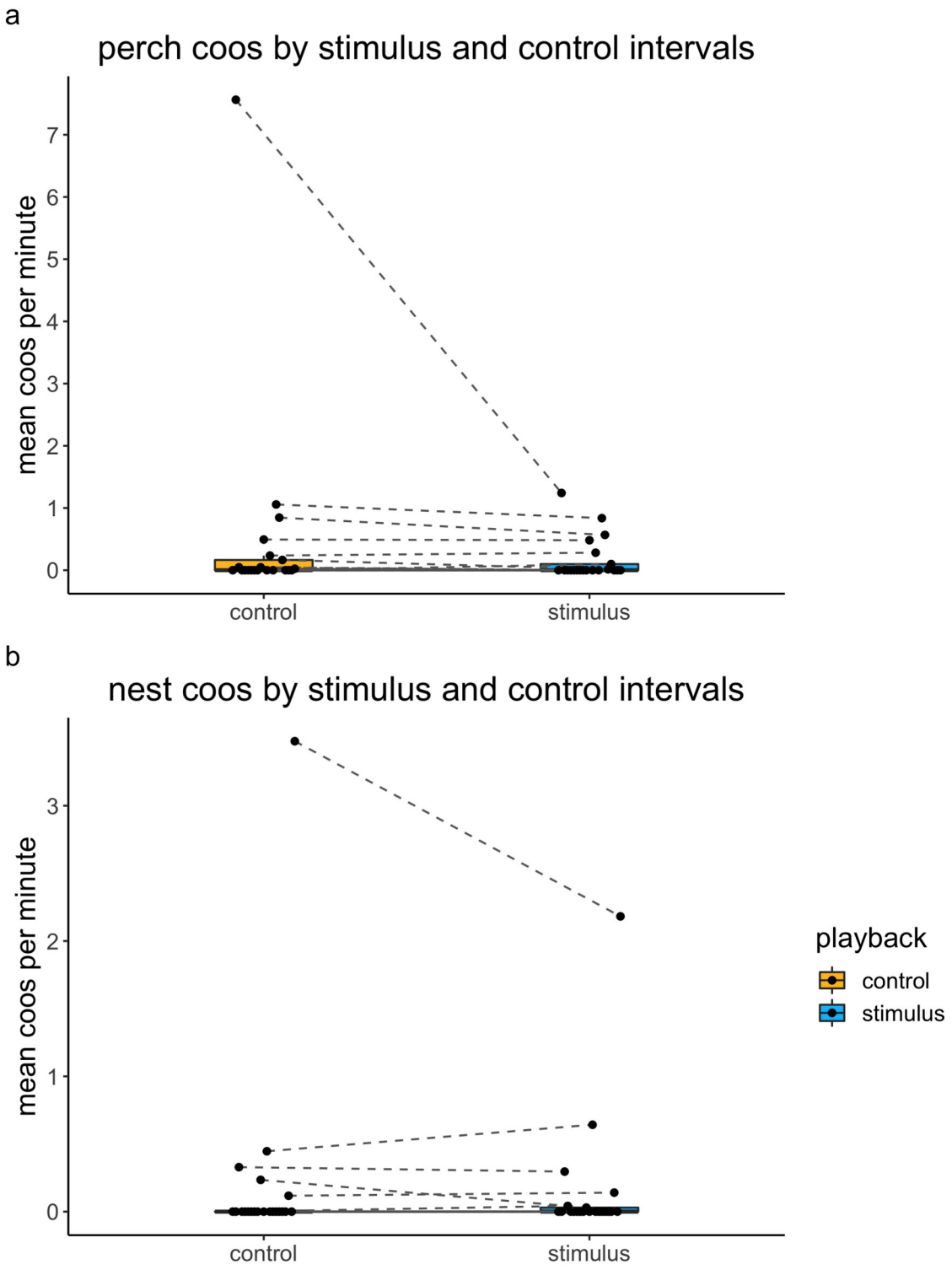

Figure 5: Boxplots and overlayed scatter plots for vocalisations. Each datapoint represents the average cooing rate from 3 or 4 days of the experiment. Connected datapoints represent the same female. Some females showed neither perch coos nor nest coos (shown as 0 values in the graph). (a) Mean perch coos per minute, (b) mean nest coos per minute.

3.2 Female response compared between conditions

Differences between conditions were analysed separately from differences between stimulus and control intervals. The results of this analysis are reported here.

Steps were observed for all conditions. Mean steps per minute (**Figure 6**) ranged from 4.574 to 117.186 (group mean = 36.04, SD = 38.27, N = 7) in the A condition, from 5.519 to 37.157 (group mean = 18.98, SD = 13.52, N = 7) in the V condition, and from 2.708 to 53.848 (group mean = 21.09, SD = 19.36, N = 7) in the AV condition. However, statistical testing showed no difference between conditions (Kruskal-Wallis rank sum test, chi-squared = 0.45269, df = 2, p = 0.7974).

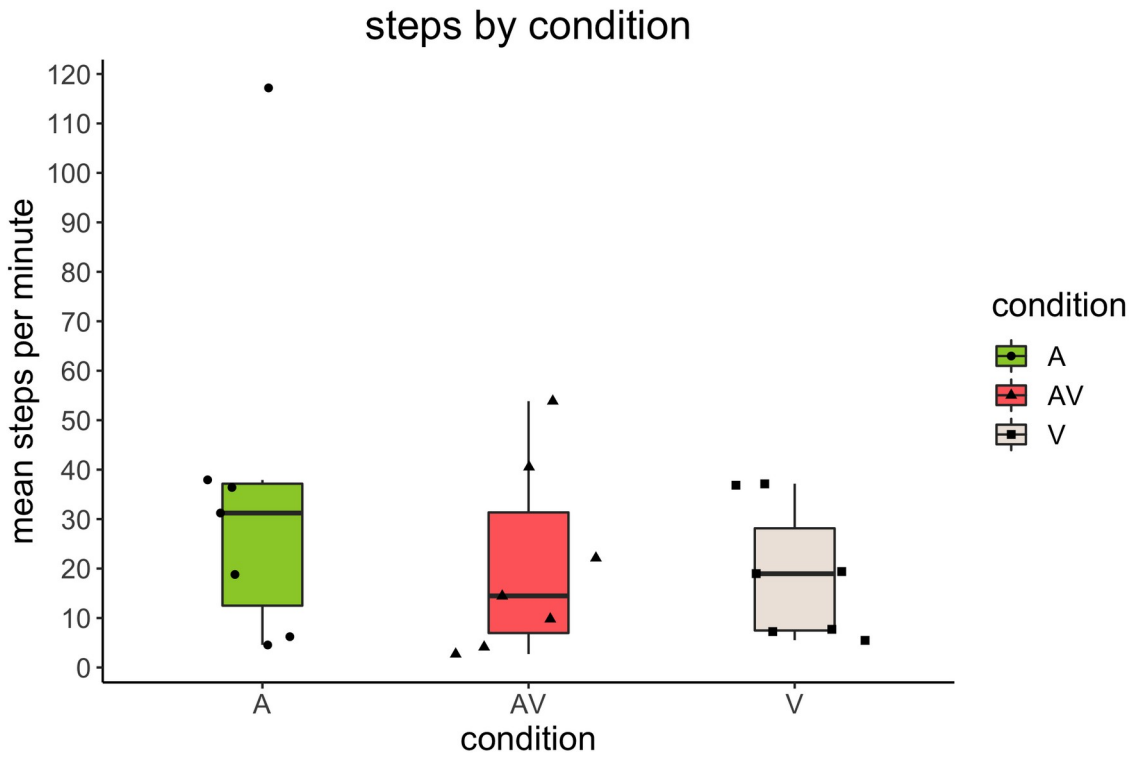

Figure 6: Boxplots and overlayed scatter plots for mean steps per minute in each condition. Each datapoint represents a different female.

Chasing was scored at least once in all conditions. While only two of seven females chased in the A condition, chasing was observed in four females in the V condition, and in five females in the AV condition. Proportion of time spent on chasing (**Figure 7**) spanned from 0 to 3.0125% (group mean = 0.85, SD = 1.45, N = 7) for the A condition, from 0 to 9.545% (group mean = 3.93, SD = 3.93, N = 7) for the V condition, and from 0 to 17.3378% (group mean = 5.43, SD = 8.15, N

= 7) for the AV condition. Kruskal-Wallis rank sum test indicated no difference between conditions (chi-squared = 2.6244, df = 2, p = 0.2692).

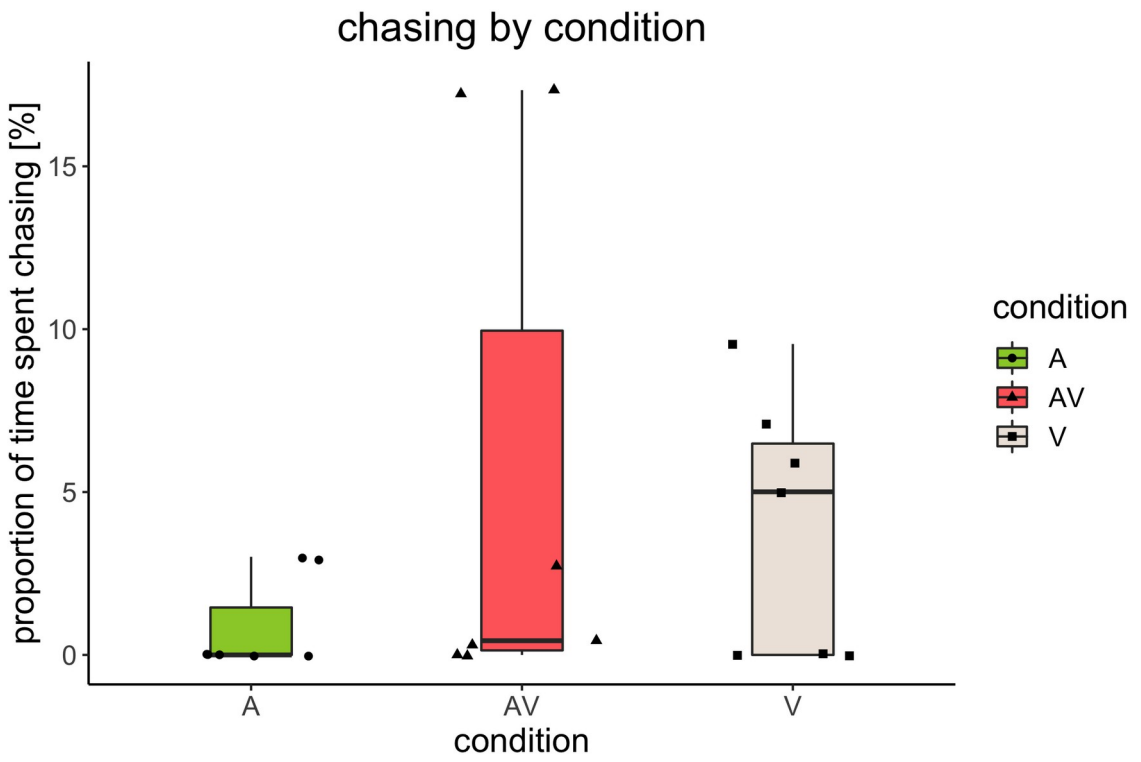

Figure 7: Boxplots and overlayed scatterplots for “chase” behaviour in each condition. Each datapoint represents a different female. Some females did not show the behaviour at all (shown as 0 values in the graph).

Preening was observed in all conditions. Similar to the comparison between stimulus and control intervals, preening was also treated as both a duration and an event for the comparison between conditions. Mean proportion of time spent on preening (**Figure 8a**) ranged from 0.2253 to 17.0911% (group mean = 6.59, SD = 6.24, N = 7) for the A condition, from 0.6565 to 19.4522% (group mean = 9.51, SD = 7.33, N = 7) for the V condition, and from 7.054 to 14.729% for the AV condition (group mean = 11.60, SD = 2.46, N = 7). Statistical testing showed no significant difference between conditions (Kruskal-Wallis rank sum test, chi-squared = 2.7681, df = 2, p = 0.2506).

Mean preening events per minute (**Figure 8b**) spanned from 0.2004 to 10.0603 (group mean = 4.66, SD = 3.62, N = 7) for the A condition, 0.4001 to 17.2537 (group mean = 9.51, SD = 5.92, N = 7) for the V condition, and from 3.767 to 21.884 (group mean = 13.71, SD = 6.57, N = 7) for the AV condition. Statistical testing indicated a significant difference between conditions (Kruskal-Wallis rank sum test, chi-squared = 6.5974, df = 2, p = 0.03693). Post hoc analysis using Dunn’s test revealed that the AV condition had significantly more

mean preening events per minute than the A condition, but that the V condition did not differ significantly from either the A or the AV condition (AV vs. A: p = 0.0331; V vs. A: p = 0.3330; V vs. AV: p = 1; p-values were adjusted for multiple comparisons using the Bonferroni method).

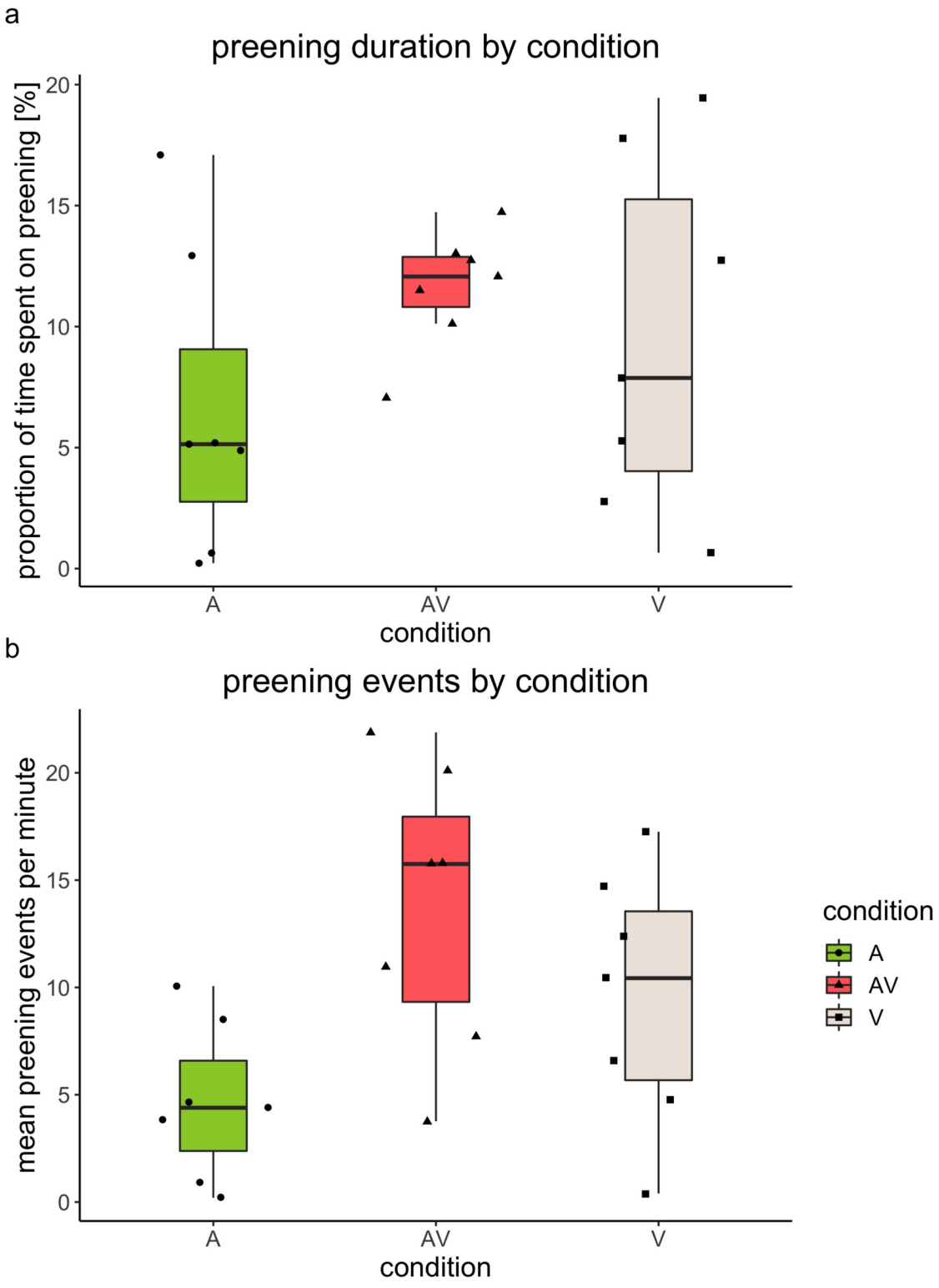

Figure 8: Boxplots and overlayed scatter plots for preening in each condition. Each datapoint represents a different female. (a) Mean proportion of time spent on preening, (b) mean preening events per minute.

Perch coos were observed in all conditions. Five of seven females perch cooed at least once in the A condition, three in the V condition, and only two in the AV condition. Mean perch coos per minute (**Figure 9a**) ranged from 0 to 3.7010 (group mean = 0.86, SD = 1.30, N = 7) in the A condition, from 0 to 0.018263 perch coos/minute (group mean = 0.0064, SD = 0.0085, N = 7) in the V condition, and from 0 to 0.07319 (group mean = 0.019, SD = 0.033, N = 7) in the AV condition. Statistical testing indicated no significant difference between conditions (Kruskal-Wallis rank sum test, chi-squared = 5.8528, df = 2, p = 0.05359).

Contrary to perch coos, nest coos were observed only in the V and AV conditions. For the V condition, nest coos were observed in four of seven females, and in the AV condition, two of seven females nest cooed at least once. Mean nest coos per minute (**Figure 9b**) ranged from 0 to 2.6674 (group mean = 0.52, SD = 0.97, N = 7) in the V condition, and 0 to 0.13197 (group mean = 0.023, SD = 0.049, N = 7) in the AV condition. Like perch coos, mean nest coos per minute showed no significant difference between conditions (Kruskal-Wallis rank sum test, chi-squared = 5.9825, df = 2, p = 0.05022).

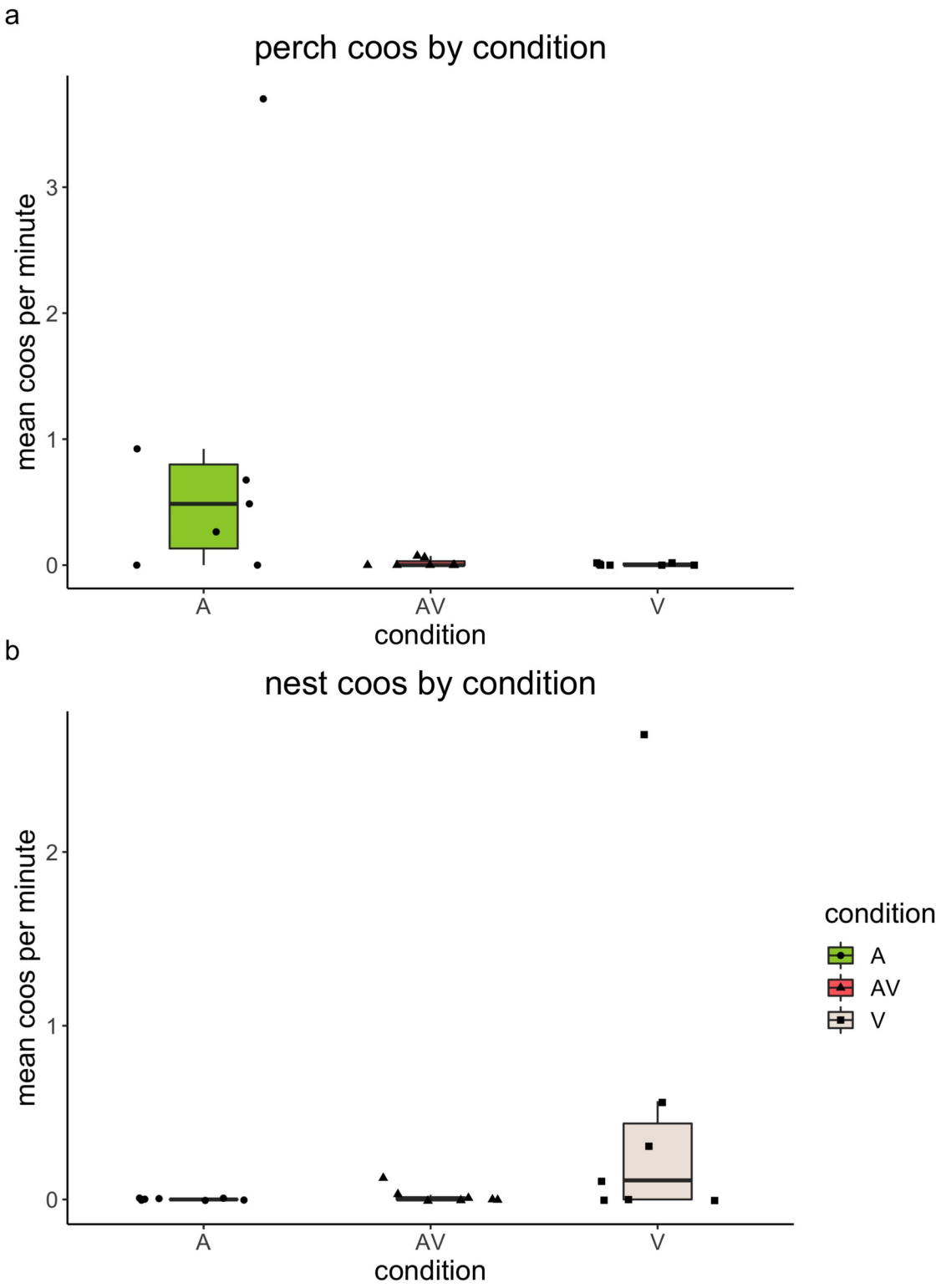

Figure 9: Scored vocalisations for each condition. Each datapoint represents a different female. Some females showed neither perch nor nest coos (shown as 0 values in the graphs). (a) Mean perch coos per minute, (b) mean nest coos per minute.

3.3 Female endocrinological response to playback

Endocrinological response to courtship playback was assessed using blood sampling to check plasma for oestradiol levels. One female was excluded from the analysis because no pre-test blood sample could be obtained. Blood oestradiol levels before testing spanned from 5.2 pg/ml (lowest detectable level: 11.82 pg/ml) to 27.78 pg/ml (group mean = 18.61, SD = 4.93, N = 20), and from 12.18 pg/ml to 35.70 pg/ml (group mean = 20.51, SD = 6.14, N = 20) after testing (**Figure 10a**). Absolute change in oestradiol concentration ranged from -6.00 to +19.56 (group mean = 1.89, SD = 6.70, N = 20), percentage change from -33% to +198.85% (group mean = 20.08, SD = 57.09, N = 20; **Figure 10b**). Negative numbers indicate a decrease in blood oestradiol concentration, positive numbers indicate an increase. Blood oestradiol levels did not differ significantly before and after testing (paired Wilcoxon signed rank test, V = 94.5, p = 0.7089). Percentage change of oestradiol levels also did not differ significantly between conditions (Kruskal-Wallis rank sum test, chi-squared = 0.88797, df = 2, p = 0.5005). **Figure 11** further illustrates blood oestradiol levels before and after testing.

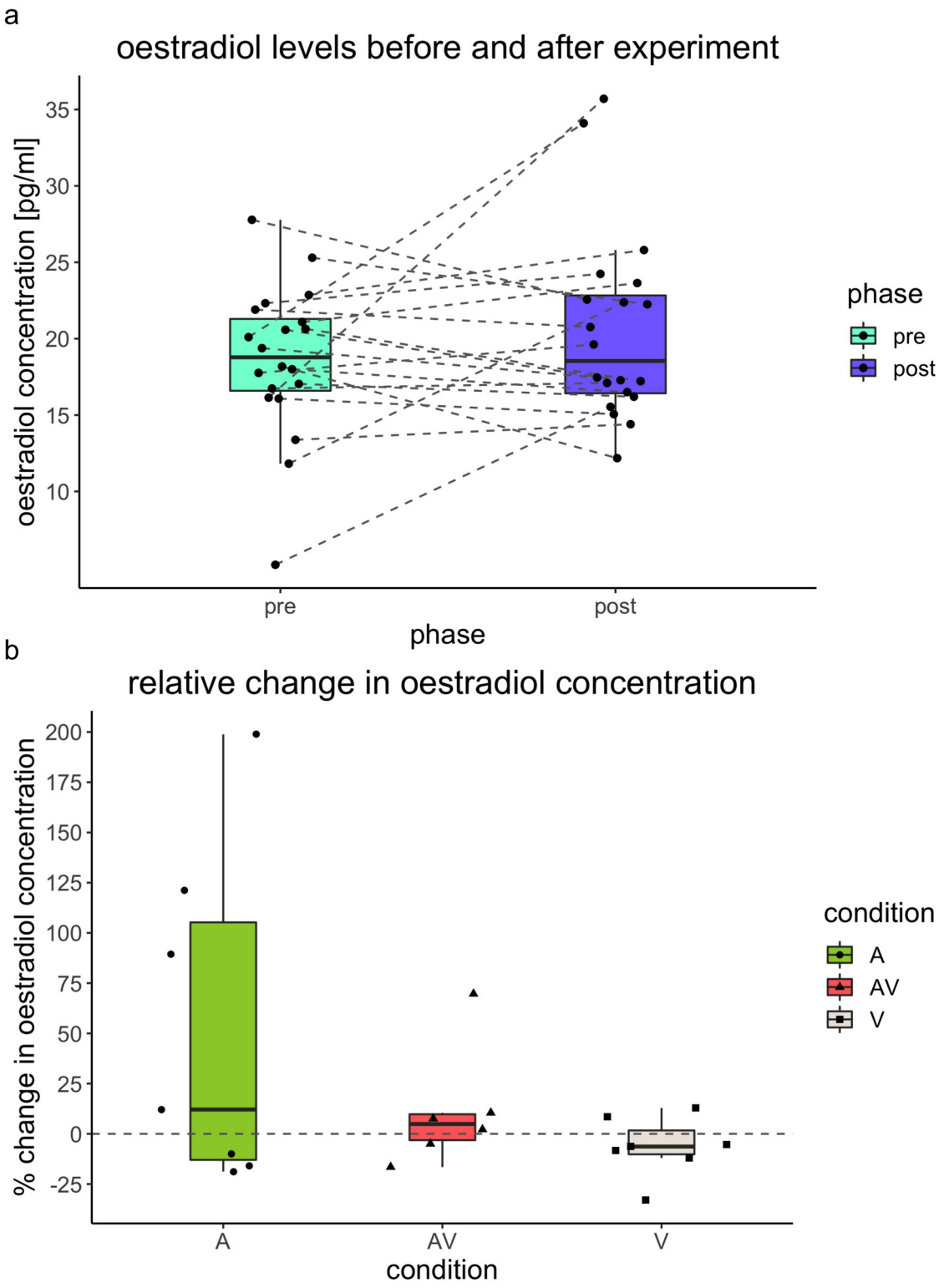

Figure 10: Boxplots and overlayed scatterplots of blood oestradiol levels. No pre-testing value could be obtained for one female. (a) Absolute oestradiol concentration before and after the experiment. Connected datapoints represent the same female. (b) Percentage change of oestradiol concentration. Each datapoint represents a different female.

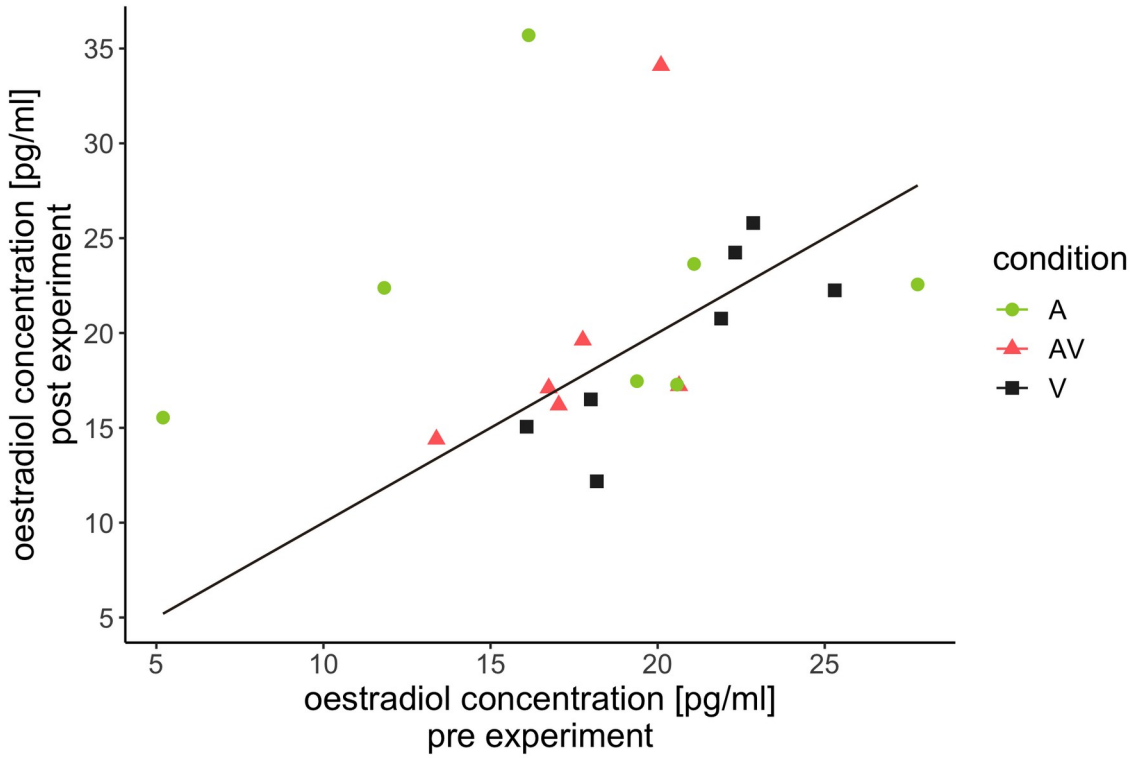

Figure 11: Oestradiol concentration pre and post experiment. Each datapoint represents a different female. Datapoints above the line indicate females with an increase in oestradiol concentration, datapoints below the line represent females that showed a decrease in oestradiol concentration.

4 Discussion

For this master's thesis, I analysed half the dataset for certain behaviours of interest, including steps, preening, and chasing, a sexually relevant behaviour for both male and female ring doves. Additionally, I analysed vocalisations for the full dataset and investigated hormonal response to playback through the use of blood oestradiol levels. This preliminary analysis was conducted to answer two main questions: (1) Can playback be used to elicit behavioural responses in ring doves, and (2) do the audio and video channels in ring dove courtship carry the same or complementary information? I can confirm the efficacy of playback in ring doves, similar to the findings of a previous study (Mitoyen et al., submitted), as chasing and preening rate were higher in stimulus than control intervals. Since chasing was observed in all conditions, the audio and visual channels likely carry redundant information in the ring dove courtship, but I am as of yet unable to confirm an enhancement effect of the multimodal courtship, as chasing did not differ significantly between conditions. Future analysis of the remaining behaviours of interest (see **Table A1**), and of the second half of the dataset will shed more light on this issue. Additionally to the behavioural response, physiological change was measured using blood oestradiol levels as a proxy for ovarian development, but there was no significant increase after repeated exposure to the courtship playback. Below I will discuss the behavioural and hormonal results in more detail, finishing with an outlook for further work with the complete dataset.

4.1 Behavioural response

4.1.1 Chasing

Chasing has long been described for males as a sexually relevant behaviour (Craig, 1909; Lovari & Hutchison, 1975), but was only recently reported in females by Mitoyen et al. (submitted) in a study featuring audio-visual playback of ring dove courtship. Like the aforementioned study, we also found significantly more chasing in the stimulus compared to the control intervals, indicating that it is a direct response to courtship and can therefore be considered a sign of sexual stimulation in the female.

Chasing was observed in all conditions, indicating that both audio and video channels can initiate the behaviour, and preliminary analysis did not yet indicate

a significant difference between conditions. Still, chasing was observed in more females in the multimodal audio-visual (five) and unimodal video (four) conditions compared to the unimodal audio (two) condition, indicating that while the audio channel alone is enough to initiate the behaviour, females may show it more only when they can see the male. It may be the case that we find a multimodal enhancement effect of chasing in future analysis.

4.1.2 Preening

The rate of preening events was overall higher during stimulus intervals compared to control intervals. This same effect has been shown in the ring dove in previous work from the dove lab (Mitoyen et al., 2021, submitted), and Cheng (1979) observed that in the initial stages of courtship, females will preen in response to the male's bow-call, indicating that preening might be sexually relevant in ring doves. Interestingly, the opposite has been reported in the closely related pigeon, where females preened less during courtship playback and more during playback of the empty setup (Partan et al., 2005).

In our study, this difference was not present when preening was tested as a duration, indicating that females preened in shorter intervals and looked up more often from preening during the courtship playback. This supports the idea that preening is a sexual signal in doves, and that females preen in shorter intervals during courtship because they are looking up to gauge the male's reaction. Short preening of the wings or area around the scapula is sometimes described as displacement preening, which is often followed by looking up at the male (Cheng, 1973). It may be that these shorter preening intervals we observed during courtship playback are equivalent to displacement preening. Further analysis of the head direction in conjunction with these preening events may clarify if the female looks up in the direction of the monitor between preening intervals and thus give insight into this observation.

Preening rates were also significantly higher in the audio-visual condition than they were in the audio condition, but this difference was again not seen when preening was treated as a duration. If the short preening intervals are indeed akin to displacement preening, females may show this behaviour only when they are able to see the male, indicating that the video channel carries additional information. Additionally, while the number of preening events in the unimodal

video condition did not differ significantly from the unimodal audio or multimodal audio-visual condition, raw data indicates that preening events are most numerous in the AV condition, and further analysis of the complete dataset may indeed reveal an enhancement effect for this behaviour.

4.1.3 Vocalisations

Neither type of vocalisation analysed for this master's thesis differed significantly between stimulus and control intervals, indicating that the immediate presence of the male is not necessary for the initiation of this behaviour. However, it may also be the case that females start vocalising only after the first stimulus interval, thus having been made aware of a conspecific's presence. They may then continue to vocalise to catch his attention, regardless of whether they can see and/or hear him in the precise moment. Analysis of when vocalisations first start should clarify the factors at work, but additional data may need to be collected, e.g. an experiment in which one group is exposed only to the control video to establish a baseline of behaviour in the experimental setup for this species.

Perch coos and nest coos showed no significant difference between conditions. However, p-values for both tests were close to significance level ($p = 0.05359$ for perch coos, and $p = 0.05022$ for nest coos), and the reason no significant effect was achieved might be because vocalisations were rarely observed, and because not all females vocalised. Further analysis of the data, for example with a zero-inflated model, might help to detect an effect. Mitoyen et al. (submitted) only featured audio-visual playback and recorded almost no vocalisations. Likewise, vocalisations were observed in more females in the unimodal conditions in our experiment than in our audio-visual condition, therefore vocalisations may just be rare in response to audio-visual playback in general.

Perch coos often occur when individuals are isolated (Davies, 1974), and in the closely related pigeon, Partan et al. (2005) observed more vocalisations from females in response to unimodal audio playback compared to unimodal video and multimodal playback. In our study, perch coos were observed in the most females in the unimodal audio condition (five of seven females), whereas fewer females showed the behaviour in the unimodal video (three) and multimodal audio-visual conditions (two), indicating that condition may have an effect on this beha-

viour. Perch cooing in the absence of the male might be an attempt by the female to indicate her location to him, especially since she is unable to physically move towards the vocalisations due to our experimental setup. Additional behaviours, such as proximity to the net separating the female from the monitor and speaker, or attempts to get through the net (see **Table A1** for "intrusion" behaviours) are beyond the scope of this thesis but will be scored as proxies for such a desire in later analysis.

Nest coos were recorded most frequently in the unimodal video condition. This type of vocalisation is usually observed when the female has been exposed to male courtship and nest soliciting for 2-3 days (Lehrman, 1964). It might be that females assigned to the V condition coincidentally showed the highest number of nest coos without the stimulus presented to them being solely responsible for it. An analysis investigating the relationship between baseline oestradiol levels and the incidence of nest coos should give further insight into this observation. If females in the unimodal video condition had the highest baseline levels for oestradiol, this would indicate an advanced physiological state unrelated to the kind of stimulus shown in the playback. However, it may also be that females in the unimodal video condition cooed more because they were deprived of the male's vocalisations, and since vocalisation plays a significant role in oviduct development (Cheng, 1986), they may be trying to balance out the male's silence with more vocalisations of their own. Previous studies (e.g. Friedman, 1977; Lambe & Erickson, 1973) have mostly focused and how the absence of the male's image influences female reproductive development, and further studies are needed to investigate how the absence of male vocalisation affects female behaviour including vocalisations as well as reproductive development.

Furthermore, nest coos were only observed in the two conditions that featured video playback, but not in the unimodal audio condition, indicating that the audio channel alone may not be enough to initiate the behaviour. This finding is in line with previous studies concerned with oviduct development: Lambe & Erickson (1973) observed that only females who saw the male's shadow laid eggs, and that oviduct development was non-existent in females that only heard the male's vocalisations. Similarly, Friedman (1977) observed that oviduct development was weakest in females that could only hear the male, more prominent in females that could hear the male and see their own reflection, and strongest in

females that could hear and see the male courting them. Since the vocalisations of the coo-bow, the nest coo, and the perch coo are all similar if not identical (Davies, 1974; Miller & Miller, 1958), the female may need the visual channel to differentiate between call types. Additionally, she may need to see the male to be sure that he is courting her and not someone else before initiating her own nest cooing, which in turn sets off oviduct development (Cheng, 1986). A future study investigating how female behavioural and endocrinological response differ between audio playback of nest coos, perch coos, and the coo-bow display may clarify whether or not females can tell these vocalisations apart through the audio channel alone, or if the video channel is essential to elicit the nest soliciting response in the female.

Another interesting aspect to consider is that females might show an increase in nest coos over experimental sessions, indicating increasing stimulation through the male's courtship display. However, I did not investigate changes over days because the full dataset was not available for all behaviours of interest. Further analysis is necessary and will be conducted once all recorded material has been scored, hopefully giving insight into this phenomenon.

Interestingly, Partan et al. (2005) found that female pigeons who were shown visual or audio-visual playback vocalised less during stimulus than control intervals, but females who only heard the male's calls vocalised more during stimulus intervals. It might be that the aforementioned study was able to find a significant effect of playback and interval type on vocalisation behaviour because the females in this study vocalised more than the females in our study. However, in addition to recording vocalisations in the setup, we also recorded vocalisations for 30 min before and after testing, providing us with additional data to investigate this issue for further effects in future analyses.

4.2 Hormone data

Ring doves undergo a number of behavioural, physiological, and anatomical changes during reproduction (described in Lehrman, 1964). In hopes of capturing these physiological changes, we measured blood oestradiol levels before and after the experiment, but were unable to show a statistically significant effect of the playback on blood oestradiol levels. Previous studies from the dove lab have shown that it is possible to elicit a rise in blood oestradiol levels through the use

of playback (Mitoyen et al., submitted). Our study followed the same protocol, i.e. females were exposed to courtship for 15 minutes every day for seven consecutive days, and, prior to the start of testing, females were isolation for 5-6 days in order to return them to a baseline hormonal state. It is therefore unlikely that courtship exposure in the playback was too short to elicit a significant change in blood oestradiol. It might be that females were already in a physiologically heightened state before the start of the experiment. This would also explain why we recorded five females laying eggs shortly before the start of the experiment, i.e. in the isolation cages. Egg laying is a behaviour that is usually only observed once the female has been paired with a male for a number of days, and represents the culmination of the ring dove courtship ritual (Lehrman, 1964). While it is unusual that ring doves reach a sexually stimulated state in the absence of males, egg laying and reproductive behaviours have been observed in the dove lab's own all-female colonies, and it stands to reason that the same happened with the females used in this experiment. However, we removed these eggs as soon as we discovered them and pushed the blood sampling back three days, which should have put the females back into a base hormonal state (Cuthbert, 1945). Future analysis of the faecal samples that were collected daily during the experiment will hopefully give us more insight into this issue.

4.3 Significance and Outlook

Multimodal signalling is often studied using cue isolation experiments. A novelty of this study was that rather than isolating the cues, we occluded them using alternate familiar stimuli. This was done in an effort to give females a plausible reason for not being able to see or hear the male, as past cue isolation studies could be criticised for not being entirely ecologically valid. In nature, a sensory channel may be occluded through some kind of obstacle, but single components of a multicomponent or multimodal signal generally do not occur on their own without reason. Although preliminary analysis did not yet reveal significant effects of condition on sexually relevant behaviours, future analysis of this dataset may be able to reveal an enhancement effect, meaning that behavioural response is stronger for multimodal compared to unimodal signals (Partan & Marler, 1999, 2005). Such effects have been reported in other animals, e.g. the wolf spider

Schizocosa ocreata (Uetz et al., 2009), and a close relative of the ring dove, the pigeon *Columba livia* (Partan et al., 2005).

Overall, even though visual playback in birds has its issues (see Fleishman & Endler, 2000; Rosenthal, 2019), the preliminary analysis indicates that females were likely to have perceived the male recordings as a live conspecific, as was found in previous work for this species (Mitoyen et al., submitted), and in the closely related pigeon (Partan et al., 2005). This indicates that the method is useful to study behavioural response in ring doves and can be used in future studies to further investigate ring dove behaviour not only in the context of courtship, but also in other behavioural contexts.

4.4 Conclusion

Preliminary analysis of half the data obtained in this experiment indicates that playback can be successfully used to elicit behavioural responses from female ring doves in response to courtship, as was found by Mitoyen et al. (submitted). Furthermore, we uncovered some promising trends of condition on female behavioural response, which may show significant effects once all available recordings are scored and analysed. Future analysis will also include some behaviours that were not analysed as part of this master's thesis (see **Table A1**), such as quivering, which has been reported by (Mitoyen et al., 2021, submitted) as a sexually relevant behaviour in doves, thus opening the possibility of more effects of condition. Finally, further insight into the physiological state of the females might be provided by the faecal samples that were taken in addition to the blood sampling.

References

Baptista, L. F., Trail, P. W., & Horblit, H. M. (1997). Columbidae. In J. del Hoyo, A. Elliott, & J. Sargatal (Eds.), Handbook of the birds of the world (Vol. 4, pp. 60–231). Lynx Editions.

Beason, R. C. (2004). What Can Birds Hear? Proceedings of the Vertebrate Pest Conference, 21(21), 92–96.

Bird, C. D., & Emery, N. J. (2008). Using video playback to investigate the social preferences of rooks, Corvus frugilegus. Animal Behaviour, 76(3), 679–687. https://doi.org/10.1016/j.anbehav.2008.04.014

Brainard, D. H. (1997). The Psychophysics Toolbox. Spatial Vision, 10, 433–436.

Candolin, U. (2003). The use of multiple cues in mate choice. In Biological Reviews (Vol. 78, Issue 4, pp. 575–595). https://doi.org/10.1017/S1464793103006158

Cheng, M.-F. (1973). Effect of ovariectomy on the reproductive behavior of female ring doves (Streptopelia risoria). Journal of Comparative and Physiological Psychology, 83(2), 221–233.

Cheng, M.-F. (1979). Progress and prospects in ring dove research: a personal view. Advances in the Study of Behavior, 9, 97–129.

Cheng, M.-F. (1986). Female cooing promotes ovarian development in ring doves. Physiology & Behavior, 37(2), 371–374.

Cheng, M.-F. (1992). For whom does the female dove coo? A case for the role of vocal self-stimulation. Animal Behaviour, 43(6), 1035–1044.

Cheng, M.-F. (2003). Vocal self-stimulation: from the ring dove story to emotion-based vocal communication. Advances in the Study of Behavior, 33, 309–354.

Cheng, M.-F. (2008). The role of vocal self-stimulation in female responses to males: Implications for state-reading. Hormones and Behavior, 53(1), 1–10. https://doi.org/10.1016/j.yhbeh.2007.08.007

Cheng, M.-F., & Silver, R. (1975). Estrogen-progesterone regulation of nest-building and incubation behavior in ovariectomized ring doves (Streptopelia risoria). Journal of Comparative and Physiological Psychology, 88(1), 256–263.

Craig, W. (1909). The Expressions of Emotion in the Pigeons: I. The Blond Ring-Dove (Turtur risorius). The Journal of Comparative Neurology and Psychology, XIX(1), 29–82.

Cuthbert, N. L. (1945). The ovarian cycle of the ring dove (Streptopelia risoria). Journal of Morphology, 77(3), 351–377.

Davies, S. J. J. F. (1974). Studies of the three coo-calls of the male Barbary dove. Emu, 74(1), 18–26.

Fleishman, L. J., & Endler, J. A. (2000). Some comments on visual perception and the use of video playback in animal behavior studies. Acta Ethologica, 3(1), 15–27.

Fleishman, L. J., Mcclintock, W. J., D'eath, R. B., Brainard, D. H., & Endler, J. A. (1998). Colour perception and the use of video playback experiments in animal behaviour. Animal Behaviour, 56(4), 1035–1040.

Friedman, M. B. (1977). Interactions Between Visual and Vocal Courtship Stimuli in the Neuroendocrine Response of Female Doves. Journal of Comparative and Physiological Psychology, 91(6), 1408–1416.

Fusani, L., Barske, J., Day, L. D., Fuxjager, M. J., & Schlinger, B. A. (2014). Physiological control of elaborate male courtship: Female choice for neuromuscular systems. Neuroscience and Biobehavioral Reviews, 46, 534–546. https://doi.org/10.1016/j.neubiorev.2014.07.017

Galoch, Z., & Bischof, H. J. (2007). Behavioural responses to video playbacks by zebra finch males. Behavioural Processes, 74(1), 21–26. https://doi.org/10.1016/j.beproc.2006.09.002

Girard, M. B., Kasumovic, M. M., & Elias, D. O. (2011). Multi-modal courtship in the peacock spider, Maratus volans (O.P.-Cambridge, 1874). PLoS ONE, 6(9). https://doi.org/10.1371/journal.pone.0025390

Higham, J. P., & Hebets, E. A. (2013). An introduction to multimodal communication. Behavioral Ecology and Sociobiology, 67(9), 1381–1388. https://doi.org/10.1007/s00265-013-1590-x

Johnstone, R. A. (1995). Honest Advertisement of Multiple Qualities using Multiple Signals. Journal of Theoretical Biology, 177(1), 87–94.

Johnstone, R. A. (1996). Multiple displays in animal communication: "backup signals" and "multiple messages." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 351(1337), 329–338.

Kleiner, M., Brainard, D., Pelli, D., Ingling, A., Murray, R., Broussard, C., & Cornelissen, F. (2007). What's new in Psychtoolbox-3? Perception, 36, 1–16. http://www.psychtoolbox.org

Korenbrot, C. C., Schomberg, D. W., & Erickson, C. J. (1974). Radioimmunoassay of Plasma Estradiol During the Breeding Cycle of Ring Doves (Streptopelia risoria). Endocrinology, 94(4), 1126–1132.

Lambe, D. R., & Erickson, C. J. (1973). Ovarian activity of female ring doves (Streptopelia risoria) exposed to marginal stimuli from males. Physiological Psychology, 1(3), 281–283.

Lehrman, D. S. (1964). The Reproductive Behavior of Ring Doves. Scientific American, 211(5), 48–55.

Liley, N. R. (1976). The role of estrogen and progesterone in the regulation of reproductive behaviour in female ring doves (Streptopelia risoria) under long vs. short photoperiods. Canadian Journal of Zoology, 54(9), 1409–1422.

Lovari, S., & Hutchison, J. B. (1975). Behavioural Transitions in the Reproductive Cycle of Barbary Doves (Streptopelia risoria L.). Behaviour, 53(1–2), 126–149.

Mangiafico, S. (2020). rcompanion: Functions to Support Extension Education Program Evaluation. https://cran.r-project.org/package=rcompanion

Miller, W. J., & Miller, L. S. (1958). Synopsis of behaviour traits of the ring neck dove. Animal Behaviour, 6(1–2), 3–8.

Mitoyen, C., Quigley, C., Boehly, T., & Fusani, L. (2021). Female behaviour is differentially associated with specific components of multimodal courtship in ring doves. Animal Behaviour, 173, 21–39. https://doi.org/10.1016/j.anbehav.2020.12.014

Mitoyen, C., Quigley, C., Canoine, V., Colombo, S., Wölfl, S., & Fusani, L. (2020) Audio-visual synchronization of multimodal courtship signals affects female sexual response in the ring dove. [Manuscript submitted for publication]. Department of Behavioural and Cognitive Biology, University of Vienna.

Mitoyen, C., Quigley, C., & Fusani, L. (2019). Evolution and function of multimodal courtship displays. Ethology, 125(8), 503–515. https://doi.org/10.1111/eth.12882

Møller, A. P., & Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology, 32(3), 167–176.

Ogle, D., Wheeler, P., & Dinno, A. (2021). FSA: Simple Fisheries Stock Assessment Methods. https://github.com/droglenc/FSA

Partan, S. R., & Marler, P. (1999). Communication goes multimodal. Science, 283(5406), 1272–1273.

Partan, S. R., & Marler, P. (2005). Issues in the Classification of Multimodal Communication Signals. The American Naturalist, 166(2), 231–245.

Partan, S. R., Yelda, S., Price, V., & Shimizu, T. (2005). Female pigeons, Columba livia, respond to multisensory audio/video playbacks of male courtship behaviour. Animal Behaviour, 70(4), 957–966. https://doi.org/10.1016/j.anbehav.2005.03.002

Pelli, D. G. (1997). The VideoToolbox software for visual psychophysics: transforming numbers into movies. Spatial Vision, 10(4), 437–442.

R Core Team. (2020). R: A Language and Environment for Statistical Computing. https://www.r-project.org/

Rosenthal, G. G. (2019). The Use of Playbacks in Behavioral Experiments. In Encyclopedia of Animal Behavior (pp. 529–534). Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.20782-6

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. Animal Behaviour, 58(5), 921–931.

Scheffer, S. J., Uetz, G. W., & Stratton, G. E. (1996). Sexual selection, male morphology, and the efficacy of courtship signalling in two wolf spiders (Araneae: Lycosidae). Behavioral Ecology and Sociobiology, 38(1), 17–23.

Smith, C. L., & Evans, C. S. (2008). Multimodal signaling in fowl, Gallus gallus. Journal of Experimental Biology, 211(13), 2052–2057. https://doi.org/10.1242/jeb.017194

Starnberger, I., Preininger, D., & Hödl, W. (2014). From uni- to multimodality: Towards an integrative view on anuran communication. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 200(9), 777–787. https://doi.org/10.1007/s00359-014-0923-1

Uetz, G. W., & Roberts, J. A. (2002). Multisensory Cues and Multimodal Communication in Spiders: Insights from Video/Audio Playback Studies. Brain, Behavior and Evolution, 59(4), 222–230.

Uetz, G. W., Roberts, J. A., & Taylor, P. W. (2009). Multimodal communication and mate choice in wolf spiders: female response to multimodal versus unimodal signals. Animal Behaviour, 78(2), 299–305. https://doi.org/10.1016/j.anbehav.2009.04.023

Ware, E., Saunders, D. R., & Troje, N. F. (2015). The influence of motion quality on responses towards video playback stimuli. Biology Open, 4(7), 803–811. https://doi.org/10.1242/bio.011270

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wickham, H. (2020). tidyr: Tidy Messy Data. https://CRAN.R-project.org/package=tidyr

Wickham, H., François, R., Henry, L., & Müller, K. (2020). dplyr: A Grammar of Data Manipulation. https://CRAN.R-project.org/package=dplyr

Appendix

Ethogram of additional behaviours

Table A1: Ethogram of additional behaviours. These behaviours were part of the scoring sheet, but not included in the analysis for this master's thesis.

category	behaviour	definition
body movements	sit	The dove remains stationary with both legs on the ground and settles down so that her stomach and/or part of her chest are touching the floor. She may also tuck one or both wings under one or both feet, and she may be tilted sideways, lying on her wing instead of her stomach.
	fly	The dove flies, i.e. moves using her wings; both feet must leave the ground.
	climb	The dove grips the net with one or both feet and stays suspended in the same place, hanging from the net and not touching the floor. One or both wings may be extended. She may also flap her wings, but she must stay in contact with the net.
	jump	The dove jumps up into the air by pushing one or both feet off the floor, but does not fly or extend her wings. Both feet must leave the ground simultaneously. Not scored when associated with "startle" behaviour. Often occurs during chasing.
vocalisations	laugh	The dove makes a sound similar to a human laugh.
	low	The dove makes a low sound, similar to humming. May differ in length.
	other	Any vocalisation that does not stem from the playback and is not described by the other vocalisation behaviours.
intrusions	monitor intrusion	The dove attempts to put her head and/or neck through the net facing the monitor by pushing against it. Not scored when the dove appears to just brush the net accidentally with the beak.
	camera intrusion	The dove attempts to put her head and/or neck through the net facing the camera by pushing against the net. Not scored when the dove appears to just brush the net accidentally with the beak.
quivering	wing quiver	The dove rapidly shakes her closed wings within a small range of motion, similar to a vibration movement. The behaviour can be very clear as well as subtle. Can be observed in a motionless, as well as in a walking dove. Also scored during vocalisation. If the tail also quivers, the behaviour is scored as a tail quiver.
	tail quiver	The dove shakes her tail (and sometimes her wings) within a small range of motion, similar to a vibration movement. The behaviour can be very clear as well as subtle. Can be observed in a motionless, as well as in a walking dove. Also scored during vocalisation.

category	behaviour	definition
interest	sex-crouch	The dove lowers herself down, and raises her shoulders, thereby slightly extending her wings. Her tail is slightly higher than her head and chest, but turned downwards. Her chest and/or abdomen may touch the floor and her eyes may be closed or half-closed. She may also flap her wings slightly.
	nest coo position	The dove holds her tail high; her head may be lowered so that her tail and head are in one line, but she may also move her head up and around. Both her feet are on the ground, but she may stand only on the tips of her feet. Her wings may twitch (at a lower frequency than the quiver), but she does not extend them like she does in the “sex crouch” behaviour. Her neck may inflate and deflate.
attention	eyes closed	The dove closes her eyes for longer than it would take to blink. Scoring starts and stops when the lid moves past the half-way point on her eye.
	eyes not visible	Scored when the dove is stationary (i.e. sitting or standing) and her head is turned in a way so that neither of her eyes are visible. Not scored when the dove is preening, nor when she is in the process of turning her head and her eyes are not visible only for a moment.
other	startle	The dove appears startled. This may include flinching, twitching her wings, or jumping away.
	mouth open	The dove opens her mouth widely, similar to a yawn.
	up-down tail	The dove tilts her tail in a downward motion so that it touches the floor and lifts it up again immediately. Not scored if associated with restoring balance. Distinct from accidentally brushing the tail on the floor while walking (no deliberate tilting).

Zusammenfassung

Vögel verwenden bei der Balz oft eine Kombination von visuellen und Audiosignalen, aber es ist unklar, ob die Signalkomponenten für einzigartige oder redundante Information kodieren. Außerdem kann sich die Reaktion auf das gesamte Signal von der kombinierten Reaktion auf einzelne Signalkomponenten unterscheiden. In dieser Studie haben wir die multimodale Signalübertragung bei der Ringeltaubenbalz untersucht, indem wir 21 Weibchen für 7 Tage hochwertige Aufnahmen von balzenden Männchen gezeigt haben. Die Weibchen wurden eingeteilt in (1) eine multimodal audiovisuelle, (2) eine unimodale Video- und (3) eine unimodale Audiogruppe. In den unimodalen Gruppen waren der Audio- (Efeu) oder Videokanal (Staubsaugergeräusch) durch alternative Stimuli verdeckt. Für diese Masterarbeit wurden bestimmte Verhaltensweisen in der Hälfte der verfügbaren Aufnahmen kodiert und die Häufigkeit zwischen den Stimulus- (Balzplayback) und Kontrollintervallen (Playback des leeres Setup) sowie Gruppen verglichen. „Chasing“, ein Verhalten, das mit sexueller Stimulation assoziiert ist, und Federputzen waren in den Stimulusintervallen signifikant höher. Die Federputzrate war in der unimodalen Audiogruppe signifikant niedriger, aber es gab keinen signifikanten Unterschied zwischen Gruppen für die anderen Verhaltensweisen, was darauf hindeutet, dass der visuelle und der Audiokanal redundante Information beinhalten. Weitere Analyse des gesamten Datensatzes könnte jedoch weitere Effekte aufdecken. Die physiologische Reaktion der Weibchen wurde anhand des Östradiolspiegels im Blut gemessen, aber es war weder der Anstieg signifikant, noch war die prozentuale Veränderung zwischen den Gruppen unterschiedlich. Insgesamt scheint das Playback erfolgreich dabei, Balz der Männchen an die Weibchen zu vermitteln. Zukünftige Analysen dieses Datensatzes werden die multimodale Signalübertragung bei Ringeltauben weiter untersuchen.