Final Technical Report

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

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1.0 EXECUTIVE SUMMARY

The objective for this study was to measure the auditory and visual physiology of Golden and Bald Eagles in order to use eagle sensory capabilities to inform the design of potential deterrent stimuli that could be used to reduce eagle/turbine collisions with wind turbines. The rationale for this approach is that sensory systems of any organism will limit the capability of that organism to perceive aspects of the world around it. Moreover, species can differ dramatically in their sensory physiology so it is important to examine these characteristics in the species of concern, rather than relying on data from similar birds. Our project consisted of two main phases. The first phase was the acquisition and analysis of visual and auditory information from Golden and Bald Eagles in rehabilitation centers. This was performed in order to identify light and sound stimuli tuned to sensitive areas in the eagle's sensory systems. The second phase of the project was to present these stimuli to both species of eagles in a behavioral experiment to identify which stimuli would be the most effective in changing the behaviors of the eagles.

Results of phase one indicated that the visual system of the Golden Eagle strongly absorbs ultraviolet light, making it unlikely the Golden Eagle (and most likely the Bald Eagle) will detect ultraviolet light signals. The Golden and Bald Eagles have differences in the sensitivities of their visual systems to light within the eye, but mathematical models indicate that both species are able to detect indigo/blue and orange/red light produced by LEDs (light emitting diodes) very well. We also found that both species of eagles have a blind spot above their head. This blind spot is particularly large in Golden Eagles due to a pronounced brow ridge above the eyes. This blind spot will result in the inability of a Golden Eagle to see something in front of it when its head is pointed down during flight – as might happen while hunting (i.e. searching the ground for prey). As such, the blind spot may increase the chance of collision with wind turbines if the eagle is actively hunting. This problem is less pronounced in Bald Eagles because their blind spot is smaller than in the Golden Eagles and their foraging strategy is different.

Results of phase one also indicated that the auditory systems of the Golden and Bald Eagles respond differently to a variety of sounds (static tones, static chords (i.e. stacked tones), and sounds with dynamic changes through amplitude modulation or frequency modulation). Both species' auditory systems responded strongly to tones across a wide range of frequencies (0.5 – 5kHz), however the Bald Eagles' auditory system was much better at processing complex sounds with dynamic rapid changes in amplitude or frequency modulation than the Golden Eagle. All of these sounds were then played with two types of noise in the background (white or pink). White noise more closely resembles the sound of wind and pink noise more closely resembles wind turbines or other sources of anthropogenic noise. Most sounds were more strongly masked by pink noise than by white noise, but several sounds (especially sounds with rapid modulation changes) showed little or no masking, indicating these were good candidate signals. However, even though rapidly changing sounds are less subject to noise masking, they are also less strongly processed by the Golden Eagle auditory system. This tradeoff does not exist in Bald Eagles because individuals of this species are very good at processing rapidly changing sounds. Given that Golden Eagle populations are at greater risk than Bald Eagle

populations, we suggest that the most efficient alerting sound stimuli used in deterrent systems should be complex sounds that do not change very rapidly.

We identified candidate light (indigo/blue and orange/red LED lights) and sound (sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack) stimuli that both eagle species sensory systems are highly sensitive to. Results of phase two, in which we presented these stimuli to eagles in a behavioral experiment, indicated that eagles behaviorally responded to all the stimuli presented, but at varying degrees. The Golden Eagles, especially, elicited higher rates of visual exploratory behavior with a flashing blue light stimulus and all sound stimuli. We therefore recommend the use of these stimuli in field-testing of light/sound eagle deterrent systems on wind turbines. The eagles showed lower rates of behavior over the course of an experiment, suggesting either that they habituated to our stimuli or were initially stressed by the setup of the behavioral tests. These results underscore the need to test for habituation effects. Nonetheless, habitation to the stimuli in these field tests would likely be reduced by the use of random presentations of the four sounds and if possible random presentation of the candidate lights.

2.0 ACCOMPLISHMENTS

Project Goals and Objectives

Golden Eagles (*Aquila chrysaetos*) and Bald Eagles (*Haliaeetus leucocephalus*) are known to be involved in collisions with wind turbines. Our primary goal of this project was to characterize the auditory and visual physiology of Golden and Bald Eagles with the ultimate intention of providing this information to engineers for the development of novel deterrent systems. These systems can be used to reduce the risk of eagle collisions with turbines by alerting eagles to the presence of wind turbines so that they may change course, or deterring them from approaching turbines while operational. We proposed a two-phase research project. First, we needed to know basic eagle sensory physiology to develop effective multimodal (auditory and visual) stimuli tuned to eagle's specific sensory capabilities. Second, we complemented the first phase of the project by running behavioral tests of prototype stimuli in wildlife rehabilitation centers to determine the responses of eagles. These two stages were necessary to ensure that the stimuli characteristics were not only conspicuous to the Golden and Bald Eagle's sensory systems, but also generated the intended response when flying near wind turbines (e.g. enhanced detection).

Technical Approach and Accomplishments Summary

The technical approach and accomplishment summaries for each research activity group are as follows. For further details, please see the Project Activities and Attachments sections.

1) IACUC Protocol and Rehab Centers (Tasks 1.1, 1.2, 6.0)

Using a Purdue University approved IACUC protocol we began contacting wildlife rehabilitation centers across the United States to conduct the measurements necessary for the project. These rehabilitation centers provided us access to Golden and Bald Eagle study subjects. We developed a set of protocols/anticipated processes that rehabilitation centers could review as a way to start a conversation about our research and provide an overview of our experimental procedures for the eagles. We established relationships with seven rehabilitation centers: Blue Mountain Wildlife (Pendleton, Oregon), California Raptor Center (Davis, California), Indiana Raptor Center (Nashville, Indiana), Liberty Wildlife Rehab Foundation (Phoenix, Arizona), Montana Raptor Conservation Center (Bozeman, Montana), Soarin' Hawk Raptor Rehabilitation (Fort Wayne, Indiana), and Wildlife Center of Virginia (Waynesboro, Virginia).

2) Building, Acquiring, and Testing Equipment (Tasks 2.0, 3.0, 4.0, 5.1, 5.2, 5.3)

To be able to measure the sensory systems of the Golden and Bald Eagles requires specialized equipment. Although we already had the equipment at Purdue University, these systems were not portable and the eagle subjects could not be brought to Purdue. We either built, borrowed, or purchased the following three necessary systems to measure the auditory and visual systems of the eagles: an anechoic chamber and auditory equipment to measure hearing; a portable microspectrophotometer to measure the sensitivity of photoreceptors in the retina; and a portable microscopy system to acquire images of the retinal tissue. We tested these three systems with various species of birds that were readily available to us before we began work on the eagles. These tests were successful and allowed us to measure the properties of the auditory and visual systems of the Golden and Bald Eagles.

3) Gathering Physiological Data (Tasks 7.1, 7.2)

We visited the seven rehabilitation centers, that we established relationships with, in over twenty-five data collection trips from January 2018 to September 2019. We acquired measurements on both species from individuals that were provided by the centers, both male and female, regardless of the age of the eagle. We successfully acquired hearing measurements using auditory evoked potentials from two Golden Eagles and six Bald Eagles. We successfully acquired visual system measurements (visual field configurations, transmittance of ocular media, density of photoreceptors, peak sensitivity of visual pigments, and absorbance of oil droplets) from fifteen Golden Eagles and twelve Bald Eagles. Our sample sizes did not allow us to analyze age or sex differences in the measurements.

4) Processing and Analyzing Physiological Data (Tasks 8.1, 8.2)

Data collected from the auditory and visual systems of the Golden and Bald Eagles needed to be processed and analyzed at Purdue University before conspicuous prototype stimuli could be developed. Processing and analysis of auditory data required development of custom code to determine the auditory responses relative to different background conditions, as well as statistical analysis to resolve patterns for the different stimuli, noise treatments, and eagle species. We found that Bald and Golden Eagles process a variety of tones (0.5 – 5kHz) very similarly. However, single tones are strongly masked by background noise in both species. When multiple tones are played simultaneously in a tone stack, the response to the individual components are quite variable depending on the composition of the stack, but noise has less of a masking effect. The response to other types of dynamically changing sounds (i.e. those with amplitude or frequency modulation) are even less subject to noise. However, the species differed substantially in their ability to process dynamic sounds: Bald Eagles processed rapid sounds as well as any species that we have tested in the past, but Golden Eagles were quite poor at processing rapidly changing sounds. This results in a tradeoff for Golden Eagles: noise effects are mitigated using dynamic sounds, but Golden Eagles are poor at processing sounds that are strongly dynamic. We therefore used sounds that were well processed by both species but ones that were dynamic enough to reduce the impact of noise on sound

processing. The following sounds were chosen as candidate signals to test in the behavioral experiments, mistuned harmonic stack, 0.4 kHz amplitude modulation (AM) with 2 kHz carrier, downward sweep (6-1 kHz in 50ms), and 70 Hz sinusoidal frequency modulation (FM) with 400 Hz depth (based on 2 kHz tone).

We processed and analyzed the visual system data collected for both eagle species. We found that both species have similar visual field configurations, a binocular field in front of their heads, and large blind spots above and behind the head of the eagles, which restricts their field of view. Both species of eagles have a violet-sensitive visual system, coupled with restricted transmittance of light through the eye, resulting in the inability to see ultra-violet light well. The results of these visual system measurements indicated, through visual modeling, that indigo/blue (410-470 nm) and orange/red (580-655 nm) LED light stimuli are maximally conspicuous to both species against a variety of visual backgrounds (blue sky, bare ground, dormant grass, green grass, and white paint [a proxy for wind turbine color]). These light stimuli were chosen as candidate signals to test in the behavioral experiments.

5) Developing and Conducting Behavioral Assays and Analysis (Tasks 9.0, 10.0, 11.0)

We conducted behavioral experiments using a stimuli playback system we developed. This system consisted of a canister with a Bluetooth controlled speaker and LED light panel that presented sound and light stimuli to the eagles. We thoroughly tested this system with falconry birds and Golden and Bald Eagles at Blue Mountain Wildlife (Oregon). A finalized behavioral experiment protocol was developed from these tests and successfully deployed at the Indiana Raptor Center and Blue Mountain Wildlife from November 2018 to September 2019. We also developed an ethogram (list of behaviors these species would exhibit) to code behavior in the videos collected during the tests and behavioral experiments that we conducted. The behaviors coded within these videos were used for the behavioral analysis, with particular attention paid to visual exploratory behavior (i.e. head movements) and stress behaviors (i.e. move, wing flap, etc.).

Behavioral analysis of the eagles revealed that Golden Eagles exhibited more visual exploratory and stress behaviors than the Bald Eagles. We found some differences in head movement rates in response to stimuli types (i.e. light, sound, light + sound). The blue flashing light stimulus (460 nm LED) was particularly alerting to the eagles, especially the Golden Eagles, indicating it is a good candidate light stimulus to deploy on wind turbine deterrent systems. All sound stimuli (sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack) were equally alerting to the eagles, indicating they are all good candidate sound stimuli to deploy on wind turbine deterrent systems.

We did notice an effect of time on the rates of behaviors exhibited during the behavioral experiments (rates decreased with time) suggesting there might be some habituation to the stimuli playback system, or perhaps more generally to the experimental environment. However, further behavioral experiments would need to be performed to confirm that the

birds habituate to the stimuli themselves. A random presentation of stimuli is likely to be best in alerting eagles to the wind turbine. A useful presentation scheme, especially for sound stimuli, would be to rotate randomly through stimuli with each broadcast for approximately one minute or so.

Conclusions

Golden and Bald eagles differ in their auditory and visual sensory physiology, making effective stimuli for use in deterrent systems difficult to identify using traditional trial and error methods. Our approach in this report allowed us to determine the physiological capabilities of the eagles to create candidate stimuli to deploy at wind turbine facilities. By measuring the sensitivities of their sensory physiology, we were able to identify areas of sensory overlap between the two species. We identified candidate light (indigo/blue [410-470 nm] and orange/red [580-655 nm] LED lights, both flashing and steady) and sound (sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack) stimuli that both eagle species' sensory systems are highly sensitive to. Results of behavioral experiments using all light stimuli we tested revealed that the eagles, especially Golden Eagles, elicited higher rates of visual exploratory behavior with a flashing blue light stimulus. All sound stimuli we tested elicited equally high rates of visual exploratory behavior from the eagles, and the Golden Eagles had higher rates on average than Bald Eagles. We had too few light + sound tests to make any definitive statements, but the data we have suggest that flashing blue light coupled with mistuned harmonics is worth testing with additional experiments. We therefore recommend the use of blue flashing lights, sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack in field-testing of light/sound eagle deterrent systems on wind turbines.

3.0 PROJECT ACTIVITIES

Project schedule for each task and milestone detailed in tables below.

		Year 1				Year 2				No-Cost Extension		
	Project Quarters →	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
		2017		2018				2019				2020
	Calendar Quarters →	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
YEAR 1												
Task 1	**Activities Approval**											
1.1	IACUC Approval											
	M1.1 Approval of IACUC protocol by Purdue University											
1.2	Develop MoUs with Rehabilitation Centers											
	M1.2 Signed MoUs with rehab centers, and state and federal regulatory agencies											
Task 2	**Devising and Building a Portable Anechoic Chamber**											
	M2.0 The portable anechoic chamber to fit Golden and Bald Eagles will be built and ready to be transported											
Task 3	**Acquiring a Portable Microspectrophotometer**											
	M3.0 The portable microspectrophotometer will be built and ready to be transported											
Task 4	**Acquiring a Portable Microscopy System**											
	M4.0 The portable microscopy system will be built and ready to be transported											
Task 5	**Testing Portable Equipment**											
5.1	Testing Anechoic Chamber and Auditory Measurements											
	M5.1 Successful test of portable anechoic chamber and auditory measurements											
5.2	Testing Portable Microspectrophotometer											
	M5.2 Successful test of the portable microspectrophotometer											
5.3	Testing the Portable Microscopy System											
	M5.3 Successful test of the portable microscopy											
Task 6	**Checking with Rehabilitation Centers**											
Task 7	**Gathering the Sensory Data**											
7.1	Gathering the Auditory Data											
	M7.1 Obtain auditory data from 5-7 individuals of each species											
7.2	Gathering the Visual Data											
	M7.2 Obtain visual data from 3-6 individuals from each species											

Black Segments = planned duration of each task, blue segments = actual duration of each task and subtask, purple triangle = point at which milestone was reached.

		Year 1				Year 2				No-Cost Extension		
	Project Quarters →	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
		2017		**2018**				**2019**				**2020**
	Calendar Quarters →	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
YEAR 2												
Task 8	**Processing and Analyzing the Sensory Data**											
8.1	Analyzing the Auditory Data											
	M8.1 Processing and analyzing of the auditory data on campus											
8.2	Analyzing the Visual Data											
	M8.2 Characterize the visual systems of Golden and Bald Eagles											
Task 9	**Developing prototype visual and acoustic stimuli for**											
9.1	Stimuli Playback System Prototype Development											
	M9.1 Develop an LED light + speaker system for playback of combinations of visual and acoustic stimuli											
9.2	Behavioral Assay Development and Testing											
	M9.2 Develop a behavioral assay to test the responses of eagles to these visual and acoustic stimuli so that it can be conducted at different rehabilitation centers in the US											
9.3	Behavioral Assay Experimental Protocol											
	M9.3 Deliver experimental protocols to funding agencies											
Task 10	**Measuring Behavioral Responses of Eagles**											
	M10.0 Gather behavioral responses from at least 6 golden eagles and 12 bald eagles											
Task 11	**Behavioral Experiment Analysis**											
	M11.0 Estimate whether Golden and Bald Eagles are attracted/repelled by combinations of visual and acoustic stimuli with different degrees of sensory conspicuousness											
Task 12	**Write Final Report**											
	M12.0 Final report and manuscript(s) for publication											

Black Segments = planned duration of each task, blue segments = actual duration of each task and subtask, purple triangle = point at which milestone was reached.

Summaries of project activities are as follows for each task, grouped according to relevant content. For complete details on each task milestone, see Attachments sections A through Q.

3.1 IACUC Protocol and Rehab Centers (Tasks 1.1, 1.2, 6.0)

Activities and Accomplishments

The main components of this project are assessing the sensory systems and behavioral responses of Golden and Bald Eagles. As such, we needed access to these species. These task objectives were to obtain IACUC approval (Task 1.1) for all physiological data collection methods and locate eagles at rehabilitation centers that we could measure and test (Task 1.2, 6.0). Using a collective 30^{+} years of experience on the techniques, we would perform with the eagles, we were quickly able to develop and write the initial IACUC protocol. This protocol was approved by Purdue University's Animal Care and Use Committee in June 2017 before the start of the project, and amended to include all project personnel with final approval on September 21, 2017.

We then needed to locate rehabilitation centers across the country that would allow us to work with their Golden and Bald Eagles. After an exhaustive and lengthy search by project personnel and funding agencies we were able to establish collaborations with seven rehabilitation facilities; Blue Mountain Wildlife (Pendleton, Oregon), Montana Raptor Conservation Center (Bozeman, Montana), Wildlife Center of Virginia (Waynesboro, Virginia), Soarin' Hawk Raptor Rehabilitation (Fort Wayne, Indiana), Liberty Wildlife Rehab Foundation (Phoenix, Arizona), Indiana Raptor Center (Nashville, Indiana), and the California Raptor Center (Davis, California). Throughout the course of the project, we built and maintained these relationships, constantly checking for the availability of both species of eagles. Thanks to the willingness of these raptor centers to participate, we were able to obtain the eagles necessary to complete the project. All necessary federal permitting was provided by Todd Katzner, with the Purdue team added as sub-permittees on his permits. In states where Todd Katzner was permitted, we were included as sub-permittees; in the other states we applied for these state permits ourselves. All necessary permits for the entire duration of the project are available upon request.

Departure from Plan and Why

At the start of the project, we initially felt that it was essential to develop signed Memorandums of Understanding between Purdue University and the various rehab centers we would be working with. We quickly realized that establishing relationships with the rehab centers is a long process that would continually evolve as the project went on and our needs changed. As a result, we never drafted formal agreements to outline our collaboration with a rehabilitation center.

Key Conclusions

All work on this project was approved by an IACUC committee and took place at rehabilitation centers around the country that we established relationships with. The outcome of these efforts allowed us to conduct the necessary testing and experiments for the completion of this project.

For additional details on these tasks, please review Attachments A and B.

3.2 Building, Acquiring, and Testing Equipment (Tasks 2.0, 3.0, 4.0, 5.1, 5.2, 5.3)

Activities and Accomplishments

The Golden and Bald Eagles that were used for this project were located across the United States. It was impossible to bring the eagles back to Purdue University, so we needed to make our lab equipment entirely portable. These task objectives were to either build, borrow, or purchase the necessary equipment and test its functionality under controlled conditions.

The first main task objective was to build a portable anechoic chamber and auditory measurement system (Task 2.0) and then test its functionality with non-eagle species at Purdue University (Task 5.1). We modeled the portable anechoic chamber and auditory measurement system after a stationary chamber located at Purdue. Building the chamber involved designing a system that would be both sturdy and anechoic, but that could be completely assembled and disassembled at multiple locations by project personnel. Once completed, we used two House Sparrows (*Passer domesticus*) to verify that the auditory measurement equipment functioned correctly and that the anechoic chamber had the proper sound dampening properties. We also successfully tested the anesthesia protocols we developed for this technique on two Canada Geese (*Branta canadensis*).

The second main task objective was to acquire (Task 3.0) and test (Task 5.2) a portable microspectrophotometer (MSP) to measure the sensitivity and absorbance properties of the Golden and Bald Eagle photoreceptors. We borrowed the portable MSP from a professor at Cornell University who custom-built the machine. We successfully tested its functionality on the Blue-black Grassquit (*Volatinia jacarina*) during a non-DOE/Avangrid funded collaboration with a researcher in Brazil.

The third main task objective was to purchase (Task 4.0) and test (Task 5.2) a portable microscopy system that would take images of the distribution and density of photoreceptors (specifically oil droplets) across the retinae of the eagles. We purchased a used demonstration Axio Scope.A1 microscope from Carl Zeiss Microscopy, LLC equipped with an X-Cite

Fluorescent lamp from Excelitas Technologies Corp and Zeiss Zen Blue 2.3 software. After many delays from Zeiss concerning part replacements and deliveries, we were able to test the microscopy system on remnant Bald Eagle and fresh Red-tailed Hawk (*Buteo jamaicensis*) retinal tissues. Results of testing indicated that a different filter set, a critical component when imaging the oil droplets within the retina, was needed for this system. After purchasing this new filter set, we successfully imaged the retinal tissue of birds with this new portable microscopy system.

Departure from Plan and Why

We had initially proposed to use European Starlings (*Sturnus vulgaris*) for all tests of the portable equipment, as they were readily available in our lab and are a non-releasable invasive species we must euthanize to comply with USDA regulations. This species also had the advantage of both hearing and vision measurements published in the literature. However, in an effort to reduce the number of animals used for this project, we salvaged tissues from other species that were available or being directly used for the project on other techniques. It was not useful to test the anesthesia methods we would employ on the eagles on a small songbird; therefore, we chose to test it on Canada Geese. Significant delays with the portable microscopy system resulted in completion of tasks 4.0 and 5.3 on month 9 instead of month 2-3 as originally planned. No eagle retinal tissue could be imaged on the portable Zeiss microscopy system in the end. We ended up using a different non-portable Olympus system to acquire the images of the oil droplets on the retinae of eagles.

Key Conclusions

The auditory measurement system, microspectrophotometer, and microscopy system were acquired, made portable, and thoroughly tested for use on the Golden and Bald Eagles within this project.

For additional details on these tasks, please review Attachments C through H.

3.3 Gathering Physiological Data (Tasks 7.1, 7.2)

3.3.1 Gathering Auditory Data (Task 7.1)

Activities and Accomplishments

We travelled to two raptor rehabilitation centers to collect auditory evoked potential measurements on anesthetized, live Bald and Golden Eagles to complete subtask 7.1. Measurements on five Bald Eagles were made at the Wildlife Center of Virginia (Waynesboro, VA) and measurements on two Golden Eagles and one Bald Eagle were made at Liberty Wildlife

(Phoenix, AZ). The experiments tested the response of the eagle auditory system to a variety of different sounds (single tones, multi-tone stacks, amplitude modulations, and linear and sinusoidal frequency modulations) in different background noise conditions (Figure 1). We tested the different sounds with a silent background and then with pink (more low-frequency components; similar to wind turbine) and white noise (even frequency distribution; similar to wind) to determine how noise, which is relevant to field conditions near turbines, affected hearing.

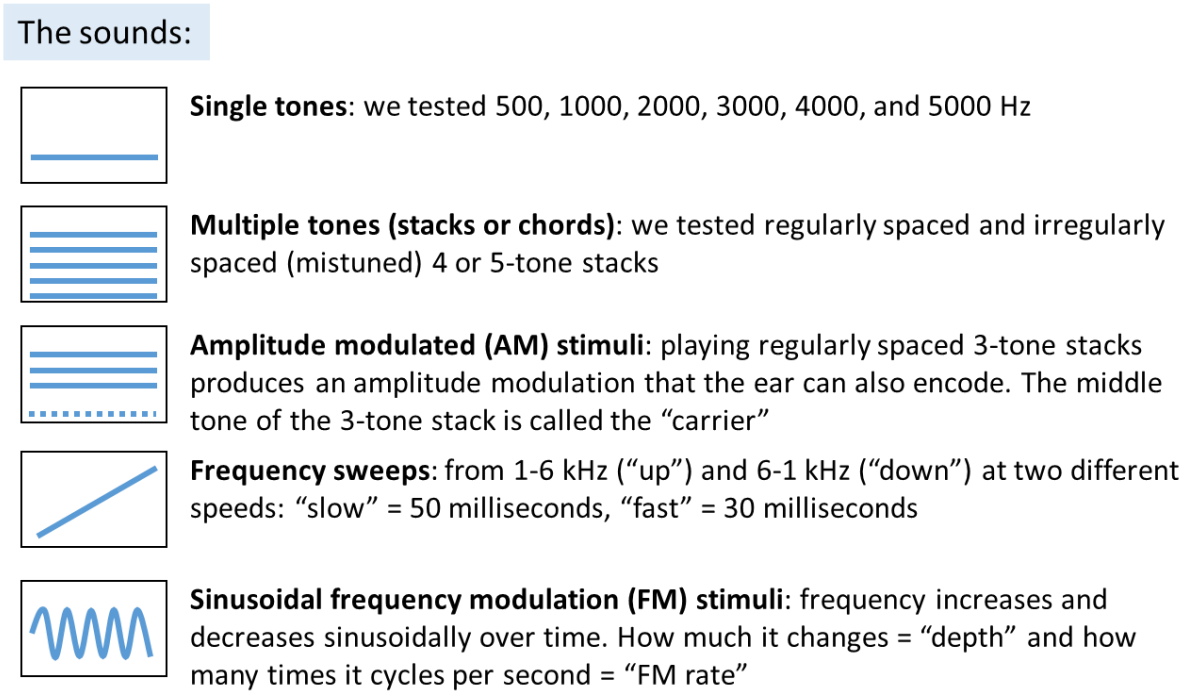

Figure 1. A summary figure of the types of sounds used as stimuli in the study. Each diagram illustrates the frequency over time of a representative stimulus in the group. For example, a single tone has a single frequency over time, and a frequency sweep has a linear change in frequency over time.

Departure from Plan and Why

The primary departure from the initial plan was that anesthesia was conducted by rehab center veterinarians instead of Dr. Jeff Ko from the Purdue Veterinary School. Dr. Ko had limited availability during our peak data collection periods because of his other commitments at Purdue University, so he helped to consult with Dr. Ernesto Dominguez at the Wildlife Center of Virginia and we collaborated on developing the anesthesia protocol and running experiments. Veterinarians and veterinary technicians performed all tasks necessary to anesthetize the eagles and to monitor eagle condition during the experiments. The research team from Purdue left judgement calls about eagle condition to the discretion of the veterinarians. The proposed equipment, stimuli, and experimental workflow were suitable for the purposes of the study.

Another departure from the proposed plan was caused by difficulty in getting access to Golden Eagles. It took a lot more time than expected to find a collaborator for the hearing work, partially because of the need for veterinary staff that would agree to use injectable anesthetics. After finding a collaborator, one of the candidate eagles took several months of rehab beyond what the veterinarians predicted, pushing data collection by several additional months. In the end, we completed auditory data collection in Month 19, instead of Month 12 as proposed.

Key Conclusions

We were successfully able to measure the auditory evoked potentials of six Bald Eagles and two Golden Eagles using the full range of stimulus sounds and noise backgrounds proposed. All eagles recovered after the two-hour experiment (See section 3.4.1 for results) of auditory measurements.

For additional details on this task, please review Attachment I.

3.3.2 Gathering Visual Data (Task 7.2)

Activities and Accomplishments

We traveled to five raptor rehabilitation centers across the United States multiple times to collect visual system measurements on Golden and Bald Eagles to complete subtask 7.2. We measured the converged and diverged visual fields of seven Golden Eagles; two at the California Raptor Center (David, California), two at Blue Mountain Wildlife (Pendleton, Oregon), and three at Liberty Wildlife (Phoenix, Arizona). We measured the converged and diverged visual fields of five Bald Eagles at the Wildlife Center of Virginia (Waynesboro, Virginia). We measured the spectral properties of the photoreceptors on five Golden Eagles; one fresh specimen from the Montana Raptor Conservation Center (Bozeman, Montana), three frozen specimens from Liberty Wildlife, and one frozen specimen from Blue Mountain Wildlife. We measured the spectral properties of the photoreceptors on five Bald Eagles; one fresh specimen from the Wildlife Center of Virginia, three frozen specimens from Blue Mountain Wildlife, and one frozen specimen from Liberty Wildlife.

We successfully measured the ocular media transmittance from three Golden Eagles, two at the Montana Raptor Conservation Center and one at Blue Mountain Wildlife. We did not have the opportunity to measure the ocular media of the Bald Eagle. Finally, we were able to image the retinae of two Golden Eagles at the Montana Raptor Conservation Center and two Bald Eagles at the Wildlife Center of Virginia.

Departure from Plan and Why

We departed from the planned visual data gathering period due to difficulties in finding fresh retinal tissue. This was especially true when attempting to measure fresh retinal tissue

using the microspectrophotometer and the microscopy system. Most rehabilitation facilities are legally and ethically obligated to immediately euthanize an eagle that is suffering. To collect most of the measurements for this project, we had to be present during euthanasia. This severely restricted the number of eagles that we had access to because of the rare circumstances we needed; a bird that needed to be euthanized, but not immediately. We were unable to attain the final Bald Eagle individual needed for the completion of the full set of visual system measurements.

Key Conclusions

We were able to acquire full measurements for the Golden Eagle and almost complete measurements for the Bald Eagle visual systems (missing ocular media measurements). Data gathered in this subtask were analyzed with results summarized in section 3.4.2.

For additional details on this task, please review Attachment J.

3.4 Processing and Analyzing Physiological Data (Tasks 8.1, 8.2)

3.4.1 Processing and Analyzing Auditory Data (Task 8.1)

Activities and Accomplishments

Processing and analysis of auditory data for subtask 8.1 required development of custom code to determine the auditory responses relative to different background conditions, as well as statistical analysis to resolve patterns for the different stimuli, noise treatments, and eagle species. Code was written in both SAS and PRAAT to process and analyze the auditory measurements of Bald and Golden Eagle auditory evoked potentials (AEPs; see Attachment I for details on experimental procedure and stimuli, and Attachment K for details on analysis). The analyses of Bald and Golden Eagle responses provided understanding of how well the eagles hear different single tones with and without background noise, multi-tone stacks with and without noise, amplitude modulations with and without noise, linear changes in frequency (sweeps) with and without noise, and sinusoidal frequency modulations at different rates with and without noise.

Bald and Golden Eagles process a variety of tones (0.5 – 5kHz) very similarly. However, single tones are strongly masked by background noise in both species. When multiple tones are played simultaneously in a tone stack, the responses to the individual components are quite variable depending on the composition of the stack. Both species are better at processing the tone stacks in noise than they are at processing single tones in noise. We also tested sounds that generated an amplitude modulation, and found that both species of eagles responded similarly to these, but there was large variation in the eagle auditory response to different amplitude

modulations. Overall, a 400 Hz amplitude modulation generated by 1.6, 2.0, and 2.4 kHz tones looked like the best sound for high stimulation of the eagle auditory system and high resistance to noise masking (Figure 2).

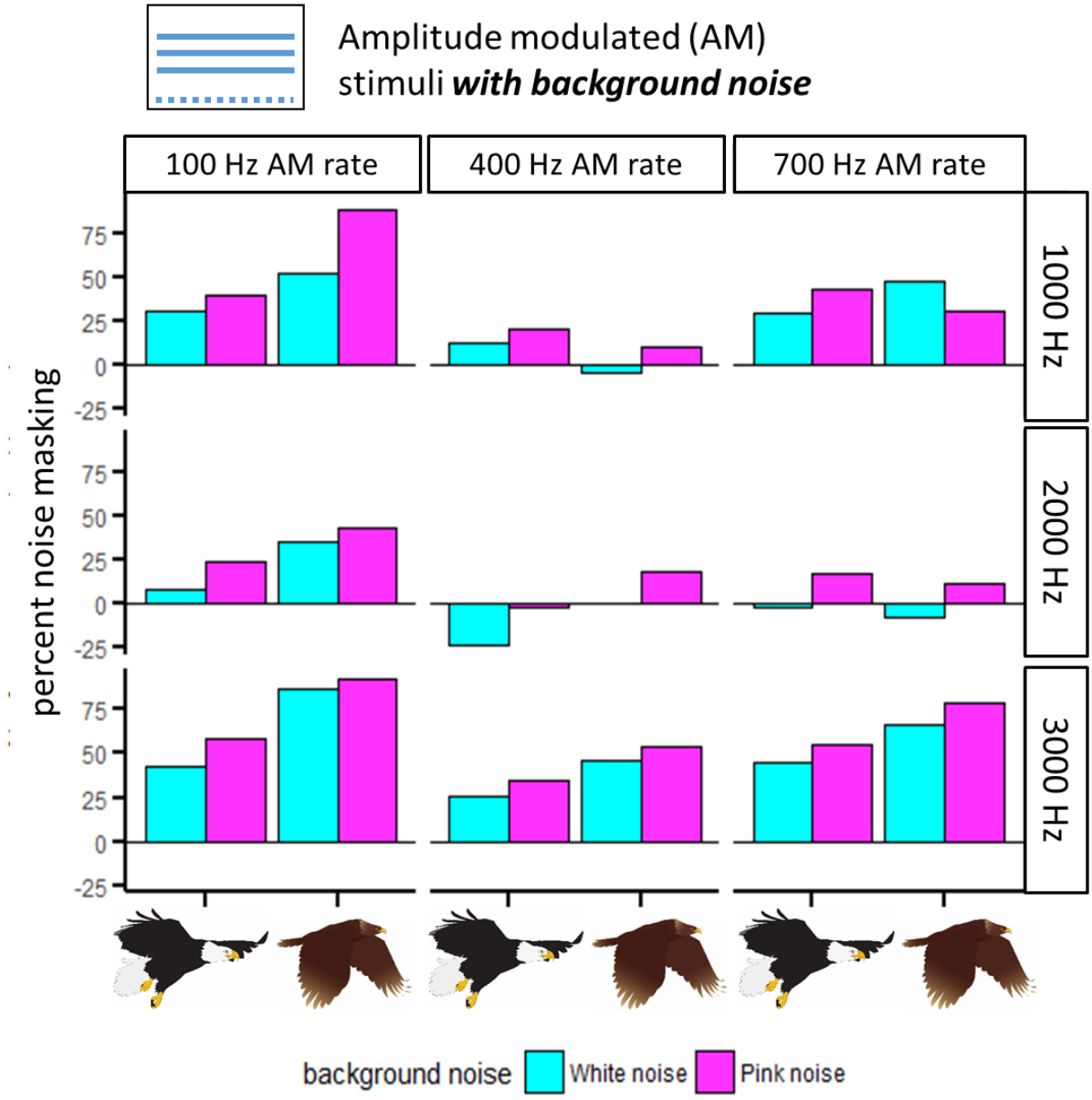

Figure 2. Most amplitude modulated (AM) stimuli are masked by background noise, and the responses of Golden and Bald Eagles are similar. The data above show the average effect of noise on the eagle's ability to hear all four components of the AM stimulus (carrier tone, high and low sideband tones, and the AM rate). The low effect of noise on several of the AM stimuli is promising, especially for 400 Hz AM with a 2000 Hz carrier, where phaselocking is strong and noise masking is low. These are good candidates for stimuli to use in the field.

In addition to sound stimuli with static frequency components, we also tested frequency modulations. Linear frequency sweeps from 1-6 kHz and 6-1 kHz were tested at two different speeds, and sinusoidal frequency modulations were tested at different modulation rates (70 and 110 Hz) and at different depths (±400Hz and ±700Hz). All sinusoidal modulations were centered around a 2000 Hz frequency. Our frequency modulations (linear and sinusoidal) varied in the rate of change of frequency, and our results show that Golden Eagles are worse than Bald Eagles at accurately processing the most rapidly changing sounds we used. However, rapid frequency modulations are resistant to noise masking in both species of eagles. The implication is that slower frequency modulations are good candidates for use in the field because they are not strongly masked by noise, and Golden Eagles should be able to hear them.

Departure from Plan and Why

Analysis and processing of auditory data proceeded as proposed with the exception of the timeline. We were significantly delayed by the lack of Golden Eagle experiments, which were not completed until Month 19. As a result, we were not able to compare the two eagle species until after Month 19, instead of starting the analysis in Month 13 as proposed.

Key Conclusions

We were able to analyze auditory evoked potential measurements for Golden and Bald Eagles to compare how well each species hears a variety of sounds. Additionally, we analyzed how hearing was affected for each sound with and without background noise. Our findings indicate that most static tone-based sounds are not good candidates for use as deterrents in the field because eagle auditory responses are strongly masked by noise for these sounds. In contrast, dynamic frequency sounds are resistant to noise with the caveat that Golden Eagles are worse than Bald Eagles at processing rapidly changing sounds. We recommend the use of the following sound stimuli for behavioral testing: mistuned harmonic stack, 0.4 kHz amplitude modulation with 2 kHz carrier, downward sweep (6-1 kHz in 50ms), and 70 Hz frequency modulation (FM) with 400 Hz depth (based on 2 kHz tone).

For additional details on this task, please review Attachment K.

3.4.2 Processing and Analyzing Visual Data (Task 8.2)

Activities and Accomplishments

After each set of visual system information was gathered, we began to process and analyze the data in subtask 8.2 to characterize the configuration of their visual fields, the type and position of their center(s) of acute vision, eye size, visual acuity, density of different types of photoreceptors, ocular media transmittance properties, peak sensitivity of visual pigments, and

absorbance of oil droplets. Golden and Bald Eagles each possess a binocular field in front of their heads, when eyes are converged, and a substantial blind area behind their heads. The Golden Eagle has a larger blind area above its head than the Bald Eagle, which, when looking down, will cause a larger blind spot in front of the flying Golden Eagle compared to the Bald Eagle (Figure 3). However, the Golden Eagle has larger eyes and higher peripheral visual acuity than the Bald Eagle, which should lead to better visual capabilities in terms of resolution of an image on the retina. Both species possess four centers of acute vision (foveae) in their visual fields (two in each eye), two projecting into the lateral visual field (subtended by one eye) and two projecting near the binocular field (subtended by both eyes) (Figure 3). These foveae will allow the eagles to resolve an image of wind turbines on the retina with high acuity.

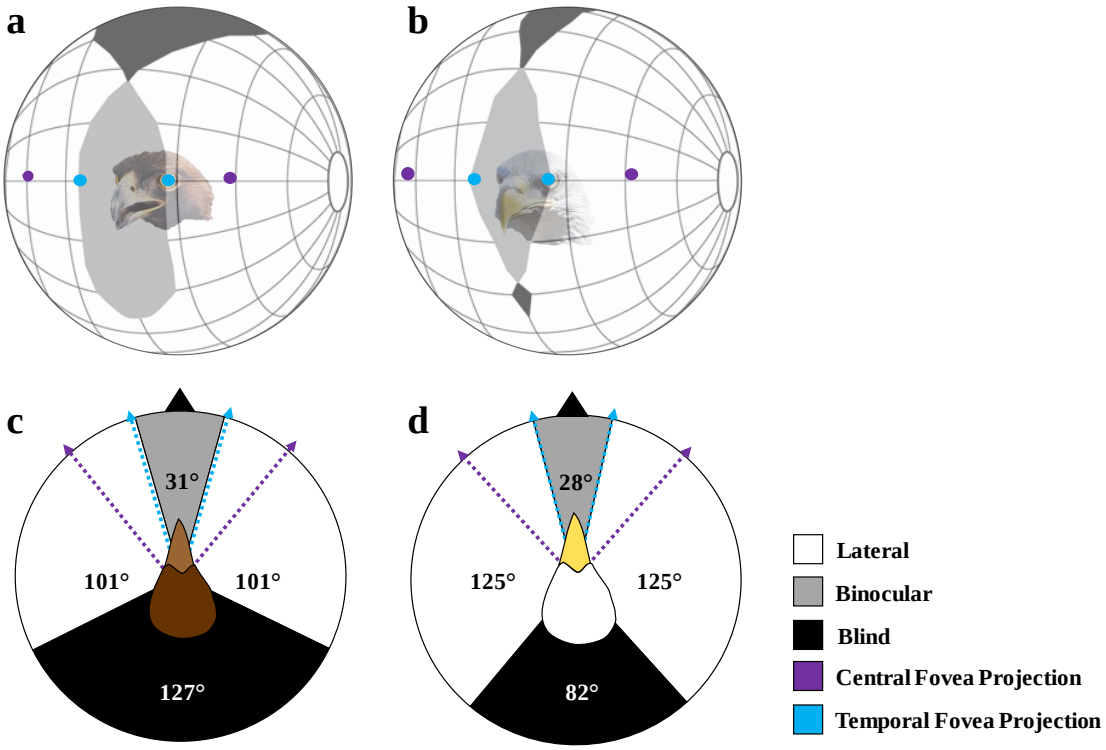

Figure 3. Visual field configurations of the Golden and Bald Eagle when eyes are converged. Spherical projections of the converged visual field around the head of the Golden Eagle (**a**) and the Bald Eagle (**b**). Black lines on the sphere are separated by 20° corresponding to the angular coordinate system used to collect the measurements. The center horizontal line is the 90° elevation used in the horizontal plane figures in panels (**c**) and (**d**). Colored dots represent the projection of the central (purple) and temporal (blue) foveae into the visual fields. Horizontal plane diagrams of the widths of the visual fields and blind area when eyes are converged in the Golden (**c**) and Bald (**d**) Eagles. Arrows represent the projection of the central (purple) and temporal (blue) foveae into the visual fields.

The Golden and Bald Eagles have similar sensitivities to different wavelengths of light, both possessing violet-sensitive visual systems (414 nm and 413 nm peak absorbance, respectively), confirmed through microspectrophotometry. This, coupled with Golden Eagle ocular media strongly filtering ultra-violet (UV) light below 383 nm, prevents the eagle from seeing UV-rich light signals. We were unable to measure the ocular media in the Bald Eagle, but predict it will be similar to other raptor species that have been measured in the literature and this study (ranging from 375-394 nm). We therefore cannot recommend the use of a UV light signal for the purposes of deterring or alerting these two eagle species from wind turbines.

Visual chromatic contrast modeling, using species-specific visual system information, revealed areas of high LED light contrast against various different visual backgrounds (blue sky, bare ground, dormant grass, green grass, and white paint [a proxy for wind turbine color]). For Golden Eagles, an indigo/blue (peak wavelength from 410-460 nm; 436-442 nm maximum conspicuousness) and orange/red (peak wavelength from 590-655 nm; 616-626 nm maximum conspicuousness) light stimuli are highly conspicuous against the various visual backgrounds. For Bald Eagles, an indigo/blue (peak wavelength from 420-470 nm; 446-448 nm maximum conspicuousness) and orange/red (peak wavelength from 580-650 nm; 606-612 nm maximum conspicuousness) light stimuli are highly conspicuous against the various visual backgrounds.

Departure from Plan and Why

We departed from the planned visual data analysis period due to difficulties in acquiring fresh retinal tissue. This was especially true when attempting to measure fresh retinal tissue using the microspectrophotometer (only measured on 1 Golden and 1 Bald Eagle) and the microscopy system (2 Golden Eagles, and 1 retina each from 2 Bald Eagles). We also analyzed data immediately after it was collected from individual eagles instead of waiting until all data were collected for both species, as originally planned.

Key Conclusions

Golden and Bald Eagles both have visual systems that allow them to view clearly visible light signals in their environment, but they are blind to objects above their head. This would be particularly problematic if the eagle is looking down while flying because they would be incapable of seeing anything in front of them under these circumstances. Moreover, this effect is stronger in Golden Eagles than in Bald Eagles. Light signals that should be maximally conspicuous/visible (as calculated via visual perceptual modeling) against a variety of backgrounds (blue sky, bare ground, green grass, dormant grass, and white of wind turbines) for both species of eagles are indigo/blue (436-448 nm) or orange/red (606-626 nm) LED lights.

For additional details on this task, please review Attachment L.

3.5 Developing and Conducting Behavioral Assays and Analysis (Tasks 9.0, 10.0, 11.0)

3.5.1 Developing Visual and Acoustic Stimuli for Behavioral Assays (Task 9.0)

Activities and Accomplishments

To be able to perform the behavioral experiments at rehab centers, we first needed to develop a portable and wireless stimulus playback system that could accommodate a variety of animal enclosures and environments (Milestone 9.1). We knew we needed the system to: 1) play both light and sound stimuli, 2) be portable, 3) be wirelessly operated and battery powered, 4) be waterproof as the system will be used outdoors, 5) easily adaptable to a variety of animal enclosures, and 6) to minimize interference by the experimenter during the tests. We decided to create a Bluetooth operated, battery powered LED light panel and speaker inside of a compact housing.

With the help of two Indiana falconry masters, we tested and refined the design of this system. The final design consisted of a PVC canister with a Bluetooth JBL speaker and an Arduino controlled Bluetooth LED light panel, operated using an Android phone. We took this stimulus playback system to Blue Mountain Wildlife in Pendleton, Oregon to test its efficacy on eagles in their care (Milestone 9.2). The test consisted of playing preliminary stimuli, chosen to be maximally conspicuous/visible to the eagle's sensory systems, at random intervals over a 45-minute period on two successive days to both Golden and Bald Eagles. The stimuli we chose at the time were the following: four candidate lights (steady or flashing) - 'ultraviolet' (385 nm), 'blue' (460 nm), 'red' (615 nm), white (broad spectrum), and four candidate sounds - mistuned tone stack, 0.4 kHz amplitude modulation with 2 kHz carrier, downward sweep (6-1 kHz in 50ms), and 70 Hz frequency modulation (FM) with 400 Hz depth (based on 2 kHz tone). The tests yielded over 300 minutes of video that were analyzed frame-by-frame using an eagle specific ethogram (list of behaviors).

Departure from Plan and Why

We began the development of this stimulus playback system with the chosen stimuli before all of the sensory system data had been collected for the Golden and Bald Eagles. The stimuli we chose were educated choices at the time based on the partial data we had, but would later be refined as we progressed with the data collection. We began this work because at the time it was uncertain if we would be able to collect the remaining data for the eagles and we needed to begin the behavioral component of this project.

Key Conclusions

The test of the stimuli playback system we developed was effective when used on raptors. This system was then ready to be deployed at various rehabilitation centers to measure the behavioral responses to various sound and light stimuli.

For additional details on these tasks, please review Attachments M and N.

3.5.2 Conducting Behavioral Assays (Task 9.0, 10.0)

Activities and Accomplishments

Using the results of the stimuli playback system test, we developed the finalized version of the experimental protocol we would employ at the rehabilitation centers (Milestone 9.3). We deployed this experimental protocol at two rehabilitation centers, Blue Mountain Wildlife (Pendleton, Oregon) and the Indiana Raptor Center (Nashville, Indiana). Using the ethogram developed in Milestone 9.2, we measured the behavioral responses of six Golden Eagles and six Bald Eagles to the playback of light and sound stimuli tuned to their auditory and visual systems (Subtask 10.0). Briefly, the behavioral experiment consisted of the deployment of two stimulus playback systems inside of the enclosure of the focal eagle(s), an acclimation period lasting at least 10 minutes, and then presentation of a series of audio and/or visual stimuli, in a random order, to the focal eagle(s). Eagle responses were recorded with video. The stimuli were presented with a variable rest period (2-5 minutes) between each stimulus to prevent the focal eagle(s) from anticipating the next signal. The cycle of stimulus and rest was repeated until all stimuli were played. The experiments were repeated on multiple days to ensure all 'light', 'sound', and 'light + sound' stimuli were tested on the same focal eagle.

Departure from Plan and Why

We were not able to measure as many Bald Eagles as we hoped because we were unable to test their behavior at the Wildlife Center of Virginia. This rehabilitation center had the most Bald Eagles in their care over the course of the project, but few were good candidates for the behavioral tests due to their underlying health conditions at the time.

Key Conclusions

We were successfully able to test and record the behavioral response of Golden and Bald Eagles to the stimuli that were maximally conspicuous to their sensory systems. For analysis of these results, please see section 3.5.3 below.

For additional details on these tasks, please review Attachments O and P.

3.5.3 Behavioral Assay Analysis (Task 11.0)

Activities and Accomplishments

To determine the effectiveness of the sound and light stimuli as deterrents or attractants to the Golden and Bald Eagles, we analyzed the videos from the behavioral experiment. Data collected from the video coding were processed in SAS v9.4 using repeated measures analysis of variance (PROC MIXED) for head movement rates and using repeated measures Poisson regression (PROC GLIMMIX) for stress vs. non-stress behaviors. Stress behaviors included flying, looking up and crouching, discharge, gaping, wing flapping, jumping or moving rapidly. All other behaviors were coded as non-stress behaviors. This list was put together by consultation with a number of raptor center personnel with a background in eagle behavior.

We focused the main analysis on head movement rates, as differences in movement rates in response to different stimulus types are a direct measure of the efficacy of each stimulus type to draw the attention of the eagle. The number of head movements during each stimulus presentation was extracted from the dataset and converted to rates by dividing the number of head movements by the duration of each separate stimulus presentation. We analyzed stress-related vs. non-stress related behaviors by counting the number of movements that were deemed stress related separately from movements that were deemed non-stress related during each stimulus presentation. These numbers were low (with many zeros) for the stress-related behaviors which is why counts were used instead of rates and Poisson regression was used instead of the more common analysis of variance models.

The statistical models included eagle species, stimulus modality (i.e., sound, light, light + sound), specific stimulus within a given modality (e.g. variance accounted for blue flashing, blue steady, red flashing, red steady, all within the light modality), time during the trial, the square of the time during the trial (note: this tests for non-linear changes in behavior during a trial). The model included all two-way interactions -- these interactions were deleted from the model in order of increasing F-value. The final model was taken as all main effects in addition to any significant two-way interactions. Head movement rates were square-root transformed to normalize residuals of the model.

We found that Golden Eagles exhibited a higher rate of visual exploratory behavior (head movements) and stress behaviors in response to the stimuli than Bald Eagles during the behavioral experiments. Although there were no statistically significant differences between the average response to all light, all sound, and all light + sound stimuli, we were able to identify specific stimuli that increased the visual exploratory behavior of the eagles. Please keep in mind that all stimuli used in the experiments elicited visual exploratory behavior, but some were more effective than others. The blue flashing stimulus (460 nm LED) was particularly alerting to the eagles, especially to the Golden Eagles. This stimulus falls within the 410-470 nm visual “sweet spot” for both eagle species against a variety of backgrounds, so we can recommend indigo/blue

light, with a peak wavelength in this range, as a stimulus for wind turbine eagle deterrent systems.

All sound stimuli (sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack) were equally alerting to the eagles, indicating they are all good candidate sounds to deploy on wind turbine deterrent systems. This is true of the analysis of head movement rates and of stress/no-stress behaviors. We had relatively few trials with light + sound stimuli so we couldn't find significant differences in behavior for these stimuli, but the blue flashing light with the mistuned harmonic stack stimulus seemed to be a good light + sound stimulus for the eagles. This stimulus would be the first light + sound stimuli we would recommend for use in wind turbine deterrent systems.

The most common stressful behaviors exhibited during the behavioral experiments were move, looking up + crouch, and wing flap. There were no significantly different stress-related behavioral responses between light, sound, and light + sound stimuli in general. However, the ratio of stress to non-stress related behaviors differed with stimuli sensory modalities (i.e. light, sound, and light + sound stimuli). The highest ratio of stress to non-stress behaviors for the Golden Eagle was in response to light stimuli, and the highest ratio for the Bald Eagle was in response to light + sound stimuli. In other words, light stimuli elicited relatively more stress behavior in Golden Eagles than sound or light + sound, and light + sound stimuli elicited relatively more stress behavior in Bald Eagles.

We also found that head movement rates, and the number of stress-related and non-stress related behaviors declined over the course of an experiment, suggesting there might be some habituation to the stimuli playback system or that there was habituation to the experimental setup itself. However, further behavioral experiments would need to be performed to confirm that this is the case. These eagles were not in a natural environment when tested, so we advise caution in directly extrapolating these results to wild populations as a whole. However, they are an informed first step in performing these tests with wild eagles at wind turbine facilities. A random assortment of stimuli is likely to be best in alerting eagles to the turbine. Our results suggest that any of the four sound stimuli are equally likely to be alerting. This fact implies that a useful presentation scheme would be to rotate randomly through those sounds with each broadcast. We suggest that the test be run varying the amount of time each stimulus is presented, with one minute per stimulus duration (as used here) as a starting point.

Departure from Plan and Why

Conducting the behavioral analysis took additional time to complete in order to effectively utilize the data we collected from the behavioral experiments. In the end we had a lower (than hoped for) sample size of eagle individuals for the behavioral component of this project.

Key Conclusions

We were successfully able to analyze the behavioral responses of the eagles to the stimuli presented during the behavioral experiment using the stimuli playback system. An increase in sample size of the eagles measured would help provide additional information on behavioral

responses to various stimuli in this semi-natural experiment context. However, we were able to identify several stimuli for use in field-testing of an eagle deterrent system. The stimuli we would recommend for use in field-testing on eagle specific wind turbine deterrent systems are as follows:

Visual Stimuli (ranked in order of effectiveness of eliciting a response in the behavioral experiment):

1) Blue flashing LED light at 1 Hz
2) Blue steady LED light
3) Red flashing LED light at 1 Hz
4) Red steady LED light

Auditory Stimuli (ranked in no particular order):

1) Mistuned harmonic stack (1.0, 2.2, 3.3, 3.6, and 4.7 kHz)
2) 0.4 kHz amplitude modulation (AM) with 2 kHz carrier
3) Downward sweep (6-1 kHz in 50ms)
4) 70 Hz frequency modulation (FM) with 400 Hz depth (based on 2 kHz tone).

We recommend a presentation scheme that would rotate randomly through these sounds, with each broadcast for approximately one minute.

For additional details on these tasks, please review Attachment Q.

4.0 PRODUCTS DEVELOPED

Presentations at Scientific Meetings:

Goller B, P Baumhardt, N Lovko, E Fernández-Juricic, and J Lucas. 2019. Measuring Golden and Bald eagle hearing for development of eagle deterrent technology. Animal Behaviour Society meeting, Chicago, IL (oral).

Goller B, P Baumhardt, E Dominguez-Villegas, T Katzner, J Lucas, and E Fernández-Juricic. 2018. Measuring eagle visual and auditory sensory perception to enhance deterrent technology for wind turbines. Wind Wildlife Research Meeting, St. Paul, MN (poster).

Goller B, P Baumhardt, T Katzner, J Lucas, and E Fernández-Juricic. 2018. Visual fields and retinal morphology of Bald and Golden eagles. Sensorium, West Lafayette, IN (poster).

Lucas JR, B Goller, P Baumhardt, T Katzner, E Dominguez, P VanWick, E Fernandez-Juricic. 2018. Using sensory information to keep eagles out of wind turbines: (1) The auditory system. Animal Behavior Society Conference, Anchorage AK, (Contributed talk).

Lucas JR, B Goller, P Baumhardt, T Katzner, E Dominguez, P VanWick, E Fernandez-Juricic. 2018. Using sensory information to keep eagles out of wind turbines: (1) the auditory system. Sensorium Conference, West Lafayette (talk).

Lucas JR, B Goller, P Baumhardt, T Katzner, E Dominguez, P VanWick, E Fernandez-Juricic. 2018 Dec 6. “Using auditory information to keep eagles out of wind turbines”. Dept of Speech Language and Hearing Sciences, Purdue University (talk).

Publications:

Goller B, P Baumhardt, T Katzner, E Fernández-Juricic, and JR Lucas. 2020. Selecting auditory deterrents for eagles on the basis of auditory evoked potentials. *In preparation*.

5.0 ATTACHMENTS

Milestone #	Milestone Description	Attachment
1.1	Approval of IACUC protocol by Purdue University	**A**
1.2	Signed MoUs with rehab centers, and state and federal regulatory agencies	**B**
2.0	The portable anechoic chamber to fit Golden and Bald Eagles will be built and ready to be transported	**C**
3.0	The portable microspectrophotometer will be built and ready to be transported	**D**
4.0	The portable microscopy system will be built and ready to be transported	**E**
5.1	Successful test of portable anechoic chamber and auditory measurements	**F**
5.2	Successful test of the portable microspectrophotometer	**G**
5.3	Successful test of the portable microscopy system	**H**
7.1	Obtain auditory data from 5-7 individuals of each species (this number would likely be higher for bald eagles given the higher availability of this species in rehabilitation centers). These traits include critical ratios (how loud sounds must be to be processed in the presence of noise), frequency sweeps and amplitude modulated sounds (particularly likely to be alerting), and processing of sounds that are of suprathreshold intensity (i.e. at about the level detectable in the field).	**I**
7.2	Obtain visual data from 3-6 individuals from each species (this number would likely be higher for bald eagles given the higher availability of this species in rehabilitation centers). These traits include visual field configuration (size of the binocular, lateral and blind areas), density of photoreceptors, peak sensitivity of visual pigments, absorbance of oil droplets, etc.	**J**
8.1	Processing and analyzing of the auditory data on campus	**K**
8.2	Characterize for golden and bald eagles: the configuration of their visual fields, the type and position of their center/s of acute vision, the density of retinal ganglion cells, eye size, visual acuity, density of different types of photoreceptors, peak sensitivity of visual pigments, and absorbance of oil droplets.	**L**
9.1	Develop an LED light + speaker system for playback of combinations of visual and acoustic stimuli	**M**
9.2	Develop a behavioral assay to test the responses of eagles to these visual and acoustic stimuli so that it can be conducted at different rehabilitation centers in the US	**N**
9.3	Deliver experimental protocols to funding agencies	**O**
10.0	Gather behavioral responses from at least 6 golden eagles and 12 bald eagles	**P**
11.0	Estimate whether golden and bald eagles are attracted/repelled by combinations of visual and acoustic stimuli with different degrees of sensory conspicuousness.	**Q**

ATTACHMENT A

Milestone 1.1

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 1.1.

Milestone 1.1 – Approval of IACUC protocol by Purdue University

Task 1.0 – Getting the IACUC protocol approved and developing Memorandums of Understanding (MoUs) with rehab centers, and State and Federal authorities (Month 0)

Task Summary:

Submitted IACUC protocol for revision within Purdue University. Once approved, we would be able to draft MoUs with rehabilitation centers, and State and Federal agencies so that everybody is aware (and approves) of every procedure we will engage in with the animals.

Subtask 1.1 – IACUC protocol approval (Month 0)

Subtask Summary: Finished writing, submitting, and getting the IACUC protocol approved by Purdue University.

Objectives

To be able to perform all necessary physiology work (both auditory and visual) and behavioral experiments at rehab centers, we first needed to write and submit a Purdue University Institutional Animal Care and Use Committee (PACUC) protocol. Once this protocol was approved, we would be able to begin work on the project.

Protocol Development and Submission

Using a collective 30^{+} years of experience on the techniques we would perform with the eagles, we were quickly able to develop and write the initial IACUC protocol. The protocol includes justifying the use of eagles for this project, the scientific rationale for the number of animals used, descriptions of the live animal work that would take place, anesthesia protocols, etc. This protocol was submitted for PACUC review on May 9th, 2017.

IACUC Approval

The protocol was approved by PACUC on June 14th, 2017. Please see approval letter.

PURDUE UNIVERSITY

Purdue Animal Care and Use Committee

To:	FERNANDEZ-JURICIC, ESTEBAN LUCAS, JEFFREY R
From:	Lori Bugher, PACUC Administrative Assistant
Date:	06 / 14 / 2017
Committee Action:	Designated Member Approval
Submission Type:	PACUC Requested Revisions
Approval Date:	06 / 14 / 2017
Protocol Number:	1705001579
Study Title:	Understanding the golden and bald eagle sensory worlds to enhance detection and response to wind turbines
Expiration Date:	06 / 13 / 2020

Your submission was reviewed and approved by the Purdue Animal Care and Use Committee(PACUC) via designated member review. The submission was approved as presented.

The PACUC office will no longer be mailing out copies of approved protocols since they are available online.

- This is the approval of new protocol 1705001579 - and the PI as listed in Coeus is Esteban Fernandez-Juricic (not Jeffrey Lucas as is listed on the form). If this is incorrect, then there needs to be an amendment filed to change the PI name.

This approval will remain in effect until: Jun 13, 2020

(This is an automated message; there is no need to respond unless you have a question or problem).

Best Regards,
PACUC Staff.

IACUC Protocol Amendments

We needed to amend the initial IACUC protocol twice throughout the project. The first amendment included the addition of personnel working on the protocol (Benjamin Goller and Patrice Baumhardt). This amendment was approved after PACUC internal review on September 21st, 2017. A second amendment to the initial protocol increased the number of eagles we could use on the project. When we first wrote the protocol, we anticipated that we would be able consistently to perform most or all of the visual system techniques on the same individual eagle. However, this was not the case and we reached the predicted sample size needed for the visual measures without completing the necessary work for the project. In most cases during the project, we were only able to perform one technique on a single eagle. This amendment was submitted on May 23rd, 2019 and approved on June 14th, 2019.

PURDUE UNIVERSITY

Purdue Animal Care and Use Committee

To:	FERNANDEZ-JURICIC, ESTEBAN LUCAS, JEFFREY R
From:	Lori Bugher, PACUC Administrative Assistant
Date:	09 / 21 / 2017
Committee Action:	Designated Member Approval
Submission Type:	Amendment
Approval Date:	09 / 21 / 2017
Protocol Number:	1705001579
Study Title:	Understanding the golden and bald eagle sensory worlds to enhance detection and response to wind turbines
Expiration Date:	06 / 13 / 2020

Your submission was reviewed and approved by the Purdue Animal Care and Use Committee(PACUC) via designated member review. The submission was approved as presented.

The PACUC office will no longer be mailing out copies of approved protocols since they are available online.

- This is the approval of amendment 1705001579A001 for addition of personnel ONLY.

This approval will remain in effect until: Jun 13, 2020

(This is an automated message; there is no need to respond unless you have a question or problem).

Best Regards,
PACUC Staff.

410 South University Street, VAHF Bldg. • West Lafayette, IN 47907
(765) 494-7206 • Fax: (765) 496-2415 • E-mail: ldsnider@purdue.edu

PURDUE UNIVERSITY

Purdue Animal Care and Use Committee

To:	FERNANDEZ-JURICIC, ESTEBAN LUCAS, JEFFREY R
From:	Lori Bugher, PACUC Administrative Assistant
Date:	06 / 14 / 2019
Committee Action:	Designated Member Approval
Submission Type:	Amendment
Approval Date:	06 / 14 / 2019
Protocol Number:	1705001579
Study Title:	Understanding the golden and bald eagle sensory worlds to enhance detection and response to wind turbines
Expiration Date:	06 / 13 / 2020

Your submission was reviewed and approved by the Purdue Animal Care and Use Committee(PACUC) via designated member review. The submission was approved as presented.

The PACUC office will no longer be mailing out copies of approved protocols since they are available online.

- This is the approval of amendment 1705001579A002

This approval will remain in effect until: Jun 13, 2020

(This is an automated message; there is no need to respond unless you have a question or problem).

Best Regards,
PACUC Staff.

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(765) 494-7206 • Fax: (765) 496-2415 • E-mail: ldsnider@purdue.edu

ATTACHMENT B

Milestone 1.2

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 1.2.

> ***Milestone 1.2 – Signed MoUs with rehab centers, and state and federal regulatory agencies***

Task 1.0 – Getting the IACUC protocol approved and developing Memorandums of Understanding (MoUs) with rehab centers, and State and Federal authorities (Month 1-19)

Task Summary:

Submitted IACUC protocol for revision within Purdue University. Once approved, we would be able to draft MoUs with rehabilitation centers, and State and Federal agencies so that everybody is aware (and approves) of every procedure we will engage in with the animals.

Subtask 1.2 – Signed MoUs with rehab centers, and state and federal regulatory agencies (Month 1-19)

Subtask Summary: Developed relationships with rehabilitation centers, and State and Federal agencies involved, and understandings about the measurements we will take on individual animals.

Objectives

At the start of the project, we initially felt that it was essential to develop signed Memorandums of Understanding (MoUs) between Purdue University and the various rehab centers we would be working with. We quickly realized that establishing relationships with the rehab centers is a long process that would continually evolve as the project went on and our needs changed. As a result, we never drafted formal agreements to outline our collaboration with a rehabilitation center. We did however develop a set of protocols/anticipated processes that rehabilitation centers could review as a way to start a conversation about our research and provide an overview of our experimental procedures for the eagles.

Initial Contact with Rehabilitation Centers

The rehab centers where eagles would be measured were initially identified by co-PI Dr. Todd Katzner, who has contacts at many of the eagle rehabilitation centers throughout the U.S. and the state and federal permits to work on eagles. At the initial proposal of the project, Dr. Katzner had guarantees from three centers, the Wildlife Center of Virginia, the Avian Conservation Center of Appalachia in West Virginia, and the Tennessee Avian and Exotic Animal Service. At the point where we were ready to start with experiments, however only the Wildlife Center of Virginia was still willing to work on the project with us. Todd Katzner provided us with an additional list of rehabilitation centers across the country that worked with raptors.

We began our own search for eagles by contacting rehab centers in Indiana starting November 2017 (Month 4). It quickly became apparent that we would not have much success in Indiana because the local rehabbers do not admit many eagles. We began working with the Wildlife Center of Virginia in March 2018 and started collecting data on Bald Eagles, but they seldom admit a Golden Eagle.

To get access to Golden Eagles, we expanded our search west to the Rockies and Pacific Coast. We called 29+ raptor centers and rehabilitators, numerous falconers, and veterinary programs at several universities (UC Davis, Washington State Univ.) to introduce the project and gauge interest in collaborations. Avangrid Renewables also contacted 9+ rehabbers across the country on our behalf and set up several phone meetings between the Purdue team and raptor rehabilitation centers. Todd Katzner also tried to set up collaborations with several of his contacts. In the end, these efforts yielded several important collaborations that provided opportunities for work with Golden Eagles: Liberty Wildlife (originally an Avangrid contact), Blue Mountain Wildlife (Purdue contact, some connections to Avangrid as well), Montana Raptor Conservation Center (Purdue contact), and UC Davis/California Raptor Center (Purdue contact). We continued to look for new collaborators until April 2019 (Month 21) at which point we shifted our focus from setting up new collaborations to completing data collection with the rehab centers where we had established collaborations, especially looking for opportunities to work with fresh retinal tissue. Concurrent with the search for collaborations, we worked to get authorization for eagle research under federal and state permits held by Todd Katzner, some of which took many months to receive.

Established Relationships with Rehabilitation Centers

We started working with Soarin' Hawk Raptor Rehabilitation (8 trips; equipment testing and tissue samples) in Ft. Wayne, IN and were able to test some of our equipment with a juvenile Bald Eagle they had in rehabilitation. Our first data collection was in collaboration with the Wildlife Center of Virginia (5 trips; hearing, visual field measurements, and tissue samples), who

generously gave us access to numerous Bald Eagles as well as storage space for our equipment. Golden Eagles were much harder to find, especially for live-animal procedures like the hearing measurements. We were able to measure visual fields of two Golden Eagles at UC Davis and the California Raptor Center (1 trip; tissue samples and visual field measurements). With Liberty Wildlife in Phoenix, AZ (1 trip) we were able to measure hearing in two Golden Eagles and one Bald Eagle, as well as measure additional Golden Eagle visual fields and collect tissue samples. In addition, we worked with Blue Mountain Wildlife (2 trips; tissue samples, visual field measurements and behavior), Montana Raptor Conservation Center (2 trips; tissue samples), and Indiana Raptor Center (6 trips; behavior) to round out the data collection. In total, we went on 25 data and tissue collection trips.

Without the generous invitations from these wildlife rehabbers and veterinarians, we would not have been able to complete this project. We are incredibly grateful for their expertise, time, assistance, facilities, and access to the animals.

ATTACHMENT C

Milestone 2.0

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 2.0.

> ***Milestone 2.0 – The portable anechoic chamber to fit Golden and Bald Eagles will be built and ready to be transported***

Task 2.0 – Devising and building a portable anechoic chamber (Month 2-5)

Task Summary:

We had an anechoic chamber at Purdue to run auditory tests on songbirds prior to our eagle study, but it could only fit relatively small to medium sized birds. Therefore, we had to design and build a new anechoic chamber with materials that would make it portable, scaling up its dimensions to accommodate the relatively large size of Golden and Bald eagles.

Objectives

In order to measure the hearing properties of the Golden and Bald Eagles, we first needed to make a portable anechoic chamber to perform the tests. We have an anechoic chamber at Purdue University, but it is not portable. We designed and built a portable anechoic chamber with the assistance of Purdue University Biology Department Maintenance personnel. This anechoic chamber needed to be both large enough to contain the eagles and all necessary equipment, and portable enough be transported across the country to various rehabilitation centers.

Materials and Design for Anechoic Chamber

We first sought the advice of a member of the Purdue University Biology Department Maintenance team. He has helped us with several large and small-scale builds over the years, and had wonderful insights that have proved instrumental for the success of many projects. On his recommendation, we used 3 mm Aluminum Composite Material (Meyer Plastics Inc., Indianapolis, IN) for the body of the chamber, mainly for its lightweight strength and the sound reduction properties of the polyethylene core. The same material was used in the past for building a stationary safety shield/case for a microscope system at Purdue. Again based on his

recommendation, extruded aluminum angle iron was used for the legs and corner attachments of the chamber, as these are lightweight but strong enough to hold the chamber together.

To make the chamber anechoic, it needed to be completely lined with acoustical foam. The stationary Purdue anechoic chamber was lined with a 4-inch (10 cm) thick Sonex convoluted acoustic foam purchased from Sound Isolation Company (Charlotte, North Carolina, USA). However, this product was no longer available on the market from any vendor. After researching various types of acoustic foam and contacting vendors about the properties and suitability to our application, we requested quotes. Unfortunately, many of the options that would both work for our application and arrive to Purdue quickly were prohibitively expensive. So we decided to use the 3" x 24" x 48" Fire Rated, one side convoluted, Acoustical Foam Panel from Sound Isolation Company. Backed with a 0.6" x 24" x 24" Fireflex Flat panel to provide additional thickness and sound reduction capabilities. Upon arrival to Purdue (after 6^{+} weeks), we noticed that the 3" acoustic panels would not work for our anechoic chamber because they were very delicate and would tear while being pulled out of the shipping containers. Therefore, we had to reorder an entirely new kind of acoustic foam, 3" x 24" x 48" UNX-3 SONEX Classic Panels from Sound Isolation Company. These thankfully were in stock, and only took 2-4 weeks to arrive.

In order to take auditory measurements with the anechoic chamber, we needed to purchase a Z-Series 3-DSP Bioacoustic System with Attenuators from Tucker-Davis Technologies, Inc. (Alachua, Florida, USA), hereafter called the TDT. This TDT system, when combined with the necessary 4-channel digitizer, headstage, and a Behringer FBQ6200HD Hi-Definition Ultragraph Pro 31-Band Equalizer, plays a series of user created sound stimuli from a speaker and simultaneously measures the neural response from the auditory brainstem of the bird being measured. The TDT can complete measurements of hundreds of sound/response cycles in a short period, making this system ideal for measuring the eagles. This reduces the amount of time that the eagles need to be under anesthesia during the procedure, thereby decreasing the chance of adverse effects to anesthesia over prolonged periods. For additional details, please review Attachment I.

Building the Anechoic Chamber

While waiting for the initial acoustic foam panels and the TDT to arrive, we began to build the anechoic chamber. Using the body sizes of the Golden and Bald eagles and dimensions of all the necessary testing equipment, we decided that the dimensions of the anechoic chamber should be a 1.22 x 1.22 x 1.22 m cube. This cube would have aluminum angle iron supports and corners, and a large door (0.91 x 0.91 m) that would fit a 0.81 x 0.81 x 0.46 m Faraday cage through. The Faraday cage was necessary to reduce the amount of electrical noise generated in the various rehab centers we would be visiting. A small notch was made to fit any wires or tubing needed for the anesthesia monitoring equipment and respirator tubing next to the door of the chamber. The supports of the anechoic chamber would rest on anti-vibration silicone gel pads

produced by Advanced Antivibration Components (Hyde Park, New York, USA), to reduce vibrations from the floor traveling into the chamber. We designed the chamber in a way that allowed a single person to repeatedly dismantle and reassemble the chamber. This was accomplished by sliding the panels of the box into tongue and grove corner brackets made using two pieces of aluminum angle iron (Figure C1).

Figure C1. Portable anechoic chamber structure after completion of the build and before application of the acoustic foam panels.

After the correct acoustic foam panels arrived, they were attached to each chamber panel, including the door, using a metal roof sealant recommended to us by the acoustic foam manufacturer. We applied the 0.6” thick foam panels to the surface of the chamber panels, and then the 3” foam panels on top, to produce a 3.6” thick acoustic foam lining within the chamber. The edges of the panels were cut at an angle to allow the panels to fit together more easily during assembly. Because the fit was so tight on the foam panels, the top of the box would not sit snugly on the chamber without some applied compression, so we used two large straps that could be ratcheted tight around the entirety of the chamber. Once the chamber with foam lining was assembled, we could place the Faraday cage (copper mesh box in Figure C2), the stimulus speaker suspended on a glassware stand, the TDT headstage and electrodes, and an infrared camera (used to observe the eagle directly during tests when the chamber was closed) into the chamber on top of the foam floor (Figure C2). Whenever possible we would fill empty spaces in the anechoic chamber with scraps of foam to help aid in the acoustics within the chamber. We used a chain and hook to hold the door of the anechoic chamber open during the initial setup at the rehabilitation centers and when checking on the eagle, as it was quite heavy after the foam was added.

Figure C2. Portable anechoic chamber complete with installed foam panels installed, Faraday cage, and testing equipment ready for measurements at a rehabilitation center.

ATTACHMENT D

Milestone 3.0

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 3.0.

> ***Milestone 3.0 – The portable microspectrophotometer will be built and ready to be transported***

Task 3.0 – Borrowing a portable microspectrophotometer from Cornell University

(Month 0)

Task Summary:

We currently have a microspectrophotometer at Purdue to measure the spectral properties of photoreceptors (visual pigments and oil droplets), but it is not portable. We borrowed a portable microspectrophotometer from Cornell University with the minimum necessary components that would allow us to transport it and set-it up in rehab centers. This was necessary because microspectrophotometry needs to be done right after retina extraction (which is done right after euthanasia) as the retinal tissue begins degrading within minutes after death. We traveled to Cornell University, trained for two days on using this portable device, and brought it back to Purdue University.

Objectives

To be able to acquire measurements on the photoreceptor sensitivities of the Golden and Bald Eagle we needed to acquire a portable microspectrophotometer (MSP). The portable MSP is able to be setup at any rehab center across the United States, accommodating the work we would undertake for this project. However, to be able to use this portable MSP, we first had to retrieve it from Dr. Ellis Loew at Cornell University and receive special training on its installation and operation.

Acquiring the MSP

To obtain the MSP from Cornell University, Patrice Baumhardt traveled by car to Ithaca, New York from Purdue University in West Lafayette, Indiana. Upon arrival, Dr. Ellis Loew showed her the main components of the custom-built MSP (Figure D1). These included the following: 1) a Tungsten lamp with housing and power converter, 2) a monochrometer with

scanning motor, 3) a microscope and base plate, 4) a series of prisms that require alignment, 4) a condenser and lens, 5) a head stage which holds a camera and a photomultiplier tube, 6) a screen to view images from the camera, 7) high voltage and computer control boxes, and 8) a laptop containing a custom made MSP control program. These components were able to fit within two cases, the first a large Pelican 1650 Series wheeled case and the second a small black Pelican 1500 Series case, enabling both to be transported on a commercial airplane as checked baggage.

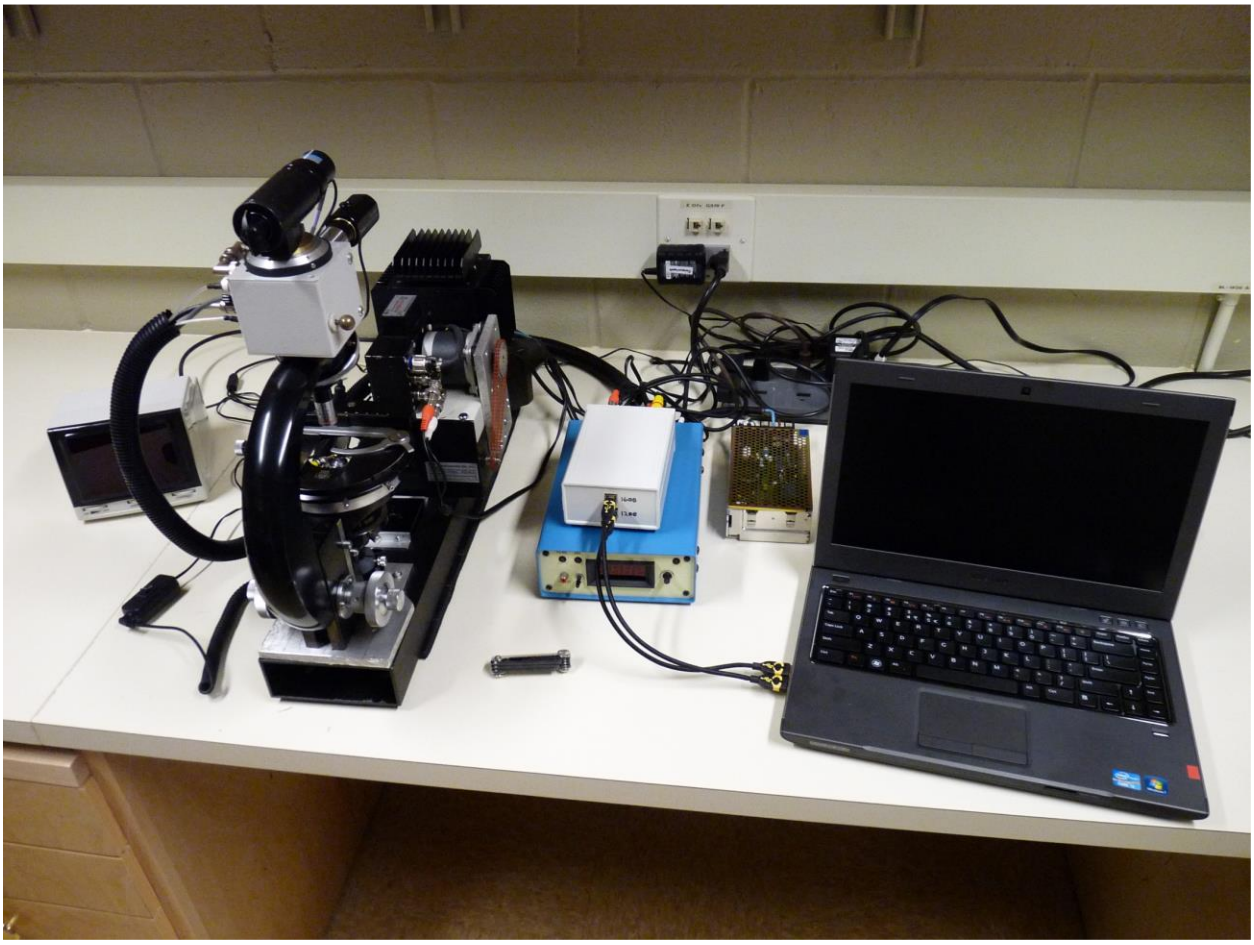


Figure D1. Portable MSP fully assembled and ready to take measurements.

Training on the Portable MSP

On day one, Dr. Ellis Loew trained Patrice Baumhardt, who had several years of previous experience working with the stationary MSP at Purdue, how to assemble the portable MSP and align the optical elements. These optical elements are critical to acquiring the measurements and require careful alignment along several points in the light path on the MSP. On day two, Dr. Loew showed Patrice how to run the new MSP Control Program software with the machine as

well as a new analysis tool, called MSPA, which is used when measuring the peak absorbance of the visual pigments. All training was video recorded for future reference. After training, the MSP was disassembled and brought back to the Purdue University campus where it awaited testing (Attachment G).

ATTACHMENT E

Milestone 4.0

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 4.0.

Milestone 4.0 – The portable microscopy system will be built and ready to be transported

Task 4.0 – Devising and building portable microscopy system (Month 2-9)

Task Summary:

We currently have a microscopy system at Purdue to take pictures of a fresh retina to determine the density of different types of photoreceptors, but it is not portable. We purchased and built a microscopy system with the minimum necessary components that would allow us to transport it and set-it up in rehab centers. This was necessary because the photoreceptors (using oil droplets as proxies) can only be seen and distinguished right after retina extraction (which is done right after euthanasia) as the retinal tissue starts degrading within minutes after death.

Objectives

To be able to acquire images of the oil droplets across the retinae of the Golden and Bald Eagle we needed to acquire a portable microscopy system. Working with Zeiss, we were able to develop a portable microscopy system that could be setup at any rehab center across the United States, accommodating the work we would undertake for this project. The microscopy system was housed in custom-made travel cases so it could be taken on commercial aircraft.

Acquiring the Portable Microscope

To develop a portable microscopy system, we first needed to purchase a microscope that would be able to take the images of the retina, including a brightfield image and an epifluorescent image. This system also needed software that could take multiple images across the retina in a grid pattern. We were able to purchase a used Carl Zeiss Microscopy, LLC (Thornwood, New York, USA) Axio Scope.A1 demo scope with Zen Blue software for almost half the cost of a new microscope (Figure E1). Unfortunately, we were not be able to use this microscope with a laptop. However, the Purdue University Science IT group was able to purchase a desktop computer that would work with the microscope, including the fire wire card

needed for the microscope's camera. Significant delays occurred due to incorrect parts sent (incorrect motorized stage) and parts on back order (fire wire card computer) from Zeiss in Germany, so the equipment was not fully ready for testing until month 9.

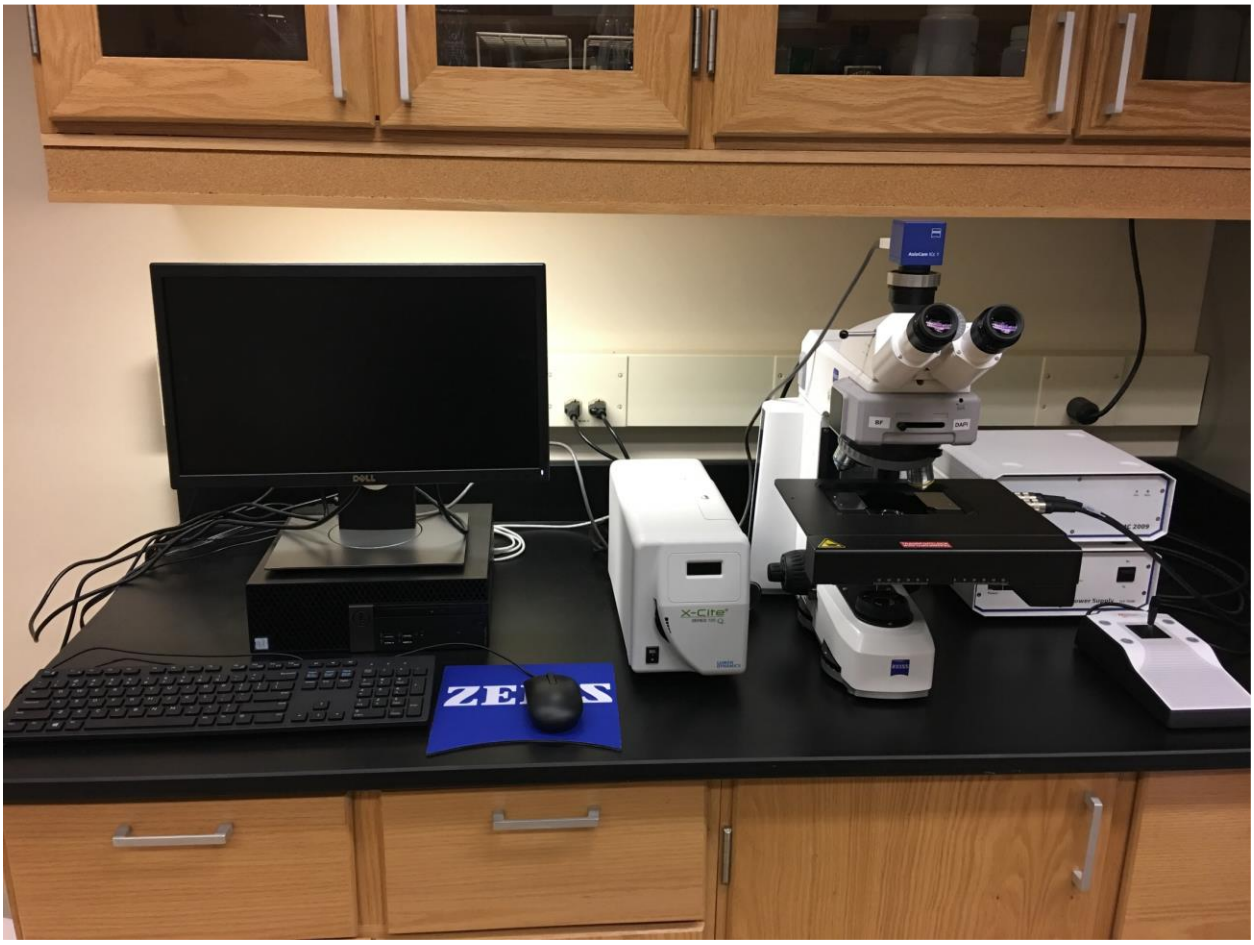


Figure E1. Portable microscope fully assembled and ready to acquire images.

Next, we needed to purchase cases to be able to transport this delicate system on an airplane. The Zeiss representative for Purdue University recommended contacting Case Design Corp. (Telford, Pennsylvania, USA), a company that had made cases for these exact demo systems in the past. Case Design Corp. was able to design cases that would accommodate our microscope and the computer we purchased. These three cases were sturdy and had large wheel rollers which made transport possible because they are quite heavy (75-90 lbs each) when loaded (Figure E2).

Figure E2. Blue cases used to transport the microscopy system to a rehabilitation center in Montana. Other cases in this image are equipment used for other visual techniques (e.g. MSP, spectrometer, etc.), and a backup Olympus microscopy system to ensure images of the retina could be acquired.

Training on the Portable Microscope

After the microscopy system was fully assembled, the Purdue Zeiss representative was able to visit our lab and installed the Zen Blue 2.3 software. The Zeiss rep. then trained us on how to use the software and microscope. During this training, we were able to explain in more detail the exact requirements we have to acquire the retina images in a way that was understandable to the Zeiss rep. Using this new information, over the course of several weeks, we were able to produce a final protocol that allowed us to successfully acquire retina images using the microscopy system and software.

ATTACHMENT F

Milestone 5.1

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 5.1.

Milestone 5.1 – Successful test of portable anechoic chamber and auditory measurements

Task 5.0 – Testing of portable equipment with an invasive bird species (Month 3-4)

Task Summary:

Using available bird species (preferably invasive) at Purdue University, we tested the anesthesia protocols and the auditory measurements using the portable anechoic chamber. These tests occurred on the Purdue campus. Similarly, we extracted bird retinas and tested on campus the portable microspectrophotometer and microscopy systems by measuring the spectral properties of the photoreceptors and the density of photoreceptors (using oil droplets as proxies).

Subtask 5.1 – Testing the anesthesia protocols and the auditory measurements on the portable anechoic chamber (Month 3-4)

Subtask Summary: Our previous work on House Sparrows includes auditory brainstem responses (ABR) derived from evoked potentials (Lucas et al. 2002). These previous results provide a baseline for testing the integrity of the portable anechoic chamber. We also measured critical ratios (auditory responses in noise) to ensure that this important aspect of our current study also worked with the chamber.

Objectives

To be able to anesthetize and take auditory measurements from the Golden and Bald Eagle we needed to test the anechoic chamber and auditory equipment on a surrogate species. Using the anechoic chamber we built (Attachment C), we tested the auditory measurement system on an invasive species and the anesthesia protocol on a large species of bird. These test measurements allowed us to verify the anechoic chamber, auditory equipment, and anesthesia protocols for use in rehabilitation centers across the United States.

Testing the Auditory Measurements

We tested the auditory measurements with two House Sparrows (*Passer domesticus*) and two Canada Geese (*Branta canadensis*). Both species were captured under U.S. Fish and Wildlife Service Permit MB143973-0 and Indiana Scientific Purposes License 18-179. All work was performed under Purdue University Animal Care and Use Committee protocol # 1111000125. These measurements involved playing a simple sound (like a single frequency tone) to an anesthetized bird and measuring the auditory brainstem response to this sound. The data collected from these measurements allowed us to confirm the equipment was functioning correctly by comparing the results with previously published data on the House Sparrow. The data also demonstrated the functionality of the anechoic chamber by how noisy the data were at certain sound frequencies. Noisy data at certain frequencies would indicate ineffective sound dampening by the acoustic foam. These tests were successful and showed that the Tucker-Davis Technologies bioacoustic system (TDT; Attachment C) and anechoic chamber were suitable for measuring the ABRs of birds, when frequencies were above 250-500 Hz.

Unfortunately, House Sparrows were too small to do full tests of the anesthesia protocols that we would be using for eagles. However, our lab was able to use the Canada Goose as a substitute species for testing the eagle anesthesia protocol with the help of Purdue University Veterinary Anesthesiologist Dr. Jeff Ko and his trainees. Dr. Ko has many years of experience with anesthesia and has helped us in the past with Canada Goose anesthesia. Dr. Ko developed an initial anesthesia protocol, that would work for both the eagles and geese, which he planned to perform with us at rehabilitation centers around the country. We tested this protocol on two geese. The protocol involved the use of an intramuscular injection of 0.20 mg/kg Butorphanol, 0.40 mg/kg Midazolam, and 0.08 mg/kg Dexmedetomidine. Isoflurane (1%) was then administered as necessary to prepare the goose for intubation. After intubation, and during the experiments, low levels of Isoflurane (0.25-0.5%) were administered to ensure the goose remained anesthetized.

The vitals of the goose were carefully monitored during the procedure, and the level of anesthesia was maintained for the time needed to complete the auditory measurements (Figure F1). Both anesthesia tests with the Canada Geese were successful. During this process, we quickly realized that it was unlikely that Dr. Ko or his trainees would be able to work with us in the future since the data collection trips could not be confined to the times they were available (a 2 week period before the beginning of the school semester). We therefore could only pursue eagles for auditory testing at rehab centers with the necessary equipment and expertise to follow the protocol Dr. Ko devised.

Figure F1. Testing the anesthesia protocol, anechoic chamber, and TDT on the Canada Goose. Panels show the vitals being monitored (**a**) on the goose under anesthesia in the anechoic chamber (**b)** and the resulting data collected on the TDT (**c**).

Understanding the Results of the Tests and Eagle Sound Stimulus Design

Auditory evoked potentials (AEPs) provide a measurement of how the auditory system – from the ear to the auditory brainstem – responds to sounds. When a sound reaches the ear of a bird, the pressure waves of the sound push against the tympanic membrane and an ossicular chain passes these pressure waves on to the cochlea. The cochlea is the sound transduction structure, which vibrates according to the pressure signal received from the ossicular chain and translates these vibrations into electrical signals in the auditory nerve. AEP responses are reported as the strength of phaselocking, which describes how well the neurons in the auditory system collectively encode a stimulus sound. For example, if the ear of an eagle is stimulated with a 2,000 Hz tone, the measured AEP should indicate that the overall firing of neurons would also have a strong 2,000 Hz phaselocking component – in essence showing that the sound had been registered by the auditory system of the eagle. Comparing phaselocking for different stimulus sounds then gives a measure of how well an eagle could process those sounds.

To find candidate sounds that are particularly alerting to eagles, we designed a suite of different sounds (from pure tones to frequency sweeps and harmonic stacks or chords, described in detail in Attachment I) that would test the eagle auditory system response to frequency changes and temporal patterns. Example AEP recordings from the Canada Geese (Figure F2), in which we tested some sample sounds like those used in eagle tests, illustrate that different types of sounds produce different auditory responses. As seen in Figure F2, Pure tones (A) give an overview of what frequencies may be particularly salient for the subject animal, for example, the goose is more sensitive to 3000 Hz frequency tones. Similarly, harmonic stacks (B) allow us to compare hearing at specific frequencies, but in the context of additional components of a chord or stack of tones, there is evidence that specific combinations of sounds change the overall response significantly. In the goose, the strength of phaselocking was similar between the three harmonic stacks tested (except in the 4000 and 5000 Hz components of one harmonic stack [yellow line]). We also tested three tone stacks that produce amplitude modulated signals (C),

and compared phaselocking to 100Hz, 400Hz, and 700Hz amplitude modulation rates. Finally, we tested frequency sweeps, where frequency increases (D) or decreases over a short period of time, which also affects both the range and the magnitude of the auditory system response even though the frequencies presented are the same across different sweeps.

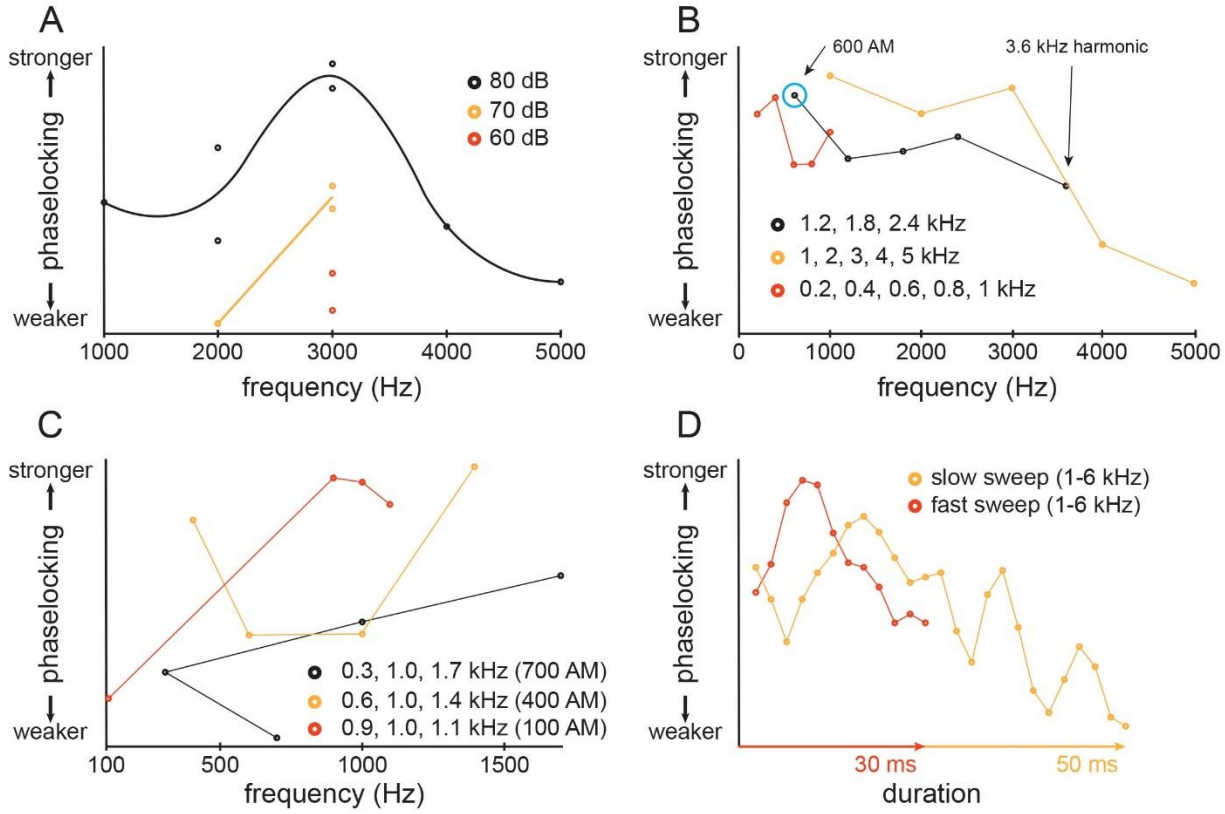


Figure F2. Auditory results collected from the two Canada Geese used to test the TDT and anechoic chamber. Descriptions of results in each panel are in the body of the text directly above. The examples illustrate that different sounds (A: tones, B: multi-tone stacks, C: amplitude modulation, D: frequency sweeps) have different auditory evoked potential phaselocking patterns that depend on the properties of the sound stimuli.

ATTACHMENT G

Milestone 5.2

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 5.2.

Milestone 5.2 – Successful test of the portable microspectrophotometer

Task 5.0 – Testing of portable equipment with an invasive bird species (Month 3-4)

Task Summary:

Using available bird species (preferably invasive) at Purdue University, we tested on campus the anesthesia protocols and the auditory measurements on the portable anechoic chamber. Similarly, we extracted retinas of birds and tested on campus the portable microspectrophotometer and microscopy systems by measuring the spectral properties of the photoreceptors and the density of photoreceptors (using oil droplets as proxies).

Subtask 5.2 – Testing the portable microspectrophotometer (Month 3-4)

Subtask Summary: We would collect measurements, using the portable microspectrophotometer, on a species of bird. By measuring its visual pigment sensitivity and oil droplet absorbance values, we can ensure that the measures are comparable to those reported in the scientific literature. If the results are comparable, and the portable unit functioning correctly, we would consider the test successful.

Objectives

To be able to measure the visual pigment sensitivity and oil droplet absorbance spectra successfully from the Golden and Bald Eagle we needed to test the portable microspectrophotometer on a more readily available species. We tested the assembly, functionality, and data output of the portable microspectrophotometer we borrowed from Dr. Ellis Loew at Cornell University (Attachment D). We used a non-invasive bird species (Blue-black Grassquit) that became readily available to us via another project in our lab.

Testing the Portable Microspectrophotometer

We had initially proposed to use the European Starling (*Sturnus vulgaris*) retina because these results have been published in the literature. We also had captive starlings in the lab that

were not releasable. However, before we could perform the work with the starlings, we had the opportunity to use the portable microspectrophotometer (MSP) on the Blue-Black Grassquit (*Volatinia jacarina*) in collaboration with a researcher at the University of Brasilia, Brazil. This trip was funded by another project and did not use any Avangrid or Department of Energy funds. All work performed was authorized under the collaborators permits. We felt that this would be an ideal test scenario because it also allowed us to see how the equipment traveled on a long plane flight, verify assembly, perform post-travel optical alignment, prepare the retinal tissues, and create a dark room in a remote location (Figure G1). A consideration we did not expect was that the electrical system in Brazil is highly variable, unstable, and at a completely different voltage from the United States. Despite the harsh electrical testing conditions and remote location, the MSP worked well and produced very clean spectra (Figure G2) from the Grassquit photoreceptor oil droplets and visual pigments.

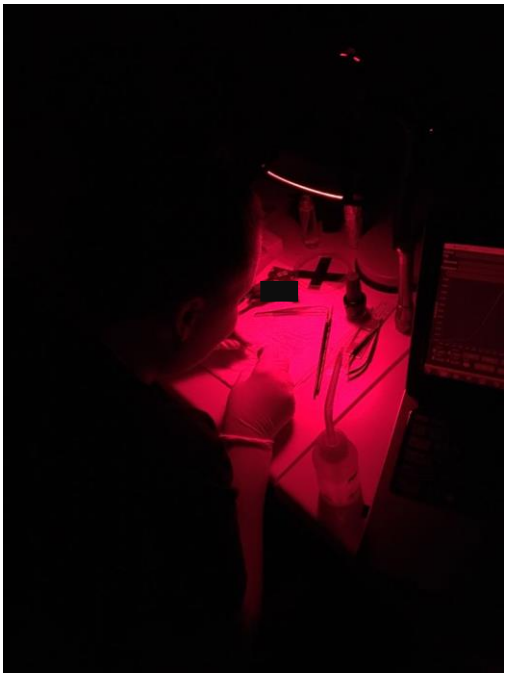


Figure G1. Portable microspectrophotometer ready for use and tissue preparations being made in a dark room in Brazil.

Analyzing the Results of the Test

When using microspectrophotometry on birds, the visual pigment spectra are the most difficult to find and measure, so we decided to look at these spectra specifically to test the functionality of the portable MSP. If the MSP is not functioning correctly, then we would not be able to measure these visual pigments, regardless of which species we tested. We were able to find and successfully measure these visual pigments, indicating that the MSP functions properly. We analyzed visual pigment spectra collected from the grassquits and compared the results with

values from other bird species published in the scientific literature. We found peak absorbance values for an ultraviolet sensitive visual [UVS] pigment at 362 nm, short-wavelength sensitive [SWS] visual pigment at 437 nm, medium-wavelength sensitive [MWS] visual pigment at 500 nm, long-wavelength sensitive [LWS] visual pigment at 573 nm, and the rod photoreceptor visual pigment at 505 nm) (Figure G2). These values fall well within peak absorbance ranges of avian visual pigments that we have measured in other species and in the scientific literature, so we considered the test successful. The portable MSP was then ready to measure the spectral properties of photoreceptors of the Golden and Bald Eagle anywhere in the United States.

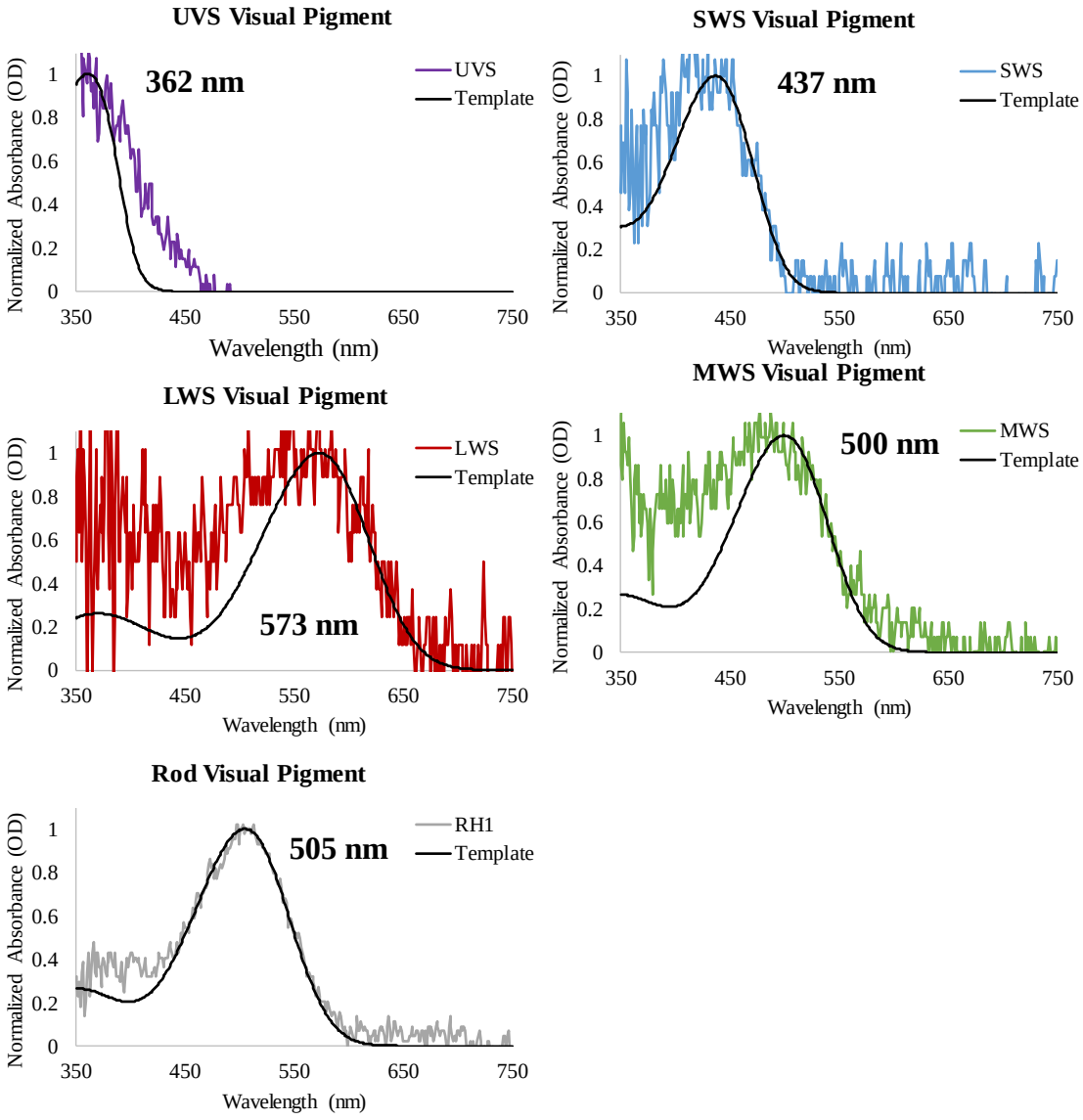


Figure G2. Normalized absorbance spectra of the various visual pigment types found in the Blue-Black Grassquit retina with peak absorbance values indicated. Black lines are fitted templates indicating both the typical shape and the peak absorbance location of the visual pigments. Sample size of one for each photoreceptor in the figures.

ATTACHMENT H

Milestone 5.3

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 5.3.

Milestone 5.3 – Successful test of the portable microscopy system

Task 5.0 – Testing of portable equipment with an invasive bird species (Month 9)

Task Summary:

Using available bird species (preferably invasive) at Purdue University, we tested on campus the anesthesia protocols and the auditory measurements on the portable anechoic chamber. Similarly, we extracted retinas of birds and tested on campus the portable microspectrophotometer and microscopy systems by measuring the spectral properties of the photoreceptors and the density of photoreceptors (using oil droplets as proxies).

Subtask 5.3 – Testing the portable microscopy system (Month 9)

Subtask Summary: We would collect measurements, using the portable microscopy system, on a species of bird. We would determine whether we could visualize the oil droplets in both bright and epifluorescent light. We would take pictures to compare the quality of the image with that of the regular microscopy system we have in the lab. If the quality were the same, we would consider the test successful.

Objectives

For a successful image acquisition of the oil droplets on the retinae of the Golden and Bald Eagles, we needed to test the portable microscopy system on fresh retinal tissue. We tested the assembly, functionality, and image output of the portable microscopy we devised with Carl Zeiss Microscopy, LLC (Attachment E). We obtained images from leftover retinal tissue of a Bald Eagle to determine if the images were of good quality for use in this project.

Testing the Portable Microscopy System

We had initially proposed to use the European Starling (*Sturnus vulgaris*) retina as they were readily available in our lab and are a non-releasable invasive species we must euthanize to comply with USDA regulations. Before we could perform the work with the starling, we had the

opportunity to acquire images from remnant Bald Eagle (*Haliaeetus leucocephalus*) retinal tissue from a recent data acquisition trip to the Wildlife Center of Virginia. We were unable to take the portable microscopy system on this data acquisition trip because it was not fully assembled (a fire wire card for the camera was still on backorder) until after our return. We took a different microscopy system (which was not portable since we could drive to Virginia) to collect images from this Bald Eagle retinal tissue during the data collection trip. Once returned from this trip, we imaged the oil droplets on the remnant retinal tissue and were successfully able to image multiple sites across the retina that remained. The images we collected were taken in both brightfield and epifluorescent light within which oil droplets can be seen (Figure H1).

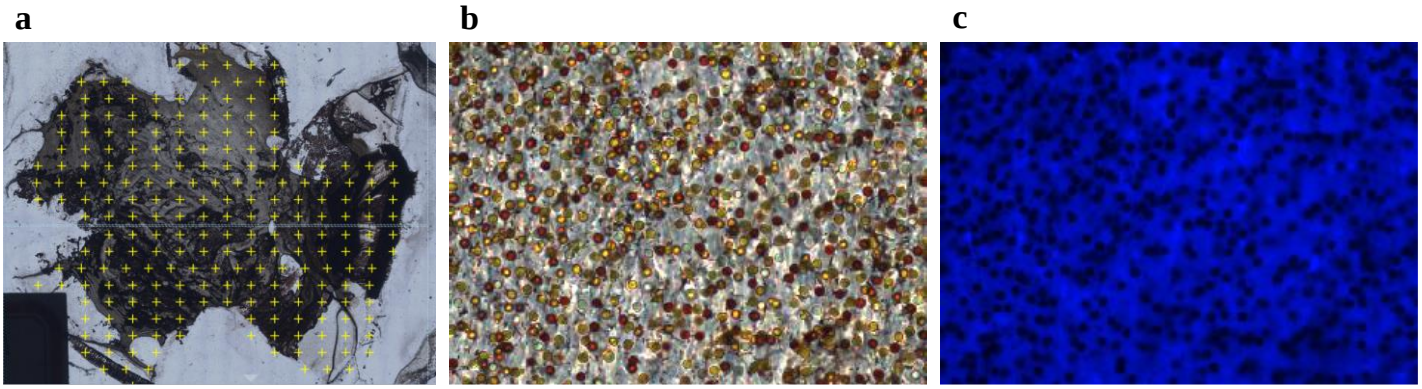


Figure H1. Images collected using the portable microscopy system. **a**) Image of the remnant Bald Eagle retinal tissue in the microscopy system, with grid markers (yellow + symbol) placed across the retinal tissue. **b**) Oil droplets viewed using the 40x objective under brightfield illumination. **c**) Oil droplets viewed using the 40x objective under epifluorescent illumination.

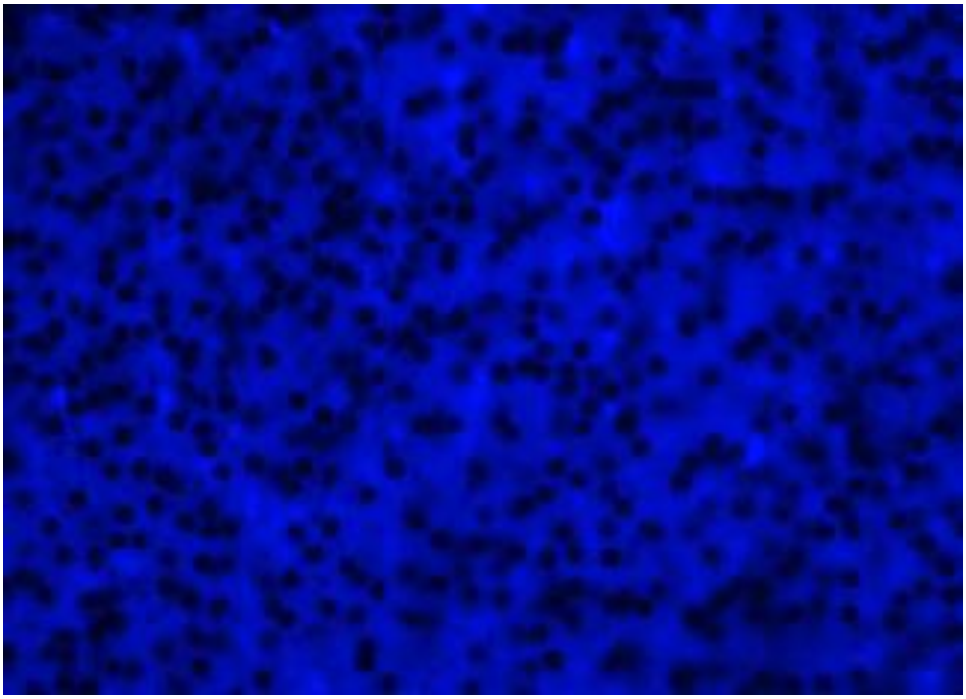
Comparing Images Taken with Purdue Microscopy Systems

At the time that the images in Figure H1 were collected, we considered the test of the portable microscopy system successful because we were able to image the oil droplets under both types of illumination. After the test, we were not given the opportunity to acquire images from fresh eagle retinal tissue for another 12 months. While we were waiting for eagle retinal tissue to image, we began acquiring images of Red-tailed Hawk (*Buteo jamaicensis*) retinal tissue. The Red-tailed Hawk was to serve as a proxy for the Golden and Bald Eagles in the event that we would have no additional opportunities to image retinal tissue from these species. We were able to take images from several Red-tailed Hawks provided to us by Soarin' Hawk Raptor Rehabilitation Center during months 17-19 of this project. Upon return of a member of the project from leave in month 20, we noticed, as we were going through the data collected, that there was an issue with the quality of the epifluorescent images.

The epifluorescent images collected on the portable microscopy system were showing the oil droplets within the image, however the oil droplets were not the right color compared to an

older Olympus microscopy system we had on campus (Figure H2). Most, but not all, of the oil droplets were showing up as dark circles (Figure H2a). We initially thought that the difference between the images from the systems was due to the difference in the microscope itself or manufacturers, but our Purdue stationary Zeiss microscopy system was able to image these oil droplets on other species (as far as we could tell at the time). We next thought that it might be a problem with the exposure time in the epifluorescent channel of the portable microscopy system, but tests with other remnant retinal tissue indicated this was not to blame.

a



b

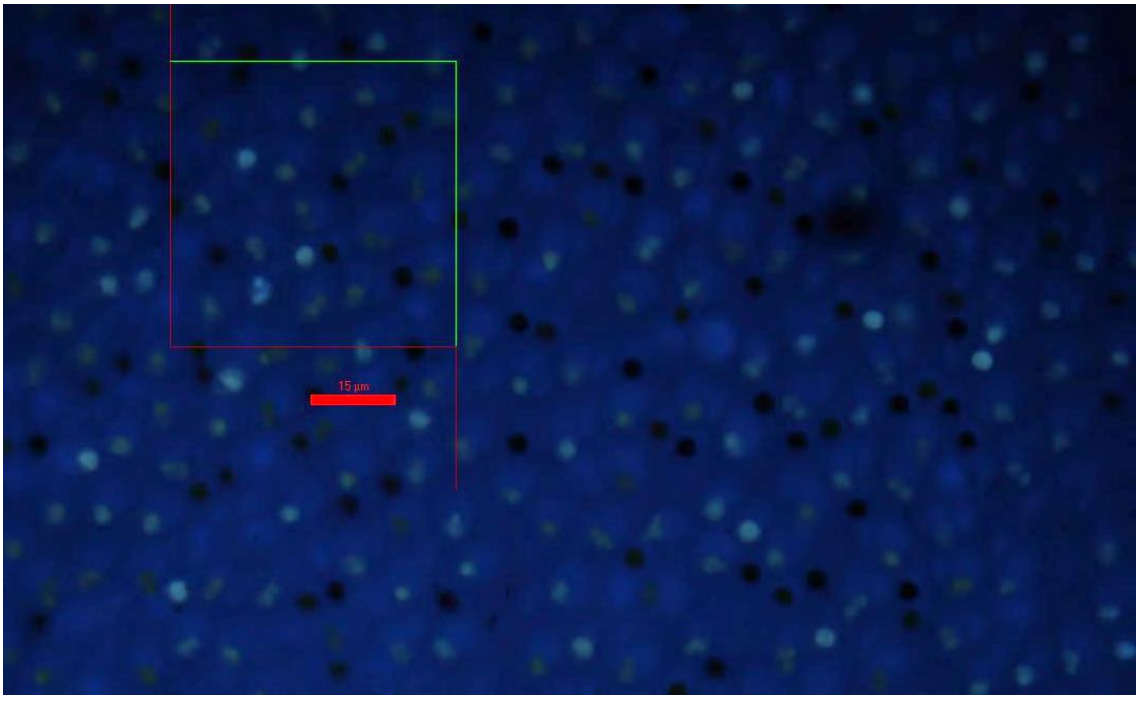


Figure H2. Epifluorescent images taken from the portable Zeiss microscopy system (**a**) and the stationary Olympus microscopy system (**b**). Red and green box in panel (**b**) is a counting frame applied to every image, please disregard for the purposes of this figure.

After multiple site visits and tests by our Zeiss representative, we found that the issue was an incorrect filter cube was purchased for both the portable Zeiss and stationary Zeiss microscopy systems (the system our portable system was modeled after). The filter cube is a special set of filters that is placed in the microscope and filters out certain wavelengths of light that reach the camera. The filter set #49 that was on both systems was filtering out the light needed to identify the oil droplets (Figure H3a). With the help of our Zeiss rep. we requested to trial a new filter set (Filter set #1) that had similar filtering characteristics as our Olympus microscopy system. Once the filter set arrived, we immediately tested it on fresh retinal tissue and were able to see the oil droplets with the correct colors. We eventually ordered a new filter set (Filter set #2 as it was more like the Olympus filter set; Figure H3b) for both of our Zeiss systems and the issue was resolved.

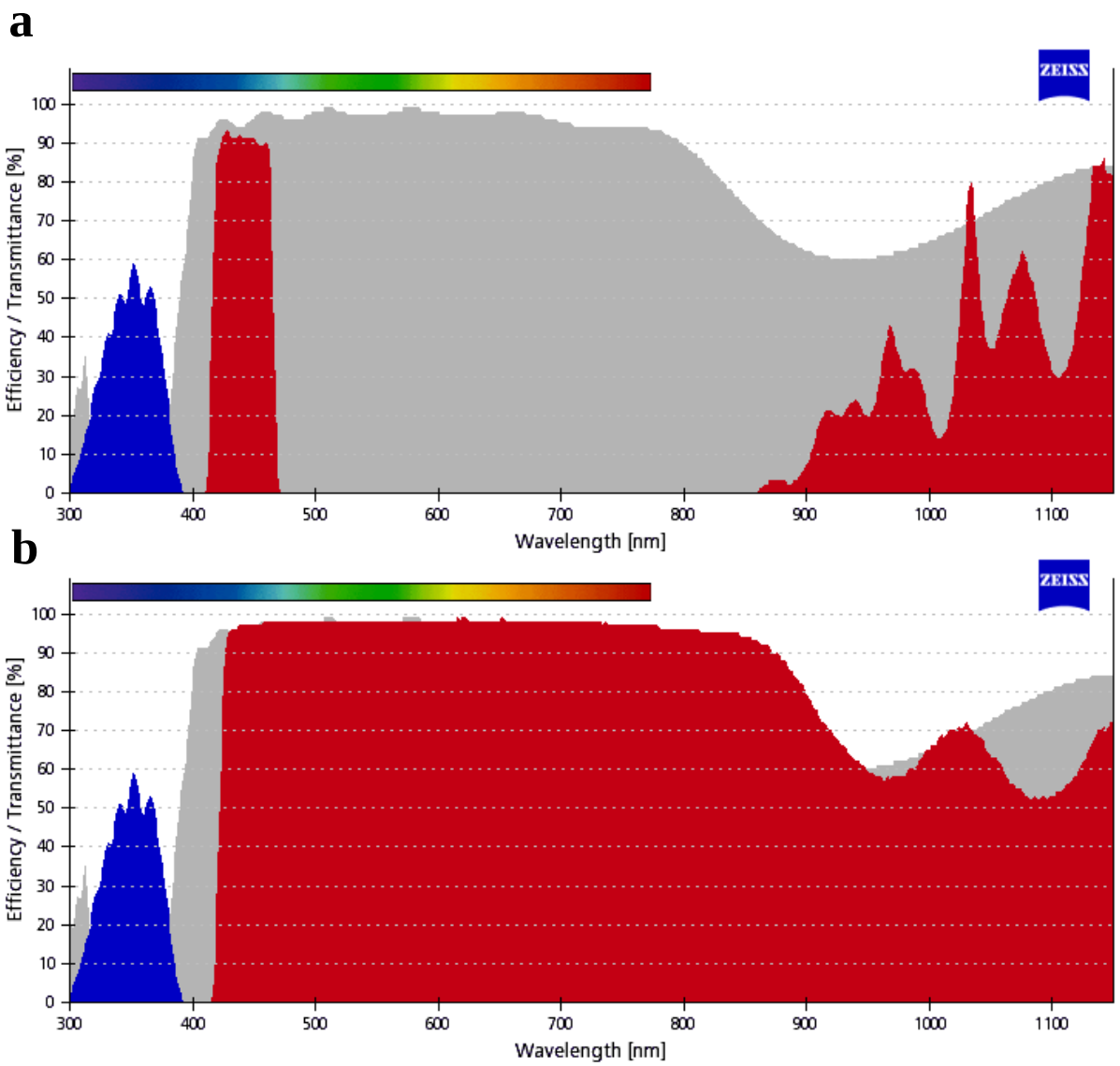


Figure H3. Carl Zeiss filter set #49 (**a**) and filter set #2 (**b**) spectra. The blue and red regions of the spectra indicate wavelengths (nm) of light that can be transmitted through the filter set. Notice the severe restriction of light in filter set #49 compared to filter set #2. Graphs obtained from the Zeiss Filter Assistant at www.micro-shop.zeiss.com/en/us/shop/filterAssistant/filtersets/

Unfortunately, by the time the new filter sets arrived, we no longer had the opportunity to image fresh retinal tissue from Golden or Bald Eagles using this portable Zeiss microscopy system. During the course of the project, however, we were able to attain images of the oil droplets on the retinas of the Golden and Bald Eagles using the old Olympus microscopy system we previously mentioned (Figure H4), which took the images of the eagle tissue correctly and had a correct filter set. This system is not meant to be portable and it was damaged during

transport on commercial aircraft. Thankfully, the damage was not significant enough to prevent the acquisition of the retinal images needed to complete the vision component of this project.

Figure H4. Old Olympus microscopy system installed at testing facility near the Wildlife Center of Virginia.

No Golden or Bald Eagle retinal tissue imaging data were lost over the course of the project due to the issue with the Zeiss microscopy system. This was because the one trip we were able to take the Zeiss system on, we also had the Olympus microscopy system (Figure H4) as a backup. We were successfully able to image the retinal tissue on the backup Olympus system during that trip.

ATTACHMENT I

Milestone 7.1

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken to gather auditory data from anesthetized eagles for the completion of Milestone 7.1.

> ***Milestone 7.1 – Obtain auditory data from 5-7 individuals of each species (this number would likely be higher for bald eagles given the higher availability of this species in rehabilitation centers). These traits include critical ratios (how loud sounds must be to be processed in the presence of noise), frequency sweeps and amplitude modulated sounds (particularly likely to be alerting), and processing of sounds that are of suprathreshold intensity (i.e. at about the level detectable in the field).***

<u>Task 7.0 – Gathering sensory data in rehab centers</u> (Month 8-26)

Task Summary:

After developing contacts with multiple rehabilitation centers that house Golden and Bald Eagles, we traveled with our equipment to the Wildlife Center of Virginia, where we stored our anechoic chamber. The post-doc and technician traveled to the Wildlife Center of Virginia multiple times and other rehabilitation center/s where we gathered the visual and auditory data needed to complete this task.

Subtask 7.1 – Gathering the auditory data (Month 8-19)

Subtask Summary: We measured the responses of the peripheral auditory system (auditory nerve and brainstem) in anesthetized eagles stimulated with a variety of different sounds. The sounds ranged from static frequency tones and stacks of tones, to highly dynamic linear and sinusoidal frequency modulations. The eagles were anesthetized during this procedure and were closely monitored by rehabilitation center veterinarians.

Objectives

Measure Auditory Evoked Potentials (AEPs) of Bald and Golden Eagles for a variety of sound stimuli under different noise conditions. AEPs show the responses of the peripheral auditory system to sound stimulation in the subject eagles. Eagles were anesthetized for the duration of the experiment, and recovered fully after these measurements. The technique used to

collect the auditory measurements is designed to minimize the impact on the subject eagles (total experiment time < 2 hours) while providing information about eagle hearing for a wide variety of sounds as well as hearing in noisy conditions.

Subjects

We measured six Bald Eagles (*Haliaeetus leucocephalus*) and two Golden Eagles (*Aquila chrysaetos*) at the Wildlife Center of Virginia in Waynesboro, VA (5 Bald Eagles; April-September 2018) and at Liberty Wildlife in Phoenix, AZ (1 Bald Eagle, 2 Golden Eagles; February 2019). These eagles were all healthy and were no longer receiving clinical treatment. They were in their final stages of rehabilitation with the goal of eventual release. Experiments were conducted in collaboration with and at the discretion of the veterinary staff of each rehabilitation facility. All work with the eagles was conducted with approval of the Purdue Animal Care and Use Committee (PACUC Protocol # 1705001579) as well as US Fish and Wildlife (Permit #: MB41892B-1) and state authorities of Virginia (Permit #: 62486) and Arizona (Permit #: SP638641).

Anesthesia

For the auditory evoked potential (AEP) measurements, the eagles were fully anesthetized with injectable and inhaled anesthesia. The mixture was necessary because inhaled Isoflurane commonly used in veterinary procedures with eagles attenuates the peripheral auditory system responses (see Thiele & Köppl, 2018). Eagles were initially anesthetized with an intramuscular injection of 0.20 mg/kg Butorphanol, 0.40 mg/kg Midazolam, and 0.08 mg/kg Dexmedetomidine. Isoflurane (1%) was then administered as necessary to prepare the eagle for intubation, followed by insertion of an intravenous (IV) or intraosseous (IO) catheter on the right leg of the bird.

Once ready with intubation tube and catheter, the eagle was moved to the experimental chamber and connected to a supply of oxygen and 0.25% isoflurane, as well as an IV. An esophageal stethoscope was used to monitor heart rate of the subject eagle from outside the anechoic chamber and a USB “night vision” webcam provided a live visual update on the animal (Figures I1-2). The oxygen and isoflurane supplies for the intubation tube (as well as a bag to allow manual respiration if necessary), stethoscope ear pieces, and IV line were all routed through openings in the Faraday cage and anechoic chamber wall to allow the veterinarian to adjust anesthesia and monitor animal condition from outside the closed anechoic chamber.

Figure I1. Veterinary staff from the rehabilitation center managed the anesthesia of the subject eagle and monitored eagle condition from outside the anechoic chamber. The setup in this figure is from the Wildlife Center of Virginia. Low doses of isoflurane were administered throughout the experiment, with periodic doses of injectable anesthetics administered by IV catheter.

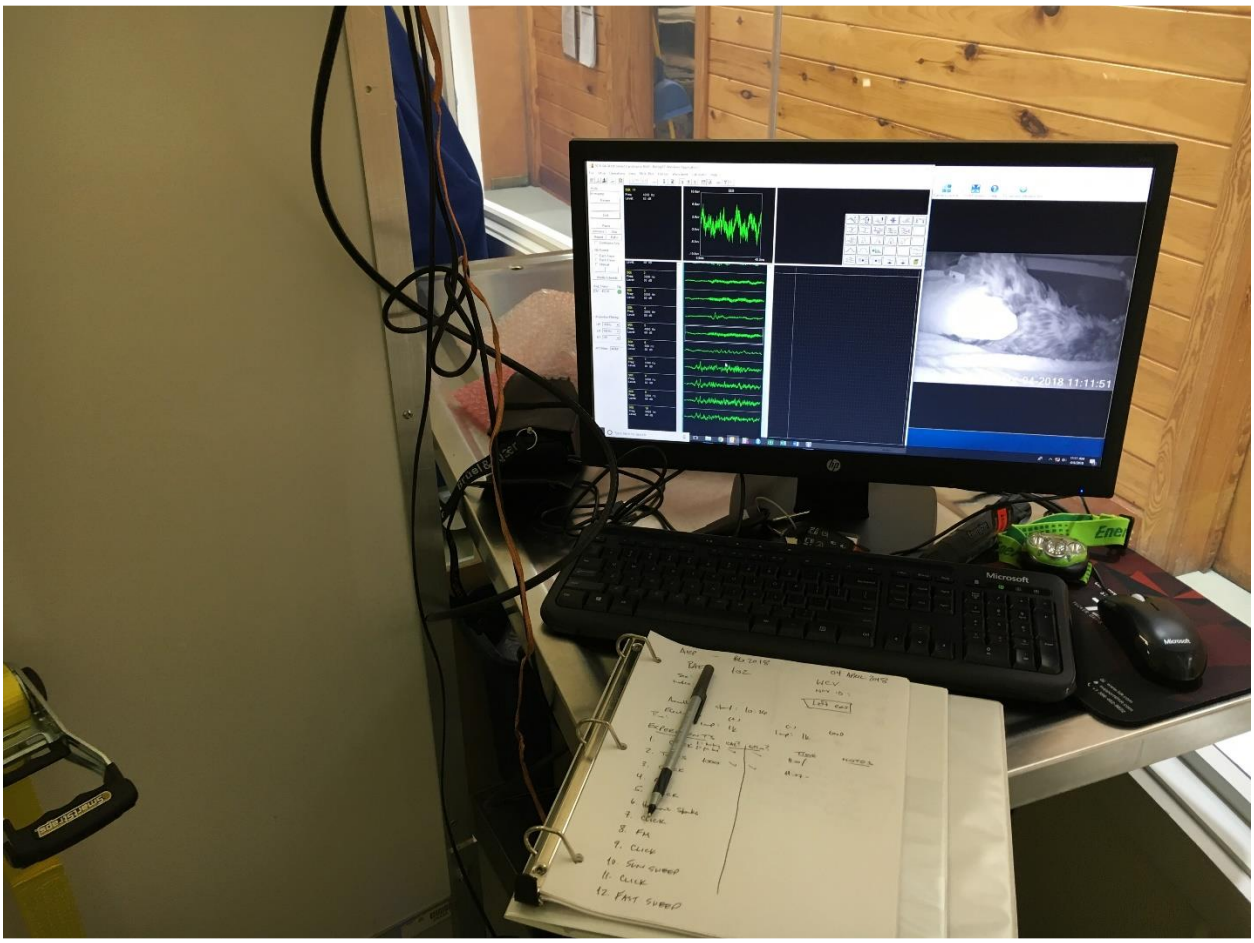


Figure I2. A view of the electronic side of the auditory evoked potential experiment in action at the Wildlife Center of Virginia. Live electrode recordings as well as completed stimulus tests are shown as green traces on the left of the computer screen in the image. A live webcam was used to monitor the subject as seen on the right of the computer monitor. The anechoic chamber is on the left of the bench with the computer.

Experiments were designed to allow a stoppage every 30 minutes for injection of half-doses of the injectable anesthetic mixture. These top-ups were administered as necessary based on the recommendation of the monitoring veterinary staff in consultation with the researcher. Auditory evoked potentials are relatively weak electrical signals, so large amplitude bursts of activity in the electrode recordings could be used as an indicator that the subject bird was no longer anesthetized fully. The injectable half-dose was delivered via the IV/IO catheter and flushed with mix of Plasmalyte (4 ml/kg/hr), Hetastarch (15 ml/kg), and Normosol (10 ml/kg). Experiments resumed after the veterinarian indicated the subject eagle's condition was stable after the fresh top-up of anesthesia. The subject was given 0.25-0.5% Isofluorane throughout the experiment to maintain stable heart rate and respiratory rate. The veterinarian was present and monitored the condition of the eagle throughout the procedure without opening the anechoic chamber.

At the end of the experiment, the eagle was quickly extracted from the experimental chamber and the intubation tube was removed. A reversal agent for Dexmedetomidine, Atipemazole (0.40 mg/kg), was administered to speed up final recovery. The total procedure from induction to recovery was approximately 3-3.5 hours with 30 min to 1 hour of preparation (intubate, position, and ensure stable condition of the animal), 1.5 hours of experiment trials in three 30-minute blocks, and 1 hour recovery post-experiment.

Auditory Evoked Potentials

Anechoic chamber

The anechoic chamber is a 1.22x1.22x1.22 m cube constructed of 3 mm thick aluminum composite material sheets (Meyer Plastics Inc., Indianapolis, IN) and 6061 aluminum 90 degree angle iron to connect and reinforce the edges. The chamber is lined with two layers of anechoic foam: first a layer of 1.5 cm Fireflex flat panels of foam, then 7.6 cm thick UNX-3 SONEX classic polyurethane foam. One side of the cube had a 0.91 by 0.91 m door that hinged at the top. Inside the chamber was a 0.81 by 0.81 by 0.46 m (l x w x h) Faraday cage made of 10 mesh copper wire (0.025 inch wire diameter) over a wooden frame. The top half could be lifted off and removed from the chamber to allow easy setup of the subject animal.

Stimulus/Recording Equipment

Experimental stimulus presentation and response recordings were controlled by an RZ6 Multi I/O Processor unit (Tucker-Davis Technologies Inc., Alachua, FL). Output from the RZ6 was passed through an Ultragraph Pro FBQ6200HD equalizer (Behringer, Willich, Germany) and then a Crown D-75 amplifier (Crown Intl., Elkhart, IN) before sounds were played from a JBL Control 25AV speaker (JBL Professional, Los Angeles, CA). Auditory evoked potentials (AEPs) were measured using a RA4LI headstage with RA4PA 4-channel Medusa Preamp (Tucker-Davis Technologies Inc., Alachua, FL) connected to the RZ6. We used 3-lead, 27 gauge, 13 mm Disposable Horizon Subdermal Needle electrodes (Rochester Electro-Medical, Lutz, FL) placed posterior to the left ear opening (+), on the crown of the head (-), and on the breast (ground) and adjusted so that impedances were under 3 kΩ. The headstage was placed next to the animal inside the Faraday cage. The speaker was positioned 45 cm above the bird's head and outside of the Faraday cage. The rest of the equipment remained outside the anechoic chamber (Figure I3).

Figure I3. The anechoic chamber was placed in a location determined by the wildlife or rehabilitation center. Electronics for generating stimuli and recording auditory responses from eagles were outside the anechoic chamber.

Program Parameters

A computer running BioSigRZ controlled both stimulus presentation and electrode recording simultaneously. Stimuli were generated using SigGenRZ.

Speaker calibration

The JBL speaker inside the anechoic chamber was calibrated in two steps. First, we loaded the CAL200K file in BioSigRZ and used the calibration tool with a PCB Model 378C01 microphone (2.0 mV). We used the calibration software within BioSigRZ to generate a calibration file. Next, we replaced the PCB microphone with a Type 2250 Hand Held Sound Level Meter with the microphone on a 2.0 m cable (Brüel & Kjaer, Naerum, Denmark). We used this sound level meter to verify calibration and, if necessary, to adjust the equalizer so that all frequencies from 100 to 8000 Hz were calibrated to within 1 dB of 80 dB SPL (Sound Pressure Level).

Stimulus Sounds

Final auditory evoked potential (AEP) recordings for each stimulus were averages of 500 repeated stimulus presentations (see Gall et al. 2011). Each type of stimulus sound (Figure I4) was grouped such that all tones were played together, followed by all amplitude-modulated sounds, etc. Between each stimulus type, we recorded the auditory brainstem response (ABR) to a series of clicks to provide a baseline measure reflecting the state of the anesthetized animal. The stimulus types were determined ahead of time to ensure the experiments were grouped into 30-minute blocks of stimuli. All stimuli except clicks had 2 ms $\cos^2$ onset/offset ramps. The stimulus types were as follows.

Noise

We used two generic forms of noise for this study: white and pink noise. White noise contains an equal amount of energy at all frequencies. The energy profile of pink noise is skewed toward lower frequencies (technically inversely proportional to frequency). White noise approximates a number of natural sources of noise (Handel & Chung 1993). Noise generated by wind through vegetation, for example, can have properties similar to white noise (Bolin 2009). However, noise profiles under many conditions tend to have more energy at lower frequencies. Pink noise is commonly used in a variety of studies to mimic noisy backgrounds (Airo et al. 1996; Schlittmeier & Hellbruck 2009; Potvin et al. 2016; Howarth & Griffin 1991). Moreover, the over-representation of lower frequencies in pink noise has been shown in measurements of wind turbine noise (Schomer et al. 2015). Note that these noise backgrounds are not meant to mimic a specific noise, but are used as a general representation of two kinds of noise profiles that are commonly found in nature.

Tones

We tested six different pure tones (0.5, 1, 2, 3, 4, 5 kHz) spanning the range of frequencies we predicted eagles would hear the best, based on previous experience with avian auditory physiology. We randomized the order of the six tones for each eagle, but all treatments within an eagle received the same six-tone order. Tones were 30 ms in duration, preceded by 10 ms background only, and played at a rate of 18.3 Hz with silence between each 40 ms stimulus. The treatments and backgrounds are as follows: each tone was presented at 80 dB SPL (Sound Pressure Level) initially with a silent background. The same order of tones was then presented at 80 dB SPL with 80 dB SPL white background noise. Finally the tones were presented at 80 dB SPL with 80 dB SPL pink background noise. This experiment was then repeated first with 70 dB SPL tones, and then 60 dB SPL tones but noise level remained 80 dB SPL. The order of background noise conditions was not varied during the experiments, partly to initially ensure that we would have data from multiple individuals for comparison even if there were issues with anesthesia. After we performed the experiments with several Bald eagles without issue, we

analyzed auditory responses and found no suggestion of a change in auditory performance over time, so we continued with the same order for our noise presentation. The consistency of noise masking across different individuals and stimulus sounds suggests that noise masking had a large effect relative to any potential adaptation of the auditory system to the stimuli.

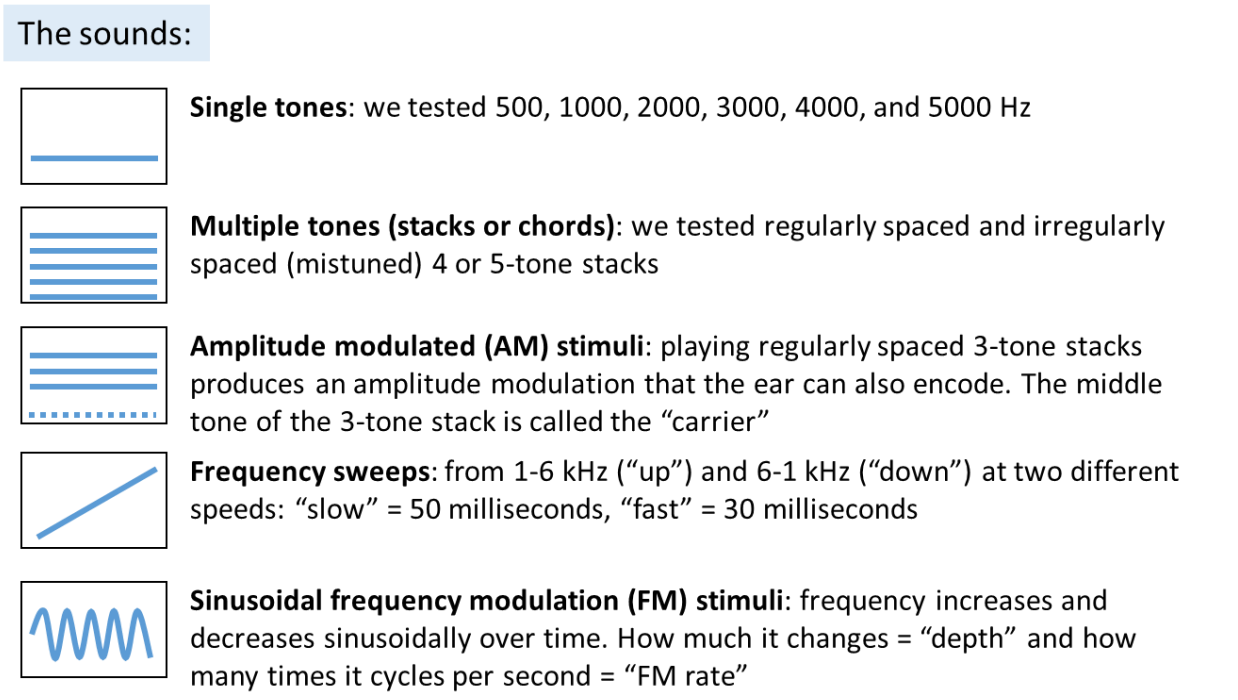


Figure I4. A summary figure of the types of sounds used as stimuli in the study. Each diagram illustrates the frequency over time of a representative stimulus in the group. For example, a single tone has a single frequency over time, and a frequency sweep has a linear change in frequency over time.

Harmonic Stacks

We tested three different stacks of tones at 80 dB SPL, first with a silent background, then with white noise, and finally with pink noise. The first stack was a 1 kHz harmonic stack containing 1, 2, 3, 4, and 5 kHz. This stack contains the 1 kHz fundamental frequency, which is equal to the spacing between consecutive tones. The second stack was a 600 Hz harmonic stack missing the fundamental frequency (600 Hz) tone. It contained 1.2, 1.8, 2.4 and 3.0 kHz tones (no 600 Hz tone, but 600 Hz spacing). The final stack was a series of five non-harmonic ("mistuned") tones (1.0, 2.2, 3.3, 3.6, and 4.7 kHz) with random spacing but otherwise in the 1-5 kHz range. Stack stimuli were 40 ms in duration starting with 10 ms background noise, then 30 ms of harmonic stack played over the background. These sounds were presented at 18.3 Hz.

Amplitude-modulation

Amplitude-modulated signals were generated by playing three equally spaced tones. The middle tone is called the carrier, and the high and low sideband tones are defined relative to the carrier. The high sideband is the carrier plus the desired amplitude modulation (AM) rate and the low sideband is the carrier minus the AM rate. We used three different carrier frequencies (1, 2, and 3 kHz) and three different AM rates (100, 400, 700 Hz) with each carrier. Each of the nine carrier-AM stimuli was presented first with a silent background, then with white noise, and finally pink noise. AM stimuli were 60 ms in duration, with 10 ms background preceding the onset of the 50 ms AM signal. These stimuli were played at a rate of 13.1 Hz.

Frequency Sweeps

Frequency sweep stimuli are rapid linear frequency changes from 1 to 6 kHz (up sweep) or 6 to 1 kHz (down sweep). We tested two different rates of frequency sweeps: either fast (30 ms) or slow (50 ms). Each sweep was preceded by 10 ms of background sound only (no noise, white noise, pink noise) such that fast sweep stimuli were 40 ms total (10+30 ms) and slow sweeps were 60 ms (10+50 ms) in duration. Fast sweeps were presented at a rate of 18.3 Hz and slow sweeps at 13.1 Hz.

Sinusoidal Frequency-modulation

Sinusoidal FM stimuli were all centered on a 2 kHz signal which frequency modulated sinusoidally at two modulation rates (70 and 110 Hz) and two depths (400 and 700 Hz – here defined as the difference between mean and minimum or maximum frequency). Stimuli were a total of 85 ms in duration with 10 ms background only (no noise, white noise, pink noise) preceding the 75 ms FM sound. These stimuli were presented at 10.1 Hz.

Clicks

We used short, 0.1 ms, broadband clicks to determine a baseline responsiveness of the subject eagle auditory system during the experiments. Clicks were alternated in phase by 180 degrees to minimize any cochlear microphonic components that may bias our measure of the Auditory Brainstem Response (ABR) elicited by the click (Hall 2007). Clicks were broadcast before and after each experiment, and before and after each top-up anesthesia injection. We primarily used the clicks as a real-time diagnostic to ensure the auditory system was responding to sound stimulation with a predicted onset response. No abnormalities were found and we do not present any further analysis of the click results as they have little functional significance.

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ATTACHMENT J

Milestone 7.2

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the collection of data for the completion of Milestone 7.2.

> ***Milestone 7.2 – Obtain visual data from 3-6 individuals from each species (this number would likely be higher for bald eagles given the higher availability of this species in rehabilitation centers). These traits include visual field configuration (size of the binocular, lateral and blind areas), density of photoreceptors, peak sensitivity of visual pigments, absorbance of oil droplets, etc.***

Task 7.0 – Gathering sensory data in rehab centers (Months 8-26)

Task Summary:

After developing contacts with multiple rehabilitation centers that house Golden and Bald Eagles, we traveled with our equipment to the Wildlife Center of Virginia, where we stored our anechoic chamber. The post-doc and technician traveled to the Wildlife Center of Virginia multiple times and other rehabilitation centers where we gathered the visual and auditory data needed to complete this task.

Subtask 7.2 – Gathering the visual data (Months 8-26)

Subtask Summary: We measured the different visual parameters including visual field configuration (size of the binocular, lateral and blind areas), density of photoreceptors, peak sensitivity of visual pigments, and absorbance of oil droplets. Visual fields were measured in live animals and the other measurements were made on salvaged tissue. After the euthanasia was performed by the rehab center personnel, we extracted both eyes, which we processed for the different visual system measurements and techniques listed above.

Objectives

In order to develop Golden and Bald Eagle specific visual stimuli or deterrents that can be deployed in wind turbine farms, we had to understand how these species see their environment. To collect this information we needed to travel to various rehabilitation centers that we had established relationships with, and measure the visual system of the Golden and Bald Eagles. While there, we attempted to measure visual field configurations (size of the binocular, lateral and blind areas) on a live restrained eagle, the density of the photoreceptors, the peak

sensitivity of visual pigments, the absorbance of oil droplets, and the transmittance of the ocular media within the eye.

Overview of Vision in Birds

Most birds allocate a lot of energy and resources to their visual system because of its importance in foraging, breeding, detecting predators, and flying. Birds have a specialized visual system that is often adapted for the needs of specific types of birds (e.g., birds that detect food items at far distances vs. birds that detect food items at close distances, etc.). This specialization results in a high degree of variation between bird species in how they see their world – a key reason why we need measurements of both Golden and Bald Eagles. To understand the relevance of these measurements as they relate to eagle vision and designing visual deterrents, we first have to understand the basic components of the avian eye and their functions.

As seen in Figure J1, light must pass through a series of structures before it can reach the photoreceptors in the retina. These semi-transparent ocular structures (cornea, lens, and vitreous humor) are collectively called the ocular media. These ocular media are vitally important for protecting the retina, but they also have optical properties that affect which wavelengths of light reach the retina. Therefore, the ocular media act as a filter of light before it reaches the photoreceptors.

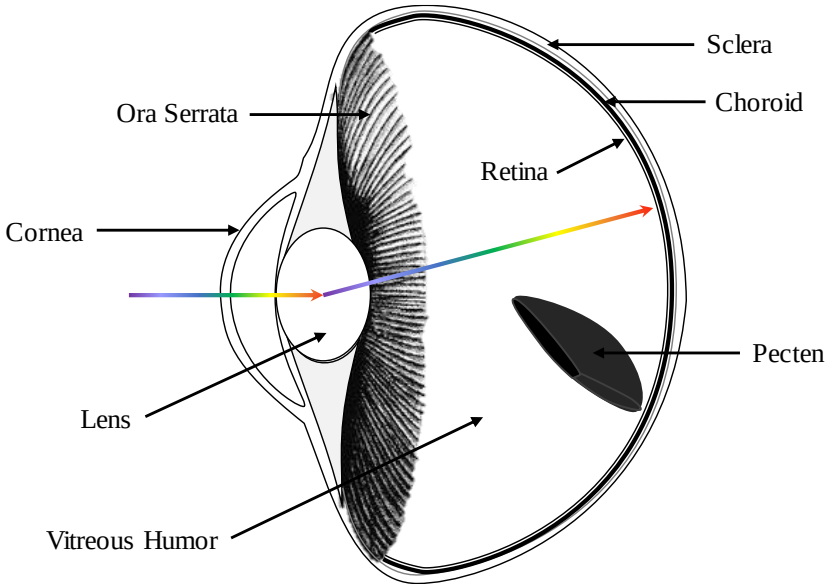

Figure J1. Diagram of the eye of a bird. Multicolored arrows represent light passing through the ocular media within the eye.

For example, humans' ocular media does not allow UV light (wavelengths <400nm) to be transmitted through the eye because UV light can be very damaging to the retina, but birds' ocular media allows UV light from ~300-400nm to reach the retina. However, the light transmitting properties of the ocular media have been shown to vary between bird species, necessitating the need to measure these properties on the Golden and Bald Eagles. Without information on the ocular media of these eagles, we would not know for example if a UV light

deterrent at 350 nm could even be seen by the eagles. We used these ocular media properties in our eagle visual modeling (i.e., mathematical algorithms to estimate how a given species visually perceives a given object) to assess the visual capacity of these species relative to different types and colors of objects.

Light that has passed through the ocular media reaches the retina and passes through several layers of cells before reaching the photoreceptors, which are responsible for converting light into a neural signal. The avian retina is composed of a highly ordered but heterogeneous mosaic of various photoreceptor cells (Figure J2) including four types of single cones (used in color vision; humans have three), double cones (used in motion and brightness detection; humans do not have double cones), and rods (used in low-light vision; humans have rods). Each of these 6 types of photoreceptor has a unique pattern of sensitivity over a particular range of light wavelengths. Collectively these photoreceptors send signals to the visual centers of the brain through the retinal ganglion cells, whose cell bodies are in the retina with axons extending to the brain.

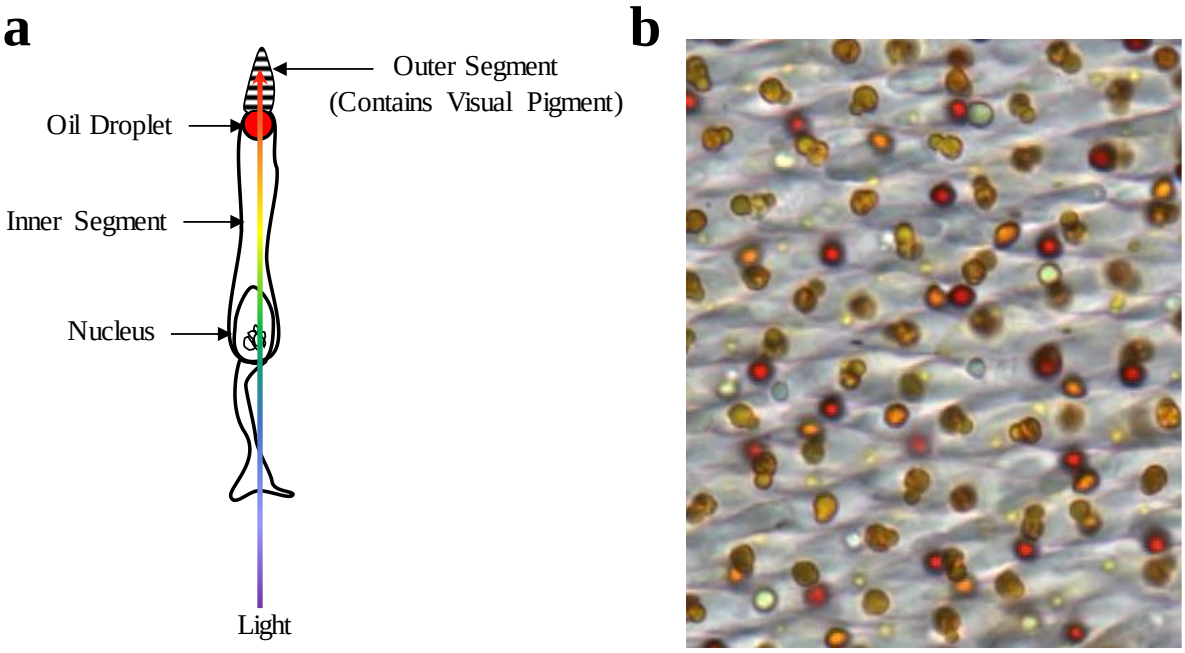

Figure J2. Photoreceptors in the avian retina. **a**) Diagram of avian single cone photoreceptor with main components labeled. Multicolored arrow shows the path that the light follows. **b**) Photograph taken of the photoreceptors in the retina of a Golden Eagle. Oil droplets are the highly visible spheres seen here in the typical mosaic pattern of photoreceptors on the retina.

In order to determine the patterns of sensitivity of the avian eye, we must first understand how the photoreceptors and their cellular components transmit and collect light. In cone photoreceptors, as seen in the figure above (Figure J2), light first passes through the main body of the cell (the inner segment) and then proceeds through a spherical carotenoid-filled organelle called an oil droplet. Carotenoids within the oil droplet filter out specific wavelengths of light

that end up reaching the outer segment (which contains the visual pigment). This filtering of light by the oil droplet fine-tunes the sensitivity of the photoreceptor (sometimes reducing the range of wavelengths to which the photoreceptor is sensitive by as much as 50-100nm) allowing for better color discrimination by the single cones used in color vision. The oil droplets contained within the photoreceptors also allow for the easy determination of photoreceptor distribution across the retina, which is incredibly variable between species. We collect this information on photoreceptor spatial distributions to be able to identify areas of acute vision, and to be able to account for neural error in our eagle visual contrast models.

The absorbance of the visual pigments does not vary across the retina (Hart 2001), but it is species specific. The sensitivity range of each type of photoreceptor forms the basis of how vision works at the level of the retina and is critical to understanding the wavelengths of light that an eagle is most sensitive to. When collecting information on the visual system in birds, it is critical that we measure the densities of each photoreceptor type and the light transmitting properties of their oil droplets, as well as the absorbance properties of the visual pigments, to incorporate properties of the eagle visual system into our identification of effective visual deterrents for wind turbines.

To identify potentially effective visual deterrents we used visual perceptual modeling to identify LEDs that are conspicuous against a given visual background from both the Golden and Bald Eagle visual system. This model requires the input of three distinct types of information; 1) the sensitivity of the eagles' visual system and the relative densities of the photoreceptors on the retina, 2) the ambient light of the environment that the eagle is in, and 3) the reflectance or radiance properties of objects or lights in the environment that the eagle will view. The results of this modeling will allow us to determine "sweet spots" for both species of eagles' visual systems. We can then select LEDs within these visual sweet spots for use in experiments to determine the behavioral responses of the eagles to these LED light stimuli.

Data Collection Methods

We were given the opportunity to measure the visual field configurations and retinal tissue measurements on 15 Golden Eagles (*Aquila chrysaetos*) and 12 Bald Eagles (*Haliaeetus leucocephalus*) sporadically from March 2018 to September 2019. These eagles were provided to us by rehabilitation centers across the United States. Due to the piece-meal nature of the tissue collection (in some instances we were able to take measurements using multiple techniques on a single eagle), please review Table J1 for a breakdown of the measurements taken for each eagle in this study and where they were acquired. All work performed with these eagles was at the consent of each rehabilitation center and approved under the Purdue University Animal Care and Use Committee Protocol # 1705001579. State and Federal Salvage permits were also in place through Co-P.I. Dr. Todd Katzner (USGS) authorizing all salvaged tissue collection and measurement.

Table J1. Table of eagle individual identity, rehab center name and location, and technique used (marked with an X). Sex and approximate age of eagle stated when known.

Species	Eagle ID	Sex	Age	Rehab Center	Location Of Center	Visual Fields	OMT	PH Densities	RGC Densities	MSP
GOEA	101	F	AD	CRC	California	X				
GOEA	102	M	AD	CRC	California	X				
GOEA	103	M	JV	MRCC	Montana				X	
GOEA	104			BMW	Oregon					X*
GOEA	105	M	JV	BMW	Oregon	X				
GOEA	106	F	JV	BMW	Oregon	X	X	X		
GOEA	107	F	JV	LW	Arizona	X				
GOEA	108	M	AD	LW	Arizona	X				
GOEA	109	M	AD	LW	Arizona	X				
GOEA	110		JV	LW	Arizona					X*
GOEA	111		JV	LW	Arizona					X*
GOEA	112		JV	LW	Arizona					X*
GOEA	113	F	AD	MRCC	Montana				X	
GOEA	114	F	AD	MRCC	Montana		X			X
GOEA	115	F	AD	MRCC	Montana		X	X		
BAEA	101	M	AD	WCV	Virginia	X		X	X	
BAEA	102		JV	WCV	Virginia	X		X	X	
BAEA	103	F	AD	WCV	Virginia	X				
BAEA	104	F	AD	WCV	Virginia	X				
BAEA	105	M	JV	WCV	Virginia	X				
BAEA	106	M	JV	MRCC	Montana				X	
BAEA	108		AD	LW	Arizona					X*
BAEA	109	F	JV	MRCC	Montana				X	
BAEA	110	F		WCV	Virginia					X
BAEA	111		AD	BMW	Oregon					X*
BAEA	112		AD	BMW	Oregon					X*
BAEA	113		JV	BMW	Oregon					X*

*Eye(s) was frozen when retrieved; only oil droplets were measured.

OMT = Ocular Media Transmittance, PH = Photoreceptors, RGC = Retinal Ganglion Cell, and MSP = Microspectrophotometry, GOEA = Golden Eagle, BAEA = Bald Eagle, F = Female, M = Male, AD = Adult, JV = Juvenile, BMW = Blue Mountain Wildlife, CRC = California Raptor Center, LW = Liberty Wildlife, MRCC = Montana Raptor Conversation Center, and WCV = Wildlife Center of Virginia.

Visual Field Configuration

We were able to measure the visual field configuration of 7 Golden Eagles (4 Male, 3 Female) and 5 Bald Eagles (2 Male, 2 Female, 1 Unknown) using an ophthalmoscopic reflex technique as in Martin (1984). We were able to measure the converged (each eye rotated to the maximum anterior position in the head) and diverged (each eye rotated to the maximum posterior position in the head) visual fields, as well as eye movements. We were unable to measure the 'at rest' visual field configuration in both the Golden and Bald Eagle as it was impossible to tell when the eye was at rest. This was due to the ability of the eagles to stare at the observer for extended periods as we were attempting to collect these data.

Measurements were collected by restraining the eagles on a custom-made platform in the center of the visual field apparatus. The platform was constructed out of a single piece of medium density fiberboard that was 38 cm by 76 cm and covered in foam, with the front corners cut off to reduce the obstruction of the platform into the visual field space (Figure J3). The platform was supported by six collapsible monopods to adjust the height and angle that the platform was held once in the apparatus. This allowed us to accommodate a wide variety of eagle body sizes. A pine beak holder covered in medical wrap tape was attached to the front of the platform and held at a 15-degree downward angle in order to maintain the resting beak angle of the eagles.

The eagle's body was wrapped with a piece of cloth to secure the wings and medical wrap tape wrapped around the feet before being placed into a foam cradle on the platform. Once on the platform, the body of the eagle was secured with Velcro straps. The eagle's head was held in place using nylon straps secured to the platform via a grommet and Velcro system. The beak was secured to the beak holder using gaffers tape so that the top of the lower mandible (straight on the beak) was held parallel to the 90° elevation within the apparatus for both species. During the visual field measurements, we constantly monitored the eagle looking for any signs of distress (e.g. nasal discharge, repeated attempts to remove head from restraints, and increase in body temperature). If we observed any signs of distress, the measurements would cease and the eagle would immediately be removed from the restraints. In some instances, we were not permitted to restrain the eagle, so the eagle was held by rehab center personnel during the measurements. Proper head orientation was ensured by personnel constantly checking the positioning of the eagles head within the visual field apparatus.

Figure J3. Visual field apparatus and eagle restraint platform fully assembled and ready for an eagle.

We measured the visual fields and eye movements in 10° increments around the head of the eagles using a Keeler Professional ophthalmoscope (accuracy of ± 0.5°) from 140°-270° elevation. These elevations are established via an angular coordinate system centered on the head of the eagle (Figure J4). The first elevation in this coordinate system is centered directly above the head of the eagle (0°), extends down to the front of the head of the eagle (90°), continues down to directly under the head of the eagle (180°), then proceeds upwards towards the back of the head (270°), until it ends directly above the head of the eagle (360°/0°). A horizontal plane, held parallel to the ground, projects through the eyes of the eagle from the 90° elevation to the 270° elevation (Martin 2007; Figure J4). We were unable to take measurements from 150°-260° due to the apparatus or body blocking the eyes of the eagles at these elevations.

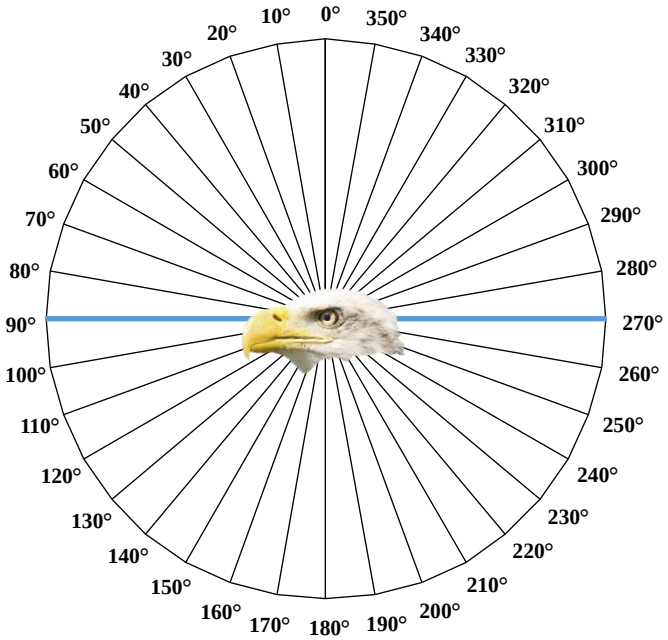

Figure J4. Elevations in the angular coordinate system around the head of the eagles. The horizontal plane is indicated by the blue line extending from the 90°-270° elevations.

We elicited eye movements from the eagles by flashing lights, making sounds (e.g. jingling keys, whistles, crinkling paper, etc.), or lightly touching the back of the eagle in the case of diverged measurements. Elicitations were necessary when we measured the converged and diverged visual field measurements. After the measurements were taken, we corrected the measurements for the size of the visual field apparatus following Martin (1984). The sizes of the visual fields including the binocular field (area where vision is subtended by both eyes), lateral fields (area were vision is subtended by a single eye), and cyclopean field (area of the binocular field + left eye lateral field + right eye lateral field) were calculated as in Fernández-Juricic et al. (2008).

Eye Size and Centers of Acute Vision

Once the eagles were euthanized by rehab center personnel, we immediately removed the eyes of the eagles, cleaned off any remaining muscle, connective tissue, and nictitating membranes, and measured the size of the eye. We measured the corneal diameter, transverse diameter, and axial length of the eyes using a caliper (Figure J5). The corneal diameter, in millimeters, is defined as the diameter of the cornea in the sclerotic ossicles (Figure J5a; red arrow). The transverse diameter, in millimeters, is defined as the diameter of the eye when viewing the front of the eye (Figure J5a; grey arrow). The axial length, in millimeters, is defined as the width of eye from the surface of the cornea to the back of the eye (Figure J5b; green arrow; Fernández-Juricic et al. 2019).

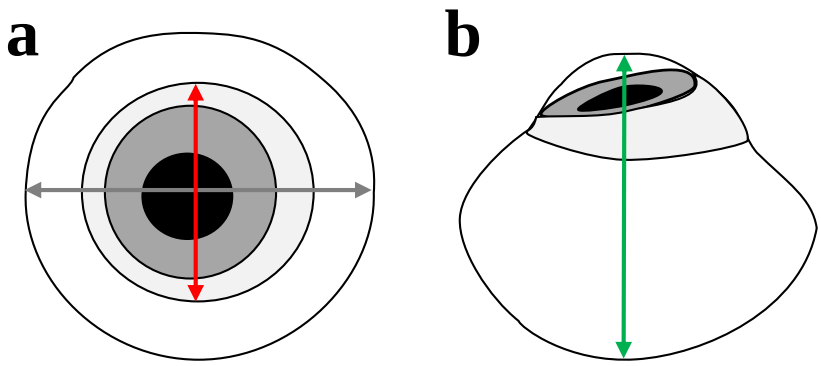

Figure J5. Diagram of the eye of a Golden Eagle. **a**) Front view of the eye with the corneal diameter (red arrow) and transverse diameter (grey arrow) indicated. **b**) Side view of the eye with the axial length (green arrow) indicated. Please note that when measuring axial length, it is measured by placing the eye on its side so that the weight of the eye does not distort the measurement.

After the eyes were measured, we hemisected the eyes and took note of any gross morphological features on the retina. If observed, we would attempt to photograph the eye. In both fresh and preserved specimens, we noted the presence of two foveae that were visible to the naked eye. We calculated the position of the fovea within the eyecup by measuring the proportional distance of each fovea from the center of the eye along the nasal-temporal axis and the dorsal-ventral axis. We were then able to take this information and project it onto the visual field maps.

Transmittance of the Ocular Media

In order to determine the amount of light transmitted to the retina of the eagles, we measured the transmittance of the ocular media of 5 eyes (3 left, 2 right) from 3 Golden Eagles (3 Females). We did not have the opportunity to measure the ocular media in the Bald Eagles. The eyes were first hemisected immediately posterior to the ora serrata (Figure J1), effectively removing the cornea and lens from the back of the eye. Hemisection occurred at this location and not the preferred back of the eye as in Fernández-Juricic et al. (2019), because the retinal tissue was needed for other techniques in this study. The front of the eye containing the cornea and lens tissue was placed in a custom-designed eye holder and the transmittance measured following Fernández-Juricic et al. (2019). We also measured the vitreous humor in the eye holder after it was removed from the eagle eyecup. The vitreous humor was removed in such a way that it remained intact in a single large piece.

Transmittance measurements were collected using an Ocean Optics Jaz spectroradiometer (Ocean Optics, Inc. Winter Park, Florida, USA) with a pulsed-Xenon lamp from 300-700 nm in

1 nm increments. The resulting transmittance spectra were then averaged together and normalized to 1 (or 100% transmittance) to determine the wavelength at which the transmittance of the light is 50% (i.e. $\lambda_{T0.5}$; Hart et al. 2000, Hart 2004; Fernández-Juricic et al. 2019). We also used a program called TableCurve 2D ™ (AISN Software, Inc. ©1989-1996) to fit a curve to the cornea-lens spectrum and used this for the visual contrast modeling.

Photoreceptor Densities

We were able to attain photographs of the oil droplets on whole-mounted retinas of 6 eyes (3 left, 3 right) from 2 Golden Eagles (2 Females) and 2 Bald Eagles (1 Male, 1 Unknown). The retinas were removed by hemisecting the eye immediately posterior to the ora serrata, thereby removing the front of the eye and exposing the retina within the eyecup. The eyecup is filled with vitreous humor, which was removed to facilitate retinal extraction. The retina was extracted by carefully separating the choroid from the sclera (Figure J1). By extracting the choroid with the retina, we are preventing mechanical damage to the retinal tissue with our dissection tools. After all choroid attachment points have been severed, the back of the pecten (a folded structure used to provide oxygen and nutrients to the retina) was separated from the sclera by slowly scratching away the connective tissue. Once released, the retina was extracted from the eyecup by pulling on the choroid. The choroid was then peeled away from the back of the retina, in some cases taking the pigmented epithelium with it (if the retina was detached from the choroid before or after hemisection, this did not work). When necessary, we attempted to brush off the pigmented epithelium from the retina as this blocks the light needed for imaging of the tissue.

We then floated the retina onto a 48 mm x 65 mm glass coverslip with phosphate buffered saline. We flattened the retina using small paintbrushes, with the photoreceptor side down to prevent disruption of the oil droplet configuration on the retina. We then applied gel superglue to the four corners of the coverslip and placed a 76 mm x 52 mm glass slide on top, ensuring that the retina was not compressed in any way and there were no air bubbles. We were then able to flip over the retina mounted on the slide so that the photoreceptor side was facing up. Pictures of the whole-mounted retina were taken to observe the location of the pecten and any other gross anatomical structures or color patterns on the retina. We use the pecten to establish the orientation that the retina maintained in the eye of the eagles.

The whole-mounted retina was then imaged using an Olympus BX51 microscope, Olympus S97809 microscope camera (Olympus Corporation of the Americas, Central Valley, Pennsylvania, USA), and Stereo Investigator 9.13 (MBF Bioscience, Williston, Vermont, USA) software following Baumhardt et al. (2014). Briefly, the retina was imaged by taking a series of images using the SRS Image Series Acquire workflow in Stereo Investigator. This workflow applies a random, orderly grid over the retina on which the software would take two images using a 40x objective, at each grid site; a brightfield image and an epifluorescence image. We

collected images on an average of 171 sites for the Bald Eagles and 390 sites for the Golden Eagles. The lower number of sites visited for the Bald Eagle was the result of a large proportion of the retina being covered with pigmented epithelium. Both the brightfield and epifluorescence images were superimposed with a 50 x 50 µm (0.0025 mm^2) counting frame within which we counted the different types of oil droplets.

We have identified five visually distinct oil droplet types from the images we collected, which we also confirmed through microspectrophotometry. We were able to count and calculate the density of these oil droplets in 10 sites for the two Bald Eagle retinas and 40 sites on a Golden Eagle retina. If a site looked like it had mechanical damage or the oil droplet configuration on the retina looked disturbed, we did not count that site. We calculated the relative densities of the oil droplets for use in the visual contrast modeling. We were also able to calculate the spatial resolving power, in cycles per degree, using the photoreceptor densities and the axial length (mm) of the eye. The spatial resolving power can be used as a proxy for the visual acuity of the eagles (Ensminger & Fernández-Juricic 2014).

Photoreceptor Sensitivities

We were able to measure the sensitivity of photoreceptor visual pigments and absorbance of oil droplets on fresh eagle tissue for one Golden Eagle (Female) and one Bald Eagle (Female). We were also given frozen eyes from four Golden Eagles (Sex Unknown) and four Bald Eagles (Sex Unknown), from which we were able to measure additional oil droplets. For fresh tissue specimens, the eagles were first dark adapted for ~2 hours before euthanasia. This was to aid in increasing the amount of visual pigments within the photoreceptor outer segments. Euthanasia and eye removal were performed in a dark room, using red flashlights, so as not to bleach the photoreceptors as the pigments they contain degrade with light. The eyes were placed in an aluminum-covered container, filled with phosphate buffered saline (PBS), and placed on ice in a refrigerator until we could begin the tissue preparations.

The eyes were hemisected immediately posterior to the ora serrata and the vitreous humor removed using small spring scissors. The retina was extracted by gently brushing it away from the eyecup using small paintbrushes. After removal, the retina was floated into a petri dish with PBS and was cut into pieces corresponding to their location on the retina (i.e. Central, Temporal, Ventral, etc.). These pieces were then put on a Corning No. 1 22 x 30 mm glass coverslip with one drop of sucrose water and one drop of PBS, to prevent desiccation and increase the length of time the outer segments remained viable for measurements. The pieces of retinal tissue were chopped on the slide using a razorblade until no large pieces remained visible. A Corning No. 1 18 x 18 mm cover slip was applied on top and the edges sealed using black nail polish. These preparations were held in a refrigerator until use to prevent tissue degradation. All dissection work and tissue preparation was performed under a dim red light when using the fresh

tissue. For frozen eyes, they were hemisected, any retinal tissue remaining in the eyecup scooped out, and the preparations made as above.

We collected measurements of the photoreceptor outer segment visual pigments and oil droplets in a dark room using the custom microspectrophotometer (MSP) acquired from Dr. Ellis Loew and described in Attachment D. The MSP measures the absorbance properties of the photoreceptor components in 1 nm increments from 350-750 nm. If measuring an outer segment with a visual pigment, we would first take a sample measurement and then bleach the visual pigment for 60 s or more with white light to confirm its identity (Liebman 1972). Oil droplets contain carotenoid pigments, which are not photo-reactive, and are easily identifiable by their spherical shape so no bleaching was necessary. Rod and medium-wavelength sensitive (MWS) single cone outer segments, which often have similar peak sensitivities, were distinguished by the morphology of the outer segment during MSP data collection. Rod outer segments are usually large, rectangular, and have easily visible striations whereas MWS single cones are smaller, have an elongated triangle shape, and have no visible striations in the outer segment (Crescitelli 1972).

We analyzed the peak absorbance (λ_{max}) of the visual pigments using a program called MSPA, created by the Dr. Ellis Loew lab using LabVIEW software. This software allowed us to compare the pre- and post-bleach absorbance spectra to both confirm that the suspected visual pigment did in fact bleach and to create a difference spectrum of the two curves. This difference spectrum was used to identify the λ_{max} for three of the four single cones and the double cone, but was not necessary for the long-wavelength sensitive single cone, or the rod photoreceptor. We analyzed the oil droplet absorbance spectra using a Matlab based program called OilDropSpec (Sesterhenn 2012, Ensminger et al. 2014). This program outputs several key parameters describing the shape of the oil droplet spectrum, such as λ_{cut}, defined as the wavelength (nm) at 100% absorbance, and λ_{mid}, defined as the wavelength (nm) at 50% absorbance (Lipetz 1984, Hart and Vorobyev 2005). These lambda parameters (λ_{max} of the visual pigment, and λ_{cut} and λ_{mid} of the oil droplets) are used to determine the sensitivities of the single and double cone photoreceptors for the eagles and is directly incorporated into the visual contrast modeling.

Visual Contrast Modeling

Using the visual system data collected for the eagles we were able to perform visual contrast modeling to determine which visual signals are highly conspicuous to both the Golden and Bald Eagles. We used the receptor-noise limited model (Vorobyev & Osorio 1998) to determine which LEDs, with peak wavelengths ranging from 300-700 nm, would be the most conspicuous against various backgrounds on a clear day. This model requires the input of three distinct types of information; 1) the sensitivity of the eagles' visual system and the relative densities of the photoreceptors on the retina, 2) the ambient light of the environment that the

eagle is in, and 3) the reflectance or radiance properties of objects or lights in the environment that the eagle will view.

The sensitivities and relative densities of the Golden and Bald Eagle photoreceptors we used are detailed in Attachment L. We used ambient light and radiance of the sky data used in Goller et al. (2018), measured on a clear day in an open grassy field on March 21st, 2015 at noon. We chose to use these data because it closely simulates the environment that wind turbines could be located. We also used 201 simulated LED spectra, from Goller et al. (2018), which had peak wavelengths that ranged from 300-700 nm in 2 nm increments to determine which LEDs were most conspicuous against the sky. In case the LED light stimulus was not placed on the top of the wind turbine (blue sky background), we decided to model the LEDs against additional visual backgrounds (bare ground, dormant grass, green grass, and a white paint [proxy for wind turbine color]) to determine if different LEDs were needed in those situations. We performed the chromatic contrast calculations in the Pavo 2.3 R package (Maia et al. 2019) following the methods used in Goller et al. (2018).

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ATTACHMENT K

Milestone 8.1

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the analysis of auditory data for the completion of Milestone 8.1.

Milestone 8.1 – Processing and analyzing of the auditory data on campus

Task 8.0 – Processing and analyzing physiological data on campus (Month 10-33)

Task Summary:

The measurements and tissues collected on Bald and Golden Eagle sensory systems at rehabilitation centers required extensive processing and analysis on campus at Purdue University. Auditory data processing and analysis required custom designed code to determine the auditory responses relative to different background conditions, as well as statistical analysis needed to resolve patterns for the different stimuli, noise treatments, and eagle species. Visual data processing and analyses required processing and measurement of retinal tissue samples, compilation of visual field data from eagle individuals, calculation of relative densities and properties of visual pigments and oil droplets, and parameterization and running perceptual visual models to determine color vision properties of the eagle visual system.

Subtask 8.1 – Processing and analyzing auditory data on Purdue University campus (Month 10-27)

Subtask Summary: Sound analysis software and custom written code in both SAS and PRAAT were used to process and analyze experimental auditory measurements of Bald and Golden Eagle auditory evoked potentials (AEPs; see Attachment I for details on Experimental Procedure and Stimuli). The analyses informed our understanding of the sensitivity of Bald and Golden Eagles to different single tones with and without background noise, multi-tone stacks with and without noise, amplitude modulations with and without noise, linear changes in frequency (sweeps) with and without noise, and sinusoidal frequency modulations at different rates with and without noise.

Objectives

To determine which sounds an eagle could hear well in a field setting, we needed to characterize the sensitivity of Golden and Bald Eagles auditory systems to a wide variety of

synthetic sounds under several noise conditions. In addition to comparing the different sound types in noise, we also compared auditory processing between eagle species. This allowed us to generate a list of sounds that are likely to be maximally processed by the eagles based on sounds that (1) Golden and Bald Eagles are sensitive, and (2) sounds for which processing was resistant to degradation or masking by background noise. The candidate sounds were then tested in behavior experiments as described in Attachments O and P.

Auditory Evoked Potential (AEP) Analysis

Auditory evoked potential (AEP) analysis provided information about how well the peripheral auditory system of eagles processed the test sound stimuli (Attachment I; Figure I4) under different noise conditions. Our stimuli fall into two categories with properties that require different types of analyses. One category is composed of one or multiple static tones. Stimuli in this category include the pure tones, harmonic and non-harmonic (mistuned) stacks, and the amplitude modulated (AM) stimuli produced with a carrier tone and two sidebands. The second category is composed of more dynamic frequency modulated (FM) tones. Stimuli in this category include the linear frequency sweeps and the sinusoidally FM tones. The auditory system will phase lock to all of these tones and AM components. Phaselocking results from populations of neurons in the auditory system firing at approximately the frequency of each tone or at the frequency of the amplitude modulation (Viemeister & Plack 1993).

Phaselocking to tones at the level of the brainstem is called the Frequency Following Response (FFR; Hall 2007). The amplitude or strength of phaselocking is a measure of the firing synchrony of brainstem neurons and the number of neurons responding to the stimulus. Auditory processing of static tones or static AM components can be characterized with a spectrum that integrates phaselocking detected with the auditory evoked potentials (AEP) over the duration of the stimulus. The auditory processing of dynamic FM tones is more complicated because phaselocking changes over time as does the amplitude of this phaselocking.

Tones, harmonic stacks, and amplitude modulated stimuli generate phaselocking at the component tones in each stimulus. Therefore, we can describe the processing of each of these stimuli using the FFR. FFR amplitude was measured using PRAAT software by first calculating the frequency spectrum of the AEP with a Fast Fourier Transform (sampling rate=40 kHz; FFT size=2048 points; frequency resolution=19.5 Hz). We then calculated the maximum amplitude (in dB) of peaks +/-50 Hz from the stimulus frequency – this maximum amplitude is the intensity of the AEP at the stimulus frequency. However, this intensity needs to be corrected for baseline neural activity (which is essentially noise in the AEP). Therefore, our estimate of phaselocking to any of the stimulus tones was calculated as the amplitude of phaselocking at the stimulus frequency minus the 95^{th} percentile of intensity of the noise floor at the stimulus frequency (see below).

The auditory system also phaselocks to strong AM components in our AM stimuli and to the amplitude envelope of the harmonic stack with the missing fundamental. AM stimuli have an AM component equal to the difference between the carrier and side band frequencies. The harmonic stack with the missing fundamental has an AM component equal to the frequency of the missing fundamental (here 600 Hz – see above). Phaselocking to the AM component of a stimulus is called the Envelope Following Response (EFR), and is measured with the same Fast Fourier Transform method described for the FFR.

The noise floor of an AEP will change with the type of masking noise (none, pink or white) broadcast with the stimulus. This change in noise floor affects our ability to distinguish the intensity of phaselocking to our stimuli from the overall level of the noise floor. In other words, we want to measure how well the stimulus sound is processed compared to the processing of the noise background to get a measure of relative phaselocking intensity. For that reason, we estimated the upper 95% confidence limit of the noise floor at the frequency of each stimulus tone or AM rate, and subtracted that number from the absolute amplitude of the AEP spectrum at the frequency of the stimulus. This was done as follows: the noise floor was first generated for each individual AEP recording using a Fast Fourier Transform (FFT) of the AEP data for frequencies 500-5000 Hz, but excluding value related peaks and shoulders for all frequencies in the stimuli within +/-100 Hz of those stimulus frequencies. The approximate average noise floor amplitude at each stimulus frequency or AM rate was estimated with a second order polynomial fit to the trimmed spectrum using Proc MIXED in SAS v9.4. Higher order polynomial equations did not provide a better fit than the second order polynomial. A separate model was generated for each eagle, stimulus type, noise type, and tone frequency (for pure tones). We also deleted outliers from the noise floor that had model residuals that were <15 dB or >15 dB. The model was rerun if outliers were identified. For example, the noise floor for a trial with a 3 kHz pure tone would be calculated from the data for 500-2900 and 3100-5000 Hz. We then estimated the 95% confidence limit of the residuals, added that to the amplitude of the noise floor at the stimulus frequency, and subtracted this sum from the amplitude of the peak of the spectrum +/-50Hz around the stimulus frequency. This value describes how much stronger the auditory response of the subject eagle was to the stimulus compared to the noise background.

For frequency sweeps and sinusoidally FM stimuli, we used an auto-correlation method implemented in the Pitch (ac) analysis function in PRAAT (v6.1.05; Boersma & Weenink 2019) to estimate phaselocking frequency as a function of time. The details of the pitch analysis for the frequency sweeps were as follows: time step=0.00025, pitch floor=1000, very accurate='yes', silence threshold=0.03, voicing threshold=0.45, octave cost=0.01, octave-jump cost=0.35, voiced/unvoiced cost=0.14, pitch ceiling=6000. The details of the pitch analysis for sinusoidally FM tones were identical to analysis of sweeps except the time step=0.000125, pitch floor=2000-modulation depth, and pitch ceiling=2000+modulation depth. The autocorrelation pitch analysis also provides an index of the relative strength of the AEP waveform which indicates the degree of periodicity of the candidate tone (here phaselocking to the stimulus tone) ranging from 0 (no

periodicity) to 1 (maximal periodicity). We evaluated the auditory response to FM stimuli using the difference between phaselocking frequency and the stimulus frequency as a function of time. Phaselocking strength was also analyzed as a function of time. Finally, we compared the slope of phaselocking frequency as a function of time with the slope of the frequency of the FM sweep itself. Note that we folded all cycles of the sinusoidally FM AEP starting at sin(0) through sin(360) and thereby analyzed a single average cycle for each sinusoidally FM stimulus. However, the first 0.015 sec were trimmed off the AEP to eliminate any onset responses and data were only included in the analysis if relative phaselocking strength was greater than zero.

Statistics

Repeated measures linear mixed models (Proc Mixed in SAS v9.4) were used to test for species and treatment effects on auditory properties. The dependent variable was relative phaselocking intensity (in dB) for the fixed frequency stimuli. Higher phaselocking values indicate better processing of the stimulus sound. A decrease in phaselocking during treatments with noise therefore means that noise negatively affected stimulus sound processing. For stimuli with either linear or sinusoidally FM we measured two different dependent variables: phaselocking strength and the frequency difference between the AEP and stimulus. Each measure was analyzed separately. For linear FM, we also analyzed the slope of the AEP phaselocking frequency as a function of time and compared that with the slope of the linear FM stimulus. Fixed effects included the stimulus frequencies (either as a tone frequency or AM frequency), time (for FM stimuli), dB level (for tones), noise background treatment, and eagle species. Individual eagles were treated as the subjects (random effects) in the repeated measures analysis and our model therefore tested for within-individual patterns instead of comparing individuals. All 3-way interaction terms were initially included and trimmed when found to be non-significant ($P>0.05$) in order of decreasing F value. Estimates presented are least squares means and standard errors calculated from the final statistical model for each stimulus (Proc Mixed, LSMEANS).

Results

Subjects

We measured six Bald Eagles (*Haliaeetus leucocephalus*) and two Golden Eagles (*Aquila chrysaetos*) at the Wildlife Center of Virginia in Waynesboro, VA (5 Bald Eagles; April-September 2018) and at Liberty Wildlife in Phoenix, AZ (1 Bald Eagle, 2 Golden Eagles; February 2019). These eagles were all healthy and were no longer receiving clinical treatment. They were in their final stages of rehabilitation with the goal of eventual release. Experiments were conducted in collaboration with and at the discretion of the veterinary staff of each rehabilitation facility. All work with the eagles was conducted with approval of the Purdue

Animal Care and Use Committee (PACUC Protocol # 1705001579) as well as US Fish and Wildlife (Permit #: MB41892B-1) and state authorities of Virginia (Permit #: 62486) and Arizona (Permit #: SP638641).

Tones

Phaselocking to single tones unsurprisingly was lower when the tones were played at lower amplitude (60 dB Sound Pressure Level = 5.79 ± 0.47, 70 dB SPL = 11.04 ± 0.63, 80 dB SPL = 15.11 ± 0.47; $F_{2,10}$ = -2.36, P = 0.040). Similarly, phaselocking to tones was lower in noisy backgrounds than in silence (no noise = 18.88 ± 0.45, pink noise = 5.05 ± 0.45; $F_{2,8}$ = 2.09, P < 0.0001) and slightly lower in pink noise than in white noise (white noise = 8.01 ± 0.45; $F_{2,8}$ = 1.75, P = 0.026). The degradation of the tone stimuli by background noise suggests that tones are not strong candidates for implementation in the field (Figure K1).

Comparing the two eagle species, Bald Eagles were generally better at phaselocking quieter (60 dB SPL) tones than Golden Eagles (Bald Eagle = 14.61 ± 0.61, Golden Eagle = 11.32 ± 1.03; $F_{2,10}$ = -2.50, P = 0.032), but only when there was no background noise. In noise, Bald Eagle adults (white noise = 9.00 ± 0.49, pink noise = 6.50 ± 0.49) again performed better than Golden Eagle adults (white noise = 4.87 ± 1.27, pink noise = 2.42 ± 1.26), but there was no difference between the juveniles of the two species. Finally, noise masked the stimulus tones more for 60 than 80 dB SPL tones, with different noise masking patterns for different tone frequencies. This complexity is shown by several significant 3-way interactions for tone phaselocking intensity: species × tone dB × noise-type ($F_{4,20}$ = 3.26, P = 0.033), species × noise × age ($F_{2,8}$ = 14.82, P = 0.0020), and tone × tone amplitude × noise ($F_{20,120}$ = 2.33, P = 0.0025).

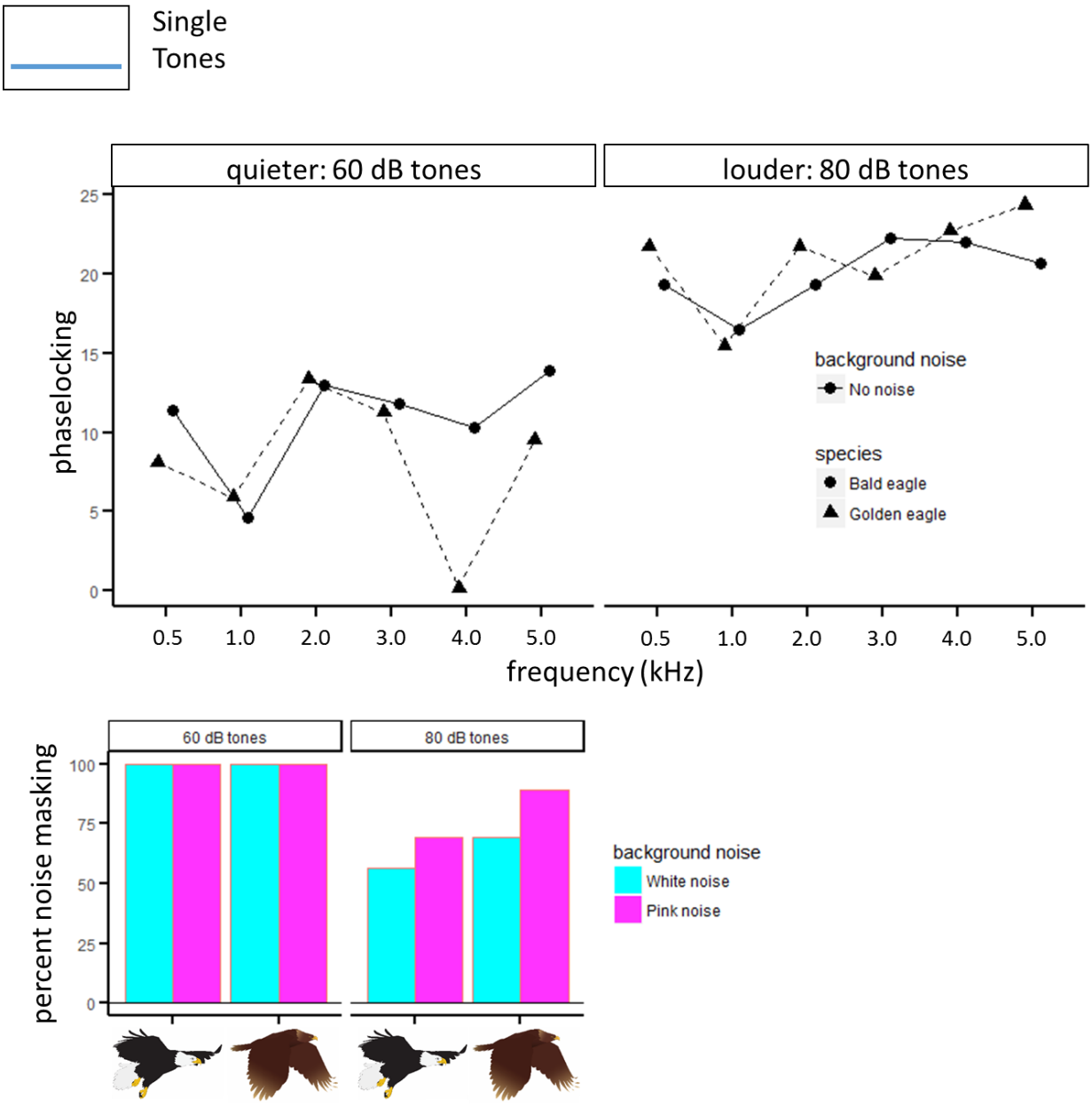

Figure K1. Golden and Bald Eagles phaselock (top data figure) to tones (played without background noise) well when tones are louder (80 dB) and have a similarly worse response when the tones are quieter (60 dB). Phaselocking is a measure of how well the eagle auditory system encodes the stimulus sound. Golden Eagles are indicated with triangles and dashed lines, Bald Eagles in circles and solid lines. Background noise (bottom figure) strongly masks the normal response to tones in both eagles. Quiet tones are fully masked (100%) while the louder tones are mostly masked by white and pink noise backgrounds. Tones are not good candidates for use in field settings where conditions are generally noisy. Golden and Bald Eagles have similar responses to tones.

Tone Stacks

The response of the auditory system of Bald and Golden Eagles to stacked tone stimuli was analyzed with a statistical model that included tone frequency, background noise type, individual eagle, species, and age effects.

Harmonic Stack

The harmonic stack was composed of five equally spaced tones ranging from 1 to 5 kHz. Component tone frequency ($F_{4,16} = 10.67$, $P = 0.0002$) and background noise type ($F_{2,10} = 64.94$, $P < 0.0001$) both affected the strength of the auditory response (Figure K2). In addition, multiple interactions between effects were significant in our model, including species × component frequency × age ($F_{4,16} = 4.05$, $P = 0.019$) and two-way interactions of species and age with noise (species×noise: $F_{2,10} = 9.92$, $P = 0.0042$; age×noise: $F_{2,10} = 7.71$, $P = 0.0094$).

Overall, noise had a strong effect on the auditory response to the harmonic stack, masking the no-noise response (13.42 ± 1.88) by 37% in white (8.45 ± 1.88) and 53% in pink (6.27 ± 1.88) noise. Adult Bald Eagles generally performed better in noise than juveniles, though this difference was not seen in the two Golden Eagle subjects (one adult, one juvenile). Without noise, Golden Eagles (15.41 ± 3.20) had higher phaselocking than Bald Eagles (11.42 ± 1.96), and both species had similar responses in white (Bald: 8.70 ± 1.96; Golden: 8.20 ± 3.20) and pink (Bald: 6.70 ± 1.96; Golden: 5.84 ± 3.20) noise.

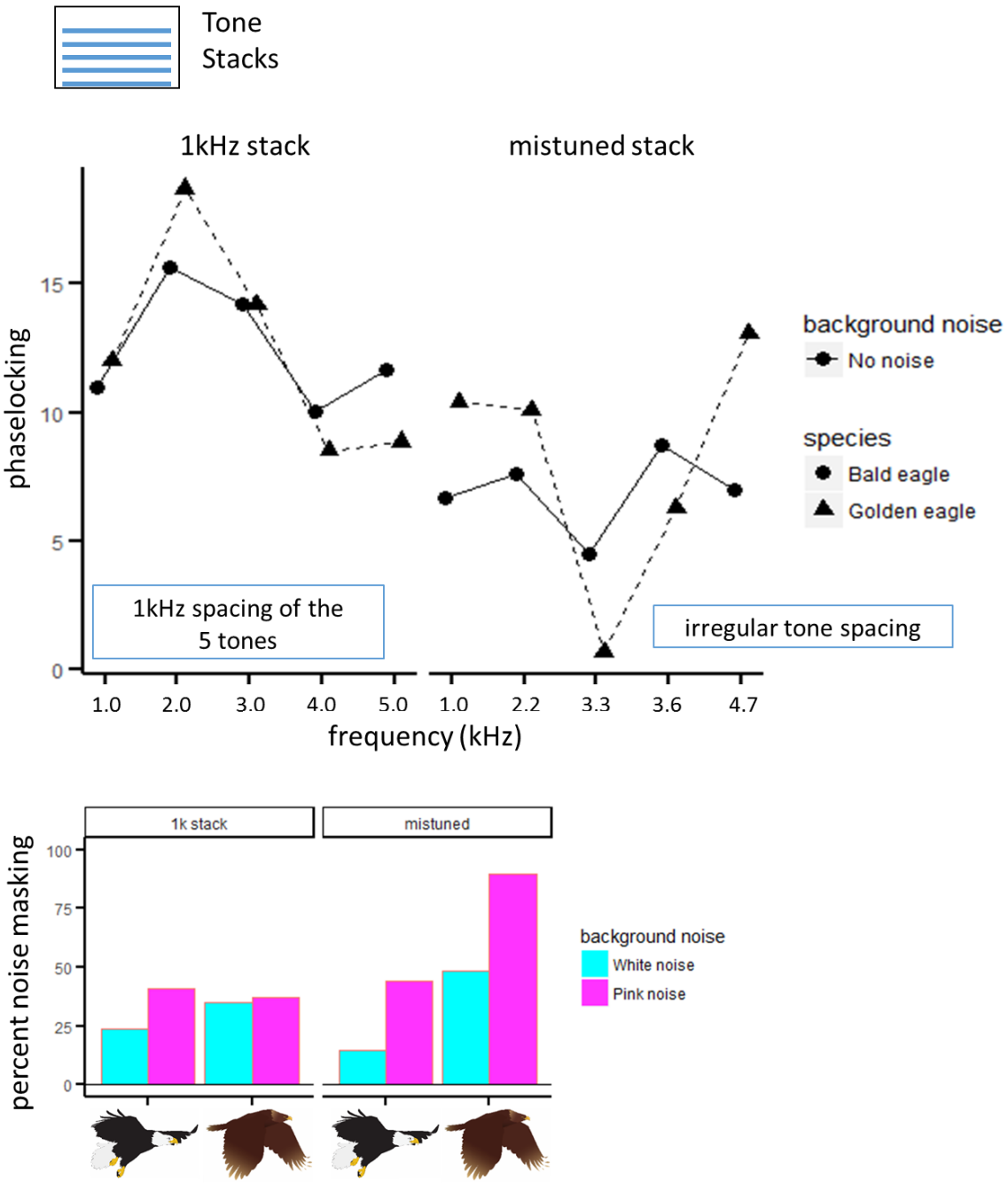

Figure K2. Similar to tones, Golden and Bald Eagles phaselock well (top data figure) to stacks of multiple tones played without background noise although there is some variation in the details of the response. Golden Eagles are indicated with triangles and dashed lines, Bald Eagles in circles and solid lines. Background noise masks the response (bottom figure) to stacks of tones in both eagles, though not as much as with single tones. Tone stacks may not be the best candidates for use in field settings but they are worth testing. Golden and Bald Eagles have similar responses to stacks of tones.

Mistuned Non-Harmonic Stack

The mistuned stack stimulus (Figure K2) contained five unequally spaced tones (1.0, 2.2, 3.3, 3.6, and 4.7 kHz). The mistuned stack was processed similarly by both eagle species, and again significantly masked by noise (no-noise: 2.14 ± 0.22, white: 1.55 ± 0.22, pink: 1.15 ± 0.22; $F_{2,14} = 5.60$, P = 0.016). A significant interaction between tone frequency and subject age ($F_{4,24} = 3.56$, P = 0.021) showed that at intermediate tone frequencies (2.2 kHz: juvenile: 0.70 ± 0.39, adult: 2.60 ± 0.31; 3.3 kHz: juvenile: 0.029 ± 0.386, adult: 1.71 ± 0.31; 3.6 kHz: juvenile: 1.16 ± 0.39, adult: 2.35 ± 0.31) the juvenile eagles had lower phaselocking than the adults. The same was not true for the lowest (1.0 kHz: juvenile: 1.56 ± 0.39, adult: 1.57 ± 0.31) and highest frequency components (4.7 kHz: juvenile: 2.23 ± 0.39, adult: 2.22 ± 0.31).

Missing Fundamental (600 Hz) Stack

The missing fundamental stack (1.2, 1.8, 2.4, and 3.0 kHz) was a harmonic stack missing the 0.6 kHz fundamental frequency resulting in a 0.6 kHz amplitude modulation (AM). Eagles had a highly variable response to the different components of the stack. Bald Eagles exhibited strong phaselocking to the 0.6 kHz AM (13.22 ± 1.16) and weaker phaselocking to 1.2 kHz (7.82 ± 1.16). In contrast, Golden Eagles showed weak processing of the 0.6 kHz signal (4.03 ± 1.97) but exhibited strong phaselocking to the 1.2 kHz component (15.27 ± 1.97). As with pure tones, noise had a significant effect on phaselocking of this stack stimulus (no noise = 10.55 ± 0.95, white noise = 8.43 ± 0.95, pink noise = 7.34 ± 0.95; $F_{2,14} = 7.32$, P = 0.0067)

Amplitude Modulated (AM) Stimuli

The AM stimuli was created by playing three equally spaced tones. For a given AM rate (*F*) the required tones were a carrier, a low sideband (carrier minus *F*), and a high sideband (carrier plus *F*). We separately analyzed the eagles' ability to phaselock to each of the three tones and to the AM component for a range of carriers (1, 2, and 3 kHz) and AM rates (100, 400, 700 Hz) in each of the noise backgrounds.

AM Rate

Generally, the strongest phaselocking to the AM envelope was measured for a 2 kHz carrier with a 400 Hz AM rate (14.12 ± 1.05). Processing of this stimulus was also least affected by noise of all of the AM stimuli (Figures K3-4).

Specifically, we found significant main effects of AM rate ($F_{2,12}$ = 47.64, P < 0.0001), carrier frequency ($F_{2,12}$ = 21.39, P = 0.0001), and noise background ($F_{2,12}$ = 99.41, P < 0.0001) on phaselocking amplitude to the AM component and the interactions of these factors as significant effects.

Bald Eagles (8.79 ± 0.54) were better at phaselocking to the AM component compared to Golden Eagles (5.80 ± 0.89; $F_{1,5}$= 8.39, P = 0.034), although this difference changed with AM rate (species × AM rate interaction: $F_{2,12}$ = 6.47, P = 0.012). In addition to the species differences, there was a significant interaction between the carrier frequency, background noise, and age on the ability to phaselock the amplitude modulation ($F_{4,24}$ = 3.32, P = 0.027). The strongest effect was in noisy conditions where adults (no noise: 12.99 ± 1.05, white: -16%, 10.92 ± 1.08, pink: -28%, 9.41 ± 1.05) exhibited less of a decrease in phaselocking of the amplitude modulation in stimuli with 2000 Hz carriers compared to juveniles (no-noise: 11.063 ± 1.295, white: -32%, 7.48 ± 1.33, pink: -35%, 7.19 ± 1.305).

AM Carrier

Phaselocking amplitude to the carrier tones would be expected to vary with differences in carrier frequency and noise background (Figures K3-4), and we find support for both factors (carrier: $F_{2,10}$ = 14.22, P = 0.0012; noise: $F_{2,12}$ = 54.86, P < 0.0001) as well as for the interaction between the two ($F_{4,24}$ = 10.11, P < 0.0001). The three-way interaction carrier frequency × noise × subject age was also significant ($F_{4,24}$ = 3.20, P = 0.031). Specifically, the 2000 Hz carrier causes stronger phaselocking (10.24 ± 1.00) than the 1000 Hz (3.77 ± 1.02) or 3000 Hz carriers (9.74 ± 0.70). Juveniles and adults have differential phaselocking to 1000 Hz (juvenile: 5.16 ± 1.59; adult; 5.26 ± 1.35), 2000 Hz (juvenile: 9.23 ± 1.53; adult: 13.72 ± 1.31), and 3000 Hz (juvenile: 16.58 ± 1.55; adult: 12.57 ± 1.33). Similarly the reduction phaselocking strength in noise relative to no noise was variable for 1000 Hz (white: juvenile= -24%, adult= -50%; pink: juvenile= -55%, adult= -36%), 2000 Hz (white: juvenile= -11%, adult= -12%; pink: juvenile= -18%, adult= -23%) and 3000 Hz carriers (white: juvenile= -51%, adult= -42%; pink: juvenile= -63%, adult= -39%)

AM High Sideband

Phaselocking strength of the high sideband showed significant carrier × noise type ($F_{4, 28}$ = 3.74, P = 0.015), carrier × AM rate type ($F_{4, 28}$ = 9.74, P < 0.0001), and AM rate × noise type interactions ($F_{4, 28}$ = 4.89, P = 0.0041). Overall, noise had a strong effect on processing of all high sidebands (no-noise: 9.95 ± 0.83; white: 6.60 ± 0.83; pink: 5.76 ± 0.83).

There was also a significant species × carrier × AM rate interaction ($F_{4, 24}$ = 7.29, P = 0.0005), as well as a significant species × AM rate interaction ($F_{2, 12}$ = 5.41, P = 0.021). Overall,

Bald Eagles performed better than Golden Eagles at phaselocking to high sidebands for 2000 and 3000 Hz carriers when the AM rate was 400 (2000 Hz Bald: 8.90 ± 1.58, Golden: 2.22 ± 2.67; 3000 Hz Bald: 5.20 ± 1.60, Golden: 1.51 ± 2.69) or 700 Hz (2000 Hz Bald: 10.30 ± 1.64, Golden: 3.81 ± 2.71; 3000 Hz Bald: 8.46 ± 1.67, Golden: 4.94 ± 2.81). However, there was no significant difference between the species at 100 Hz AM rate (2000 Hz Bald: 12.12 ± 1.64, Golden: 11.29 ± 2.71; 3000 Hz Bald: 10.15 ± 1.64, Golden: 9.04 ± 2.71). For 1000 Hz carrier stimuli, Bald Eagles had stronger phaselocking for 100 Hz AM rate (Bald: 7.71 ± 1.67; Golden: 5.54 ± 2.81) and 700 Hz (Bald: 9.24 ± 1.64; Golden: 0.70 ± 2.71), but Golden Eagles were far better than Bald Eagles for the 400 Hz AM rate (Bald: 6.63 ± 1.60, Golden: 16.16 ± 2.69).

AM Low Sideband

Phaselocking to the low sideband was significantly affected by three main effects: AM rate ($F_{2,12} = 4.99$, $P = 0.0265$), noise background ($F_{2,14} = 39.43$, $P < 0.0001$), and carrier tone ($F_{2,12} = 12.98$, $P = 0.0010$). Four interaction terms were also significant: carrier tone × noise ($F_{4,28} = 4.98$, $P = 0.0037$), AM rate × noise ($F_{4,28} = 5.33$, $P = 0.0026$), species × carrier tone ($F_{2,12} = 8.67$, $P = 0.0047$), and AM rate × age ($F_{2,12} = 7.17$, $P = 0.0089$). Notably, phaselocking of the low sidebands was particularly poor for stimuli with a 1000 Hz carrier (2.59 ± 1.18) compared to 2000 (9.44 ± 1.10) and 3000 Hz (8.00 ± 1.18). In addition, both types of background noise decreased phaselocking strength to the low sideband (no-noise: 8.95 ± 0.84; white: -35%, 5.84 ± 0.84; pink: -41%, 5.24 ± 0.84), especially for the 1000 Hz carrier stimulus.

Golden Eagles generally had higher phaselocking strength compared to Bald Eagles for stimuli with a 2000 Hz carrier (Bald: 7.33 ± 1.12; Golden: 11.55 ± 1.88), but Bald Eagles had stronger phaselocking at 1000 Hz (Bald: 3.93 ± 1.20; Golden: 1.24± 1.99) and 3000 Hz (Bald: 10.88 ± 1.20; Golden: 5.12 ± 1.99).

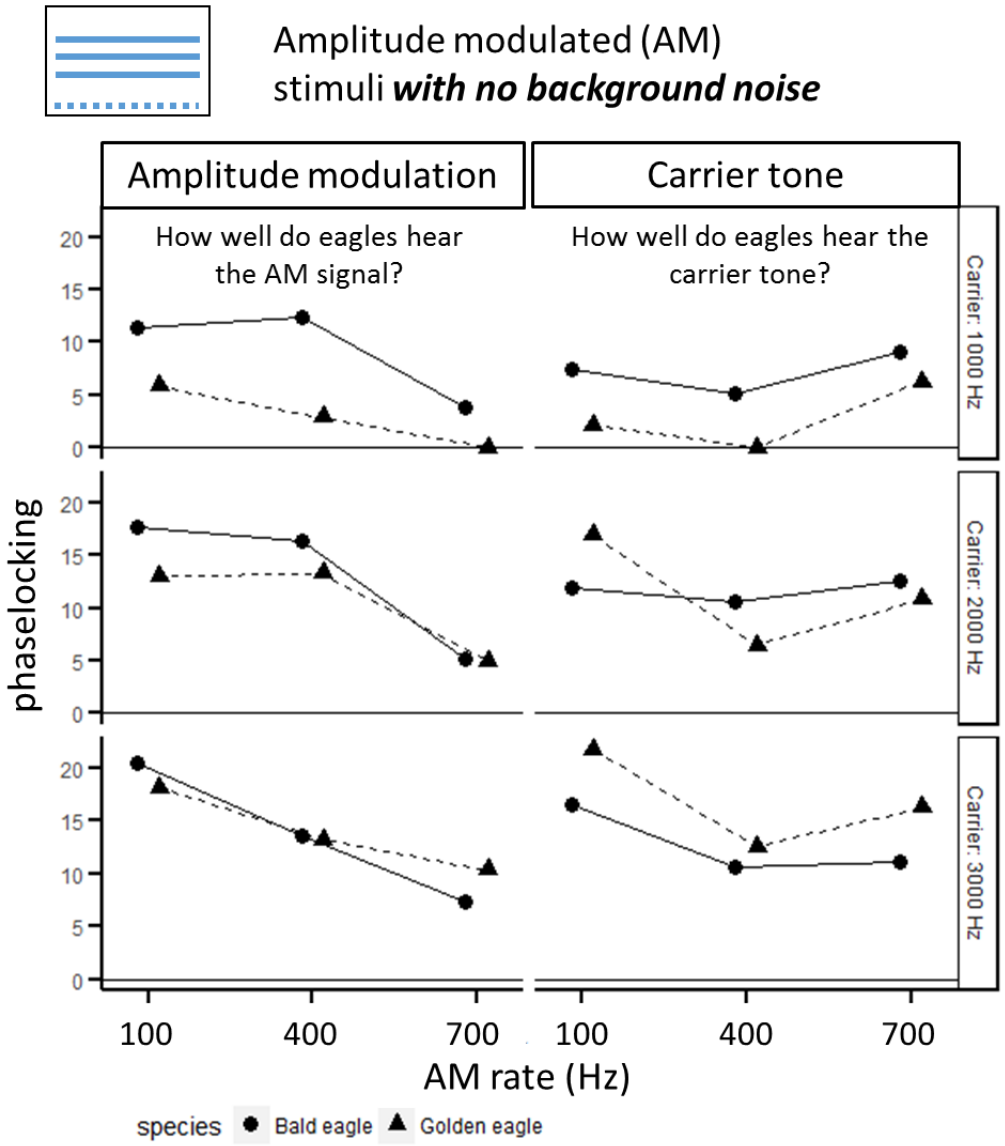

Figure K3. The stimuli with amplitude modulation (AM) are played with a chord consisting of three tones: a middle "carrier" tone, a higher sideband tone, and a lower sideband tone. The sideband tones are the same frequency distance from the carrier and produce an AM equal to that distance. For example a three tone set composed of a 2000 Hz carrier with 1600 Hz and 2400 Hz sideband tones (-/+ 400 Hz) yields an AM rate of 400 Hz. The auditory system of the eagle phaselocks to the three tones and also to the AM rate. Above, we show how Golden and Bald Eagles phaselock to the AM rate and to the carrier tone. We show data from the nine combinations we tested: three different carrier tones (3 rows: 1000, 2000, 3000 Hz) and with three different AM rates for each (x-axis: 100, 400, 700 Hz). Golden and Bald Eagles perform similarly for each of these combinations, although Golden Eagles appear to have more trouble hearing the AM component and the carrier tone with a 1000 Hz carrier. They also seem to be better than Bald Eagles at processing the 3000 Hz carrier. These data are all from treatments without background noise.

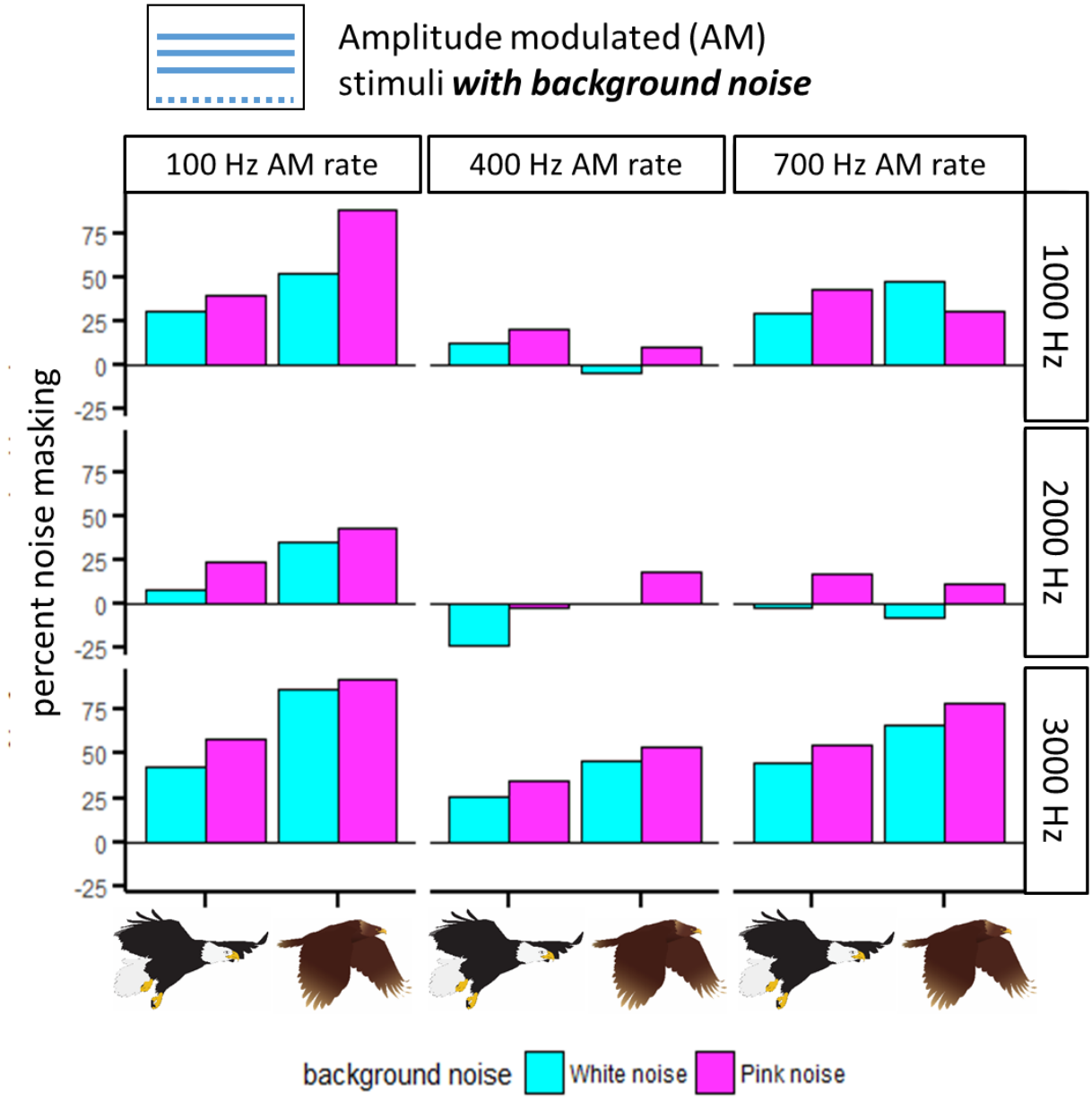

Figure K4. Most amplitude modulated (AM) stimuli are masked by background noise, and the responses of Golden and Bald Eagles are similar. The data above show the average effect of noise on the eagle's ability to hear all four components of the AM stimulus (carrier tone, high and low sideband tones, and the AM rate). The low effect of noise on several of the AM stimuli is promising, especially for 400 Hz AM with a 2000 Hz carrier, where phaselocking is strong (Figure K3) and noise masking is low. These are good candidates for stimuli to use in the field.

Linear Frequency Modulated (FM) Stimuli (Sweeps)

Slope of Phaselocking Frequency Relative to Stimulus Frequency

We compared the slope of the auditory response of the eagles (slope of AEP frequency as a function of time) to the slope of each sweep stimulus (slope of stimulus frequency as a function of time). The difference between the AEP slope and stimulus slopes (Figure K5) was significantly affected by the type of sweep (up/down + fast/slow: $F_{3,76} = 31.69$, $P < 0.0001$), eagle species ($F_{1,76} = 4.44$, $P = 0.038$), and the interaction between the two ($F_{3,76} = 11.24$, $P < 0.0001$). Interestingly there was no significant effect of background noise on the slope difference ($F_{2,76} = 0.07$, $P = 0.931$). Across species, the mismatch between the AEP slope and the stimulus slope was greatest for the fast upsweep (fast upsweep difference = 37.60 ± 3.12 kHz/s; fast down-sweep difference = 17.09 ± 3.14 kHz/s; slow up-sweep difference = 2.95 ± 2.29 kHz/s; slow down-sweep difference = 5.48 ± 2.29 kHz/s).

Golden Eagles had stronger mismatched slopes than Bald Eagles for upsweeps (fast upsweep: Golden difference = 54.41 ± 5.71 kHz/s, Bald difference = 20.80 ± 2.32 kHz/s; slow upsweep: Golden difference = 5.13 ± 3.96 kHz/s, Bald difference = 0.76 ± 23.16 kHz/s). In contrast, Bald Eagles had stronger mismatched slopes than Golden Eagles for fast down-sweeps (fast down-sweep: Golden difference = 10.12 ± 5.71 kHz/s; Bald difference = 24.07 ± 2.51 kHz/s). Neither species showed a significant mismatch for slow down-sweeps (Golden difference = 4.86 ± 3.96 kHz/s, Bald difference = 6.09 ± 2.32 kHz/s).

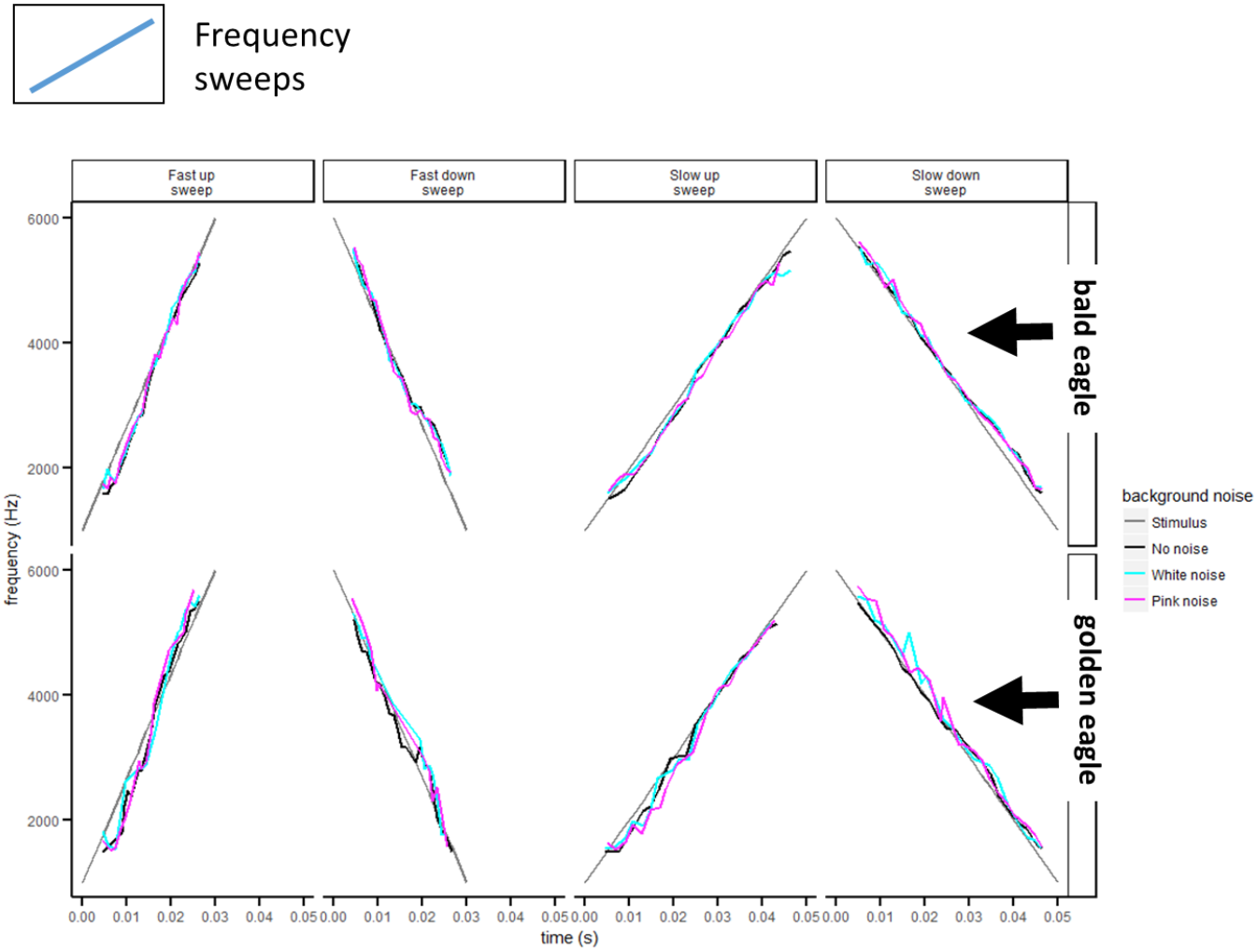

Figure K5. Frequency sweeps are rapid changes in frequency either from 1000 Hz to 6000 Hz (up sweep) or from 6000 Hz to 1000 Hz (down sweep). We tested two different speeds of these sweeps; one lasting 30 ms ("fast") and the other 50 ms ("slow"). The gray line above indicates the stimulus at each point in time for Bald Eagles in the top row, and Golden Eagles in the bottom row. The black and colored lines indicate the average eagle response to the stimuli in the various background noise treatments. Bald Eagles follow the stimulus quite well, with little effect of noise (the lines overlap well). Golden Eagles are not as good at following the sweep stimulus, although their performance is similarly unaffected by noise.

Phaselocking Strength During the FM Sweep

Phaselocking strength was generally higher without noise than in white or pink noise (fast up: $F_{2,12} = 3.13$, $P = 0.081$; fast down: $F_{2,10} = 19.51$, $P = 0.0004$; slow up: $F_{2,14} = 68.56$, $P < 0.0001$; slow down: $F_{2,14} = 6.21$, $P = 0.012$). Comparing the two species, Bald Eagles generally exhibited stronger phaselocking than Golden Eagles to all but the fast/down sweep (fast up: $F_{1,4}$

= 38.43, P = 0.0034; fast down: $F_{1,3}$ = 1.63, P = 0.292; slow up: $F_{1,5}$ = 19.55, P < 0.0069; slow down: $F_{1,4}$ = 7.92, P = 0.048). Significant time × species interactions (fast up: $F_{1,1422}$ = 28.83, P < 0.0001; fast down: $F_{1,1304}$ = 8.21, P = 0.0042; slow up: $F_{1,2264}$ = 10.68, P = 0.0011; slow down: $F_{1,3682}$ = 11.09, P = 0.0009) show that Golden Eagles have weaker phaselocking in response to the lower portions of the frequency sweep stimuli, and that both species are similar at the higher frequency portions.

Sinusoidal Frequency Modulated Stimuli

We used two indices to characterize eagle auditory responses to sinusoidal FM: the difference between phaselocking frequency and stimulus frequency, and phaselocking strength to the sinusoidal FM stimuli.

Frequency Difference

We found no significant effect of background noise on the difference between eagle phaselocking frequency and the sinusoidally FM stimulus for any of the stimuli (70 Hz FM/400 Hz depth: $F_{2,14}$ = 1.92, P = 0.184; 70 Hz FM/700 Hz depth: $F_{2,14}$ = 0.67, P = 0.527; 110 Hz FM/400 Hz depth: $F_{2,14}$ = 0.55, P = 0.589; 110 Hz FM/700 Hz depth: $F_{2,14}$ = 0.68, P = 0.521). The frequency difference varied with time for all stimuli tested (70/400 Hz: $F_{19,114}$ = 9.88, P < 0.0001; 70/700 Hz: $F_{19,114}$ = 5.72, P < 0.0001; 110/400 Hz: $F_{19,113}$ = 13.6, P < 0.0001; 110/700 Hz: $F_{19,114}$ = 8.42, P < 0.0001) as well as species (70/400 Hz: $F_{1,6}$ = 30.75, P = 0.0015; 70/700 Hz: $F_{1,6}$ = 20.55, P = 0.0040; 110/400 Hz: $F_{1,6}$ = 56.67, P = 0.0003; 110/700 Hz: $F_{1,6}$ = 37.95, P = 0.0008) and the interaction of species × time (70/400 Hz: $F_{19,114}$ = 2.03, P = 0.0120; 70/700 Hz: $F_{19,114}$ = 2.45, P = 0.0019; 110/400 Hz: $F_{19,113}$ = 2.08, P = 0.0097; 110/700 Hz: $F_{19,114}$ = 4.16, P < 0.0001). In general, Golden Eagles were consistently too low during the higher-frequency portions of the sinusoidal FM, whereas the Bald Eagle auditory system followed the stimulus sound more closely (Figure K6). Phaselocking rates were higher than the stimulus frequency for both eagle species during low-frequency portions of the stimulus.

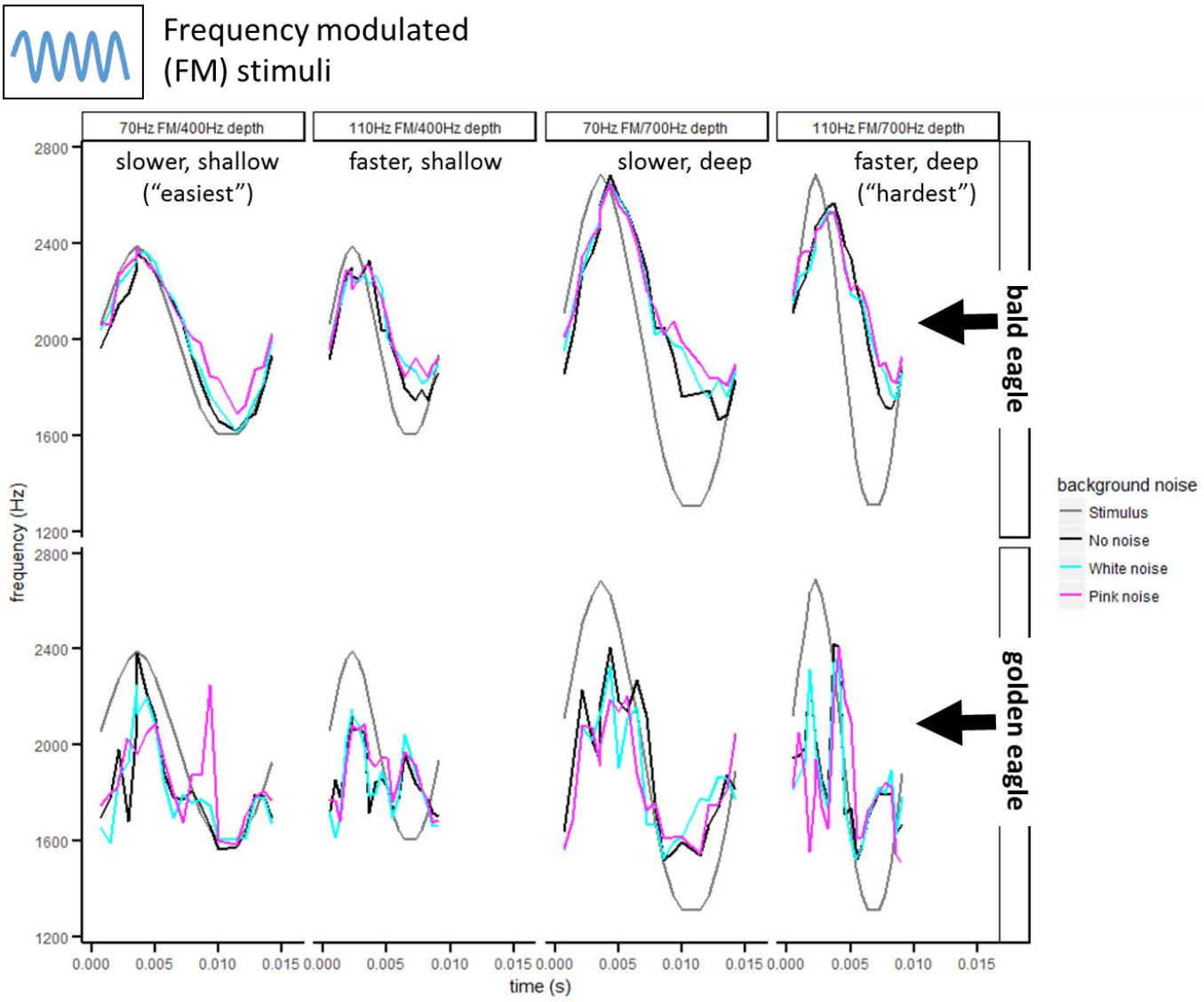

Figure K6. Frequency modulated (FM) stimuli are sinusoidal oscillations in frequency (similar to many back-to-back up and down sweeps). These are described using two quantities: 1) the rate at which frequency cycles (FM rate) and 2) the range of frequencies the sound sweeps through in a single cycle (depth). We tested four different FM stimuli composed of either 70 Hz or 110 Hz FM rates and either 400 Hz or 700 Hz depth. Our slowest FM stimulus therefore cycles from 2000 Hz up to 2400 Hz, down to 1600 Hz and back to 2000 Hz at a rate of 70 times per second. Our fastest moves from 2000 Hz to 2700 Hz to 1300 Hz to 2000 Hz at 110 times per second. Bald Eagles (top row) are surprisingly good at following these frequency changes (note: the frequency of the stimulus sound is in gray), although their performance drops for the faster FM stimuli (they start to mis-represent the low frequencies). Golden Eagles exhibit poor performance for all these stimuli and do not process them well. There is little effect of noise on auditory responses to FM.

Strength Results

Phaselocking strength for sinusoidally FM stimuli decreased significantly for all but the fastest modulating stimulus in the presence of background noise (70/400 Hz: $F_{2,14} = 27.27$, $P < 0.0001$; 70/700 Hz: $F_{2,14} = 10.99$, $P = 0.0013$; 110/400 Hz: $F_{2,14} = 11.41$, $P = 0.0011$; 110/700 Hz: $F_{2,14} = 2.90$, $P = 0.0882$). Phaselocking strength also varied significantly with time during the cycle for all but the fast/shallow stimulus (70/400 Hz: $F_{19,114} = 2.45$, $P = 0.0019$; 70/700 Hz: $F_{19,114} = 2.84$, $P = 0.0003$; 110/400 Hz: $F_{19,113} = 1.55$, $P = 0.0823$; 110/700 Hz: $F_{19,114} = 2.97$, $P = 0.0002$). There was generally no main effect of species on phaselocking strength (70/400 Hz: $F_{1,6} = 3.35$, $P = 0.117$; 70/700 Hz: $F_{1,6} = 0.00$, $P = 0.946$; 110/400 Hz: $F_{1,6} = 8.44$, $P = 0.0271$; 110/700 Hz: $F_{1,6} = 0.26$, $P = 0.628$), but all stimulus combinations had a significant time × species interaction (70/400 Hz: $F_{19,114} = 1.86$, $P = 0.0236$; 70/700 Hz: $F_{19,114} = 2.07$, $P = 0.0098$; 110/400 Hz: $F_{19,113} = 2.84$, $P = 0.0003$; 110/700 Hz: $F_{19,114} = 4.67$, $P < 0.0001$). In general, Bald Eagles had much higher phaselocking strength for high frequency than Golden Eagles, and the species showed similar phaselocking strengths at lower frequencies.

Summary of Results

Bald Eagle hearing results

1) Bald Eagles can hear a broad range of tones (we measured 0.5 to 5.0 kHz), but tones are strongly masked by background noise.
2) More complex sounds are less masked by tones.
3) Sounds that include rapid changes in frequency are encoded very well in the Bald Eagle ear showing they have excellent hearing!
4) Overall we can point to a number of sounds: mistuned harmonic stacks, frequency or amplitude modulated sounds, and slow frequency sweeps as good candidates for behavioral testing on the basis that they are 1) heard by Bald Eagles and 2) are not strongly affected by background noise.

Golden Eagle hearing results

1) Golden Eagles can hear a wide range of tones (we measured 0.5 to 5.0 kHz), but these tones are strongly masked by background noise and therefore not good candidate stimuli.
2) Golden Eagles can hear sounds that are more complex well, as long as they do not include fast changes in frequency.

3) Sounds with rapid frequency changes like sinusoidal frequency modulation or frequency sweeps are not encoded well by the Golden Eagle auditory system, even without background noise.

4) Overall our Golden Eagle data suggest that: mistuned harmonic stacks (1.0, 2.2, 3.3, 3.6 and 4.7 kHz), amplitude modulated sounds with a 2000 Hz carrier tone and a 400 Hz modulation rate, and slow frequency sweeps (6 to 1 kHz in 50 ms) and sinusoidally frequency modulated tones (70 Hz FM with 400 Hz depth) are potential candidates for behavioral testing on the basis that they are 1) heard by Golden Eagles and 2) are not strongly affected by background noise

General auditory results

Our study investigated how well Bald and Golden Eagles hear a variety of different sounds and tested their hearing under different background noise conditions. The results inform us about the ability of eagles to hear various sounds, and also how likely the eagles are to hear them when there is background noise. We found that Bald Eagles have excellent hearing that rivals some of the best songbirds measured to date, but that Golden Eagles are not as good at processing sounds with rapid frequency changes. Both species have similar responses to the different sounds when there is background noise: processing of complex sounds is less affected by noise than processing of simple sounds. These results suggest that complex sounds are good candidates for deterrents in field settings, but must be selected carefully if implemented to target Golden Eagles, because Golden Eagles are not particularly good at processing some types of complex sounds. We did not have the power to detect hearing differences based on sex, age, or prior history of lead poisoning.

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ATTACHMENT L

Milestone 8.2

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the analysis of data for the completion of Milestone 8.2.

> ***Milestone 8.2 – Characterize for golden and bald eagles: the configuration of their visual fields, the type and position of their center/s of acute vision, the density of retinal ganglion cells, eye size, visual acuity, density of different types of photoreceptors, peak sensitivity of visual pigments, and absorbance of oil droplets.***

Task 8.0 – Processing and analyzing physiological data on campus (Months 8-29)

Task Summary:

The measurements and tissues collected on Golden and Bald Eagle sensory systems at rehabilitation centers required extensive processing and analysis on campus at Purdue University. Auditory data processing and analysis required custom designed code to determine the auditory responses relative to different background conditions, as well as statistical analysis to resolve patterns for the different stimuli, noise treatments, and eagle species. Visual data processing and analysis required processing and measurement of retinal tissue samples, compilation of visual field data from eagle individuals, calculation of relative densities and properties of visual pigments and oil droplets, and parameterization and running perceptual visual models to determine color vision properties of the eagle visual system.

Subtask 8.2 – Processing and analyzing of the visual data (Months 8-29)

Subtask Summary: We used the processing and analytical tools available to us to estimate the configuration of their visual fields, the type and position of their center/s of acute vision, the density of retinal ganglion cells, eye size, visual acuity, density of different types of photoreceptors, peak sensitivity of visual pigments, and absorbance of oil droplets.

Objectives

In order to develop Golden and Bald Eagle specific visual stimuli or deterrents that can be deployed in wind turbine farms, we had to understand how these species see their environment. We have collected various parameters of the visual systems of these species that now need to be analyzed. The analysis of the visual system information will directly lead to the

visual contrast modeling we will use to determine the visual conspicuousness of various light stimuli candidates.

Visual Data Analysis Results

We were given the opportunity to measure the visual field configurations and retinal tissue measurements on 15 Golden Eagles (*Aquila chrysaetos*) and 12 Bald Eagles (*Haliaeetus leucocephalus*) sporadically from March 2018 to September 2019. These eagles were provided to us by rehabilitation centers across the United States. Due to the piece-meal nature of the tissue collection, please review Table L1 (same as Table J1 in Attachment J, presented again here for ease of viewing) for a breakdown of the measurements taken for each eagle in this study. All work performed with these eagles was at the consent of each rehabilitation center and approved under the Purdue University Animal Care and Use Committee Protocol # 1705001579. State and Federal Salvage permits were also in place through Co-P.I. Dr. Todd Katzner (USGS) authorizing all salvage tissue collection and measurement.

Table L1. Table of eagle individual identity, rehab center name and location, and technique used (marked with an X). Sex and approximate age of eagle stated when known.

Species	Eagle ID	Sex	Age	Rehab Center	Location Of Center	Visual Fields	OMT	PH Densities	RGC Densities	MSP
GOEA	101	F	AD	CRC	California	X				
GOEA	102	M	AD	CRC	California	X				
GOEA	103	M	JV	MRCC	Montana				X	
GOEA	104			BMW	Oregon					X*
GOEA	105	M	JV	BMW	Oregon	X				
GOEA	106	F	JV	BMW	Oregon	X	X	X		
GOEA	107	F	JV	LW	Arizona	X				
GOEA	108	M	AD	LW	Arizona	X				
GOEA	109	M	AD	LW	Arizona	X				
GOEA	110		JV	LW	Arizona					X*
GOEA	111		JV	LW	Arizona					X*
GOEA	112		JV	LW	Arizona					X*
GOEA	113	F	AD	MRCC	Montana				X	
GOEA	114	F	AD	MRCC	Montana		X			X
GOEA	115	F	AD	MRCC	Montana		X	X		
BAEA	101	M	AD	WCV	Virginia	X		X	X	
BAEA	102		JV	WCV	Virginia	X		X	X	
BAEA	103	F	AD	WCV	Virginia	X				
BAEA	104	F	AD	WCV	Virginia	X				
BAEA	105	M	JV	WCV	Virginia	X				
BAEA	106	M	JV	MRCC	Montana				X	
BAEA	108		AD	LW	Arizona					X*
BAEA	109	F	JV	MRCC	Montana				X	
BAEA	110	F		WCV	Virginia					X
BAEA	111		AD	BMW	Oregon					X*
BAEA	112		AD	BMW	Oregon					X*
BAEA	113		JV	BMW	Oregon					X*

*Eye was frozen when retrieved; only oil droplets were measured.

OMT = Ocular Media Transmittance, PH = Photoreceptors, RGC = Retinal Ganglion Cell, and MSP = Microspectrophotometry, GOEA = Golden Eagle, BAEA = Bald Eagle, F = Female, M = Male, AD = Adult, JV = Juvenile, BMW = Blue Mountain Wildlife, CRC = California Raptor Center, LW = Liberty Wildlife, MRCC = Montana Raptor Conversation Center, and WCV = Wildlife Center of Virginia.

Visual Field Configuration

The visual fields of Golden and Bald Eagles look similar to visual fields of other raptors that have previously been described (O'Rourke et al. 2010; Potier et al. 2018). With eyes converged (each eye rotated to the maximum anterior position in the head) on the horizontal plane, Golden and Bald Eagles have a narrow binocular field in front of their head and a large blind area behind the head. With eyes diverged (each eye rotated to the maximum posterior position in the head), they have a blind area that extends from in front of their head to the back of the head.

On the horizontal plane, the size of the binocular field with eyes converged was 31.39° ± 1.95° for the Golden Eagle (Figure L1c) and 28.31° ± 2.30° for the Bald Eagle (Figure L1d), this was the elevation (90°) of the maximum binocular width for the Bald Eagle (Figure L1b). The Golden Eagle maximum binocular width, with eyes converged was at 110° elevation with a 31.62° ± 2.79° wide binocular field (Figure L1a). The binocular field extended from 140° (below the beak) to 60° (above the beak), with an average width of 25.53° ± 3.10° for the Golden Eagle with eyes converged (Figure L1a and 3a). The binocular field extended from 120° (below the beak) to 50° (above the beak), with an average width of 16.11° ± 3.27° for the Bald Eagle with eyes converged (Figure L1b and 3b).

On the horizontal plane, the size of the lateral field was 100.61° for the Golden Eagle (Figure L1c) and 124.84° for the Bald Eagle (Figure L1d). The average width of the lateral fields was 112.99° ± 3.84° for the Golden Eagle and 129.70° ± 2.10° for the Bald Eagle with eyes converged. On the horizontal plane, the size of the cyclopean field (total visual field width) was 232.60° for the Golden Eagle (Figure L1c) and 278.00° (Figure L1d) for the Bald Eagle with eyes converged. The average width of the cyclopean fields was 253.21° ± 4.87° for the Golden Eagle (Figure L1c) and 277.00° ± 4.27° for the Bald Eagle (Figure L1d). The reduced lateral and cyclopean fields in the Golden Eagle compared to the Bald Eagle were due to the skull blocking the visual fields behind the head.

On the horizontal plane, the size of the blind area with eyes converged was 127.4° ± 1.58° for the Golden Eagle (Figure L3a) and 82° ± 8.92° for the Bald Eagle (Figure L3b), this was the elevation (270°) of the maximum blind area width for the Golden Eagle (Figure L3a). The Bald Eagle maximum blind area width, with eyes converged was at the 290° elevation (behind the head) with a 92.67° ± 1.46° wide blind area (Figure L3b). The blind area extended from 50° (above the beak) to 270° (behind the head), with an average width of 74.38° ± 8.92° for the Golden Eagle (Figure L3a). The blind area extended from 40° (above the beak) to 270° (behind the head), with an average width of 43.53° ± 8.22° for the Bald Eagle (Figure L3b). The larger blind area above the head in the Golden Eagle will prevent the eagle from seeing directly ahead when looking down during flight

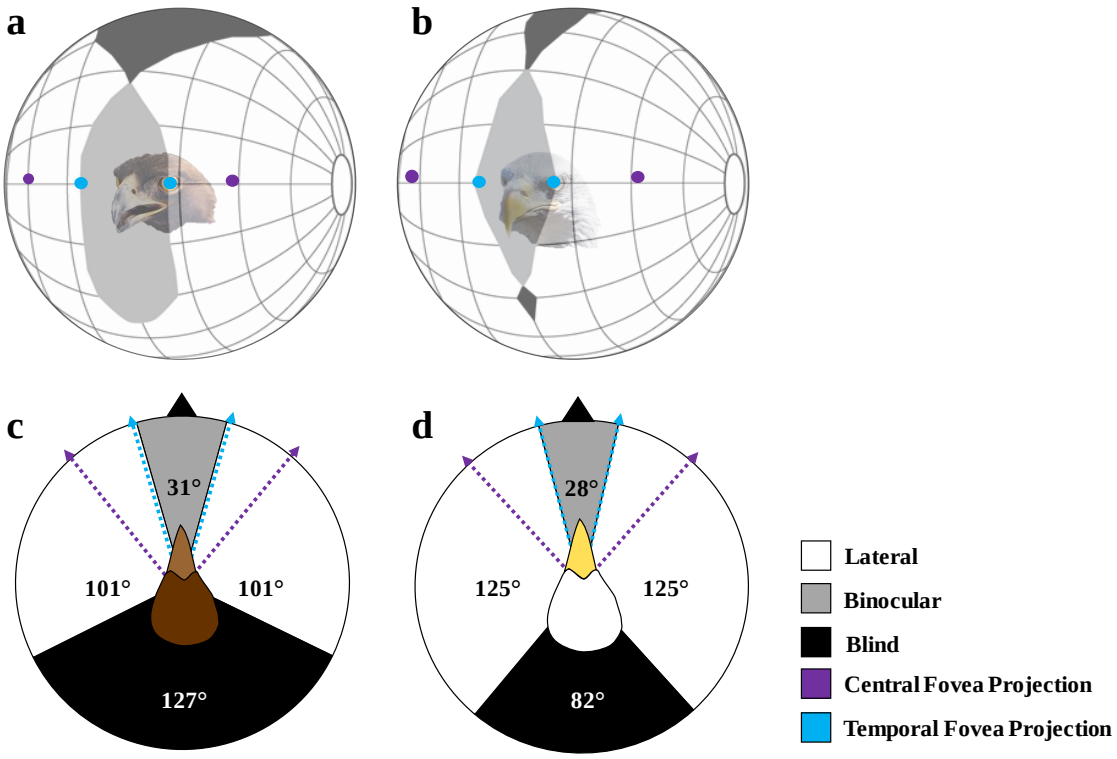

Figure L1. Visual field configurations of the Golden and Bald Eagle when eyes are converged. Spherical projections of the converged visual field around the head of the Golden Eagle (**a**) and the Bald Eagle (**b**). Black lines on the sphere are separated by 20° corresponding to the angular coordinate system used to collect the measurements. The center horizontal line is the 90° elevation used in the horizontal plane figures in panels (**c**) and (**d**). Colored dots represent the projection of the central (purple) and temporal (blue) foveae into the visual fields. Horizontal plane diagrams of the widths of the visual fields and blind area when eyes are converged in the Golden (**c**) and Bald (**d**) Eagles. Arrows represent the projection of the central (purple) and temporal (blue) foveae into the visual fields.

On the horizontal plane, the binocular field was completely abolished when the eyes diverged, producing a blind area width of 6.09° ± 2.33° for the Golden Eagle (Figure L2a and c) and 28.05° ± 2.11° for the Bald Eagle (Figure L2b and d). The blind area extended from 140° (below the beak) to 270° (behind the head) around the head, for both species (Figure L3a and b). On the horizontal plane, the size of the lateral field was 136.01° for the Golden Eagle (Figure L2c) and 127.80° for the Bald Eagle (Figure L2d) with eyes diverged. The cyclopean field was abolished with the eyes diverged for both species.

On the horizontal plane, the size of the blind area behind the head, with eyes diverged, was 81.89° ± 10.87° for the Golden Eagle (Figure L3a) and 76.35° ± 16.61° for the Bald Eagle

(Figure L3b), this was the elevation (270°) of the maximum blind area width for the Bald Eagle. The Golden Eagle maximum blind area width, with eyes diverged was at 280° elevation (behind the head) with an 89.65° ± 19.57° wide blind area (Figure L3a). The average width of the blind area was 42.98° ± 6.51° for the Golden Eagle and 27.89° ± 2.95° for the Bald Eagle with eyes diverged. For the full results of the visual field retinal margins measured when eyes are converged and diverged at all elevations measured around the head, please see Figure 3a and b.

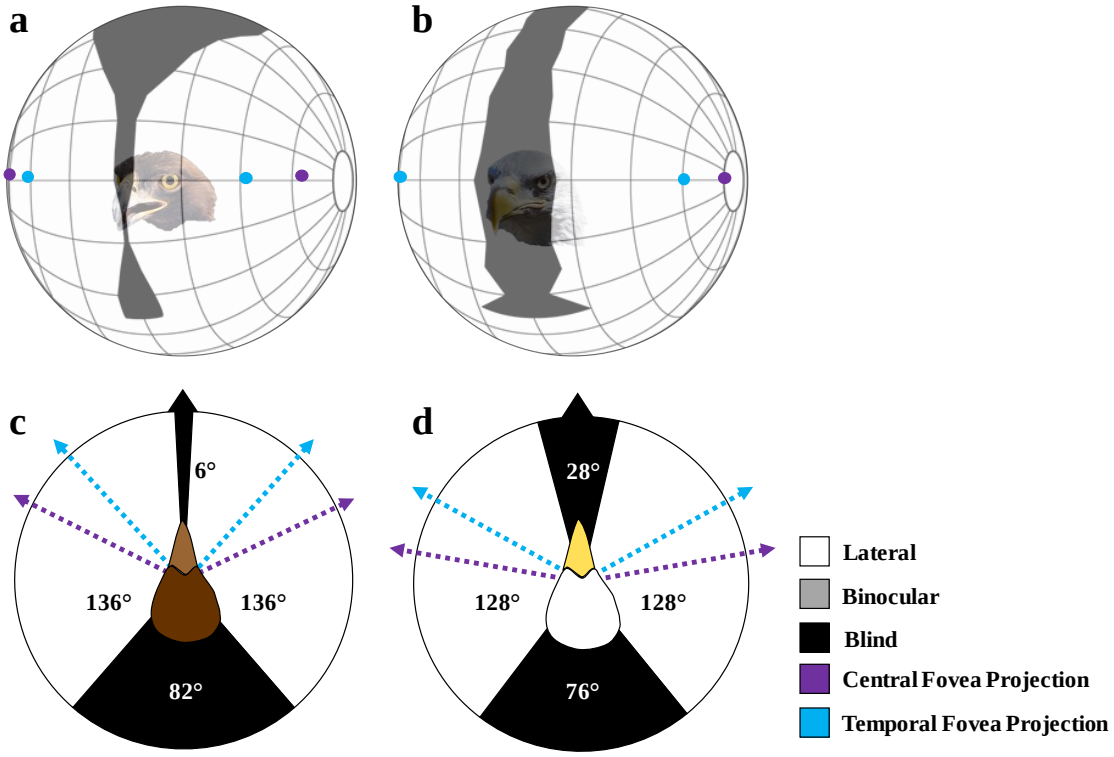

Figure L2. Visual field configurations of the Golden and Bald Eagle when eyes are diverged. Spherical projections of the diverged visual field around the head of the Golden Eagle (**a**) and the Bald Eagle (**b**). Black lines on the sphere are separated by 20° corresponding to the angular coordinate system used to collect the measurements. The center horizontal line is the 90° elevation used in the horizontal plane figures in panels (**c**) and (**d**). Colored dots represent the projection of the central (purple) and temporal (blue) foveae into the visual fields. Horizontal plane diagrams of the widths of the visual fields and blind area when eyes are diverged in the Golden (**c**) and Bald (**d**) Eagles. Arrows represent the projection of the central (purple) and temporal (blue) foveae into the visual fields.

We quantified the eye movement amplitude by comparing eye position between converged and diverged visual field retinal margins. Both Bald and Golden Eagles have a high degree of eye movement relative to other raptors studied to date (Figure 3c and d; O'Rourke et al. 2010). The implication is that these two species can change their visual configuration (i.e.,

relative size of binocular vs. lateral vision) to a larger degree than other raptors species, which could give them a greater flexibility to see different elements of their visual environment. For the Golden Eagle, the largest amplitude eye movement was 40.85° at the 130° elevation (Figure L3c). For the Bald Eagle, the largest eye amplitude movement was 56.36° at the 90° elevation in the horizontal plane (Figure L3d). The average amplitude of eye movements across all elevations measured was 21.88° ± 2.74° for the Golden Eagle and 27.67° ± 3.66° for the Bald Eagle.

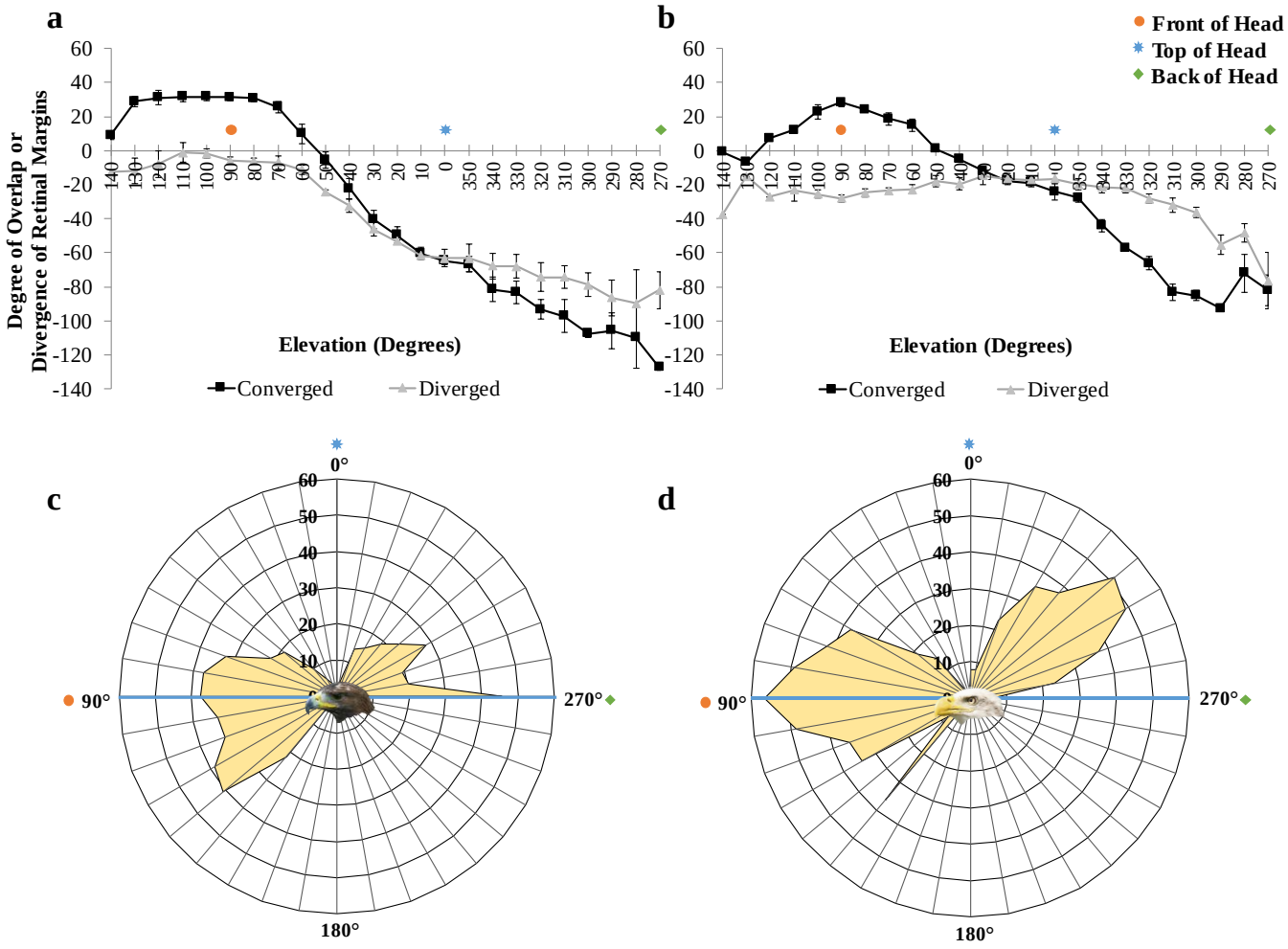

Figure L3. The degree of retinal binocular overlap or divergence in the Golden (**a**) and Bald (**b**) Eagle used to determine the amplitude of eye movements (in degrees) for the Golden (**c**) and Bald (**d**) Eagle. The blue line in the eye movement amplitude panels (**c**) and (**d**) indicates the position of the horizontal plane. Colored shapes indicate elevations in the front (orange circle), top (blue asterisk), and back (green diamond) of the eagle head. In panels (**a**) and (**b**) positive values indicate areas of binocular overlap and negative values indicate blind areas in the visual field. These two panels are a 2D graphical representations of the data presented in figures L1 and L2 (panels a and b; 3D spherical plots) and allow us to view the results from across all elevations measured around the head of the eagles.

Eye Size and Centers of Acute Vision

We were able to measure 12 eyes (5 left, 7 right) from 7 Golden Eagles (3 Females, 1 Male, 3 Unknown). The average eye size measurements were as follows: corneal diameter of 20.67 ± 0.27 mm, transverse diameter of 34.94 ± 0.21 mm, and axial length 31.40 ± 0.25 mm. We were also able to measure 13 eyes (6 left, 7 right) from 8 Bald Eagles (2 Males, 1 Female, 5 Unknown). The average eye size measurements were as follows: corneal diameter of 19.50 ± 0.21 mm, transverse diameter of 31.88 ± 0.17 mm, and axial length 27.18 ± 0.28 mm. Using two-tailed T-tests assuming equal variance, we found that the Golden Eagle eye is significantly larger than the Bald Eagle for all metrics measured (corneal diameter, $t_{23} = 3.41$, p = 0.002; transverse diameter, $t_{23} = 11.57$, p <0.001; axial length, $t_{19} = 11.06$, p <0.001). When comparing with other predatory bird species in the literature, the log axial length to log body mass for the Golden and Bald Eagle are the highest that have been measured (Figure L4). This is an indicator that they will likely have high acuity vision.

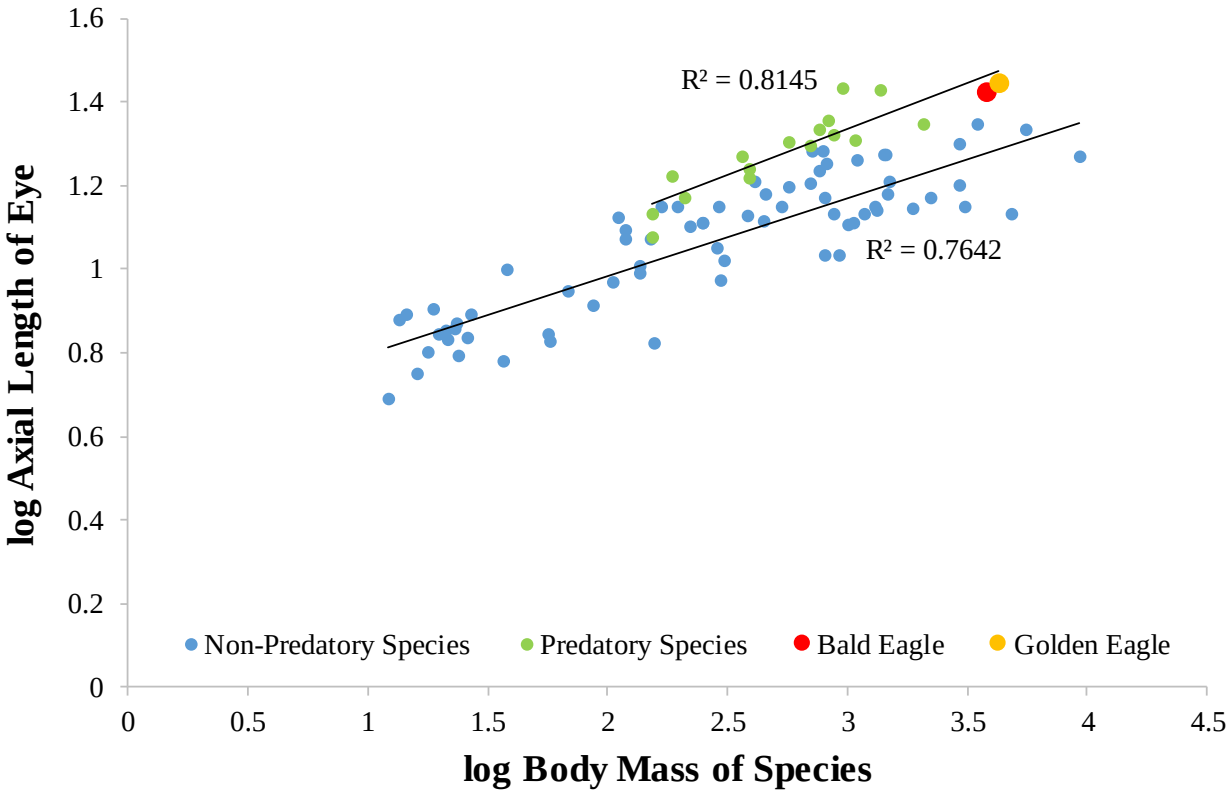

Figure L4. The comparison of the log transformed axial length versus log transformed body mass of avian species, both predatory (green) and non-predatory (blue), and for the Golden (yellow) and Bald (red) Eagles. R^2 values indicate the fit of the trend lines for the predatory and non-predatory species.

While measuring the eyes of both the Golden and Bald Eagles, we noticed a strong asymmetry in the nasal-temporal plane of the eye. This asymmetry presented as the cornea and iris slanting anteriorly towards the nasal side of the eye of the eagle (Figure L5). This has been noted previously in Golden Eagles (Murphy and Dubielzig 1993), but to our knowledge has not been noted in the Bald Eagle. This slant is thought to aid in increasing the frontal field of view for the eagles by converging the optical elements in the eye itself and not by changing the placement of the eye in the skull orbit (Murphy and Dubielzig 1993).

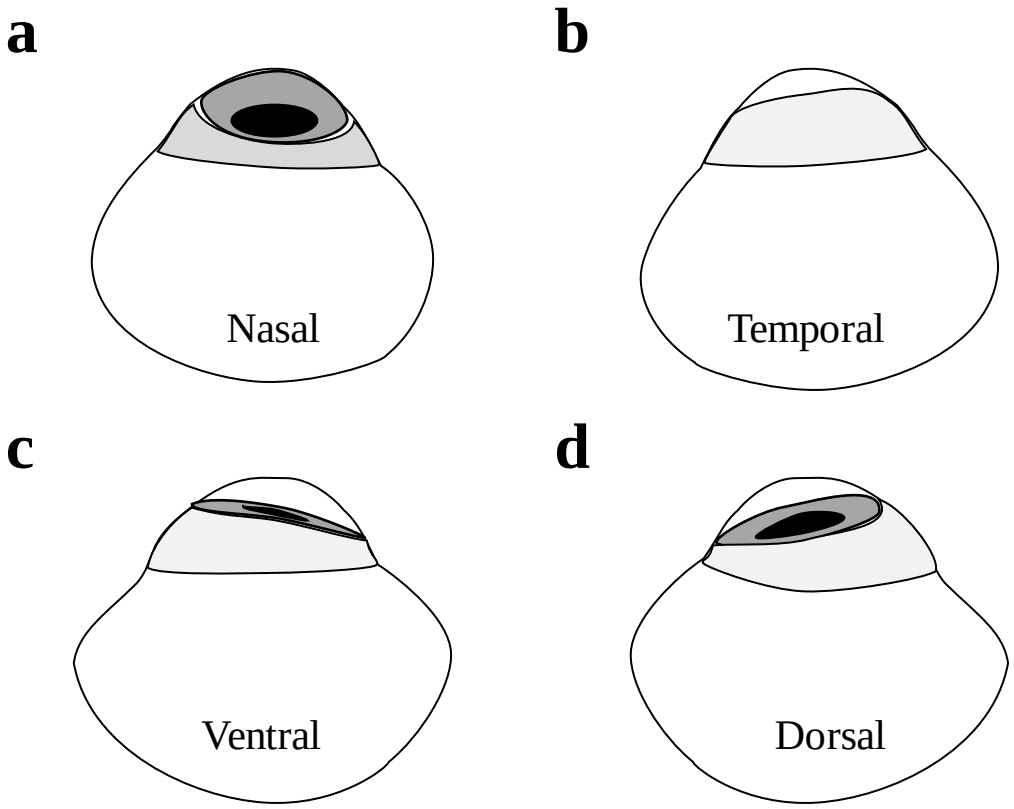

Figure L5. Diagrams of the right eye of a Golden Eagle showing the corneal asymmetry viewed from multiple vantage points; Nasal side (**a**), Temporal Side (**b**), Ventral Side (**c**), and Dorsal Side (**d**).

Upon inspection of the hemisected eye, the two foveae of the Golden and Bald Eagle are clearly visible on the retina for both fresh and preserved specimens (Figure L6; Potier et al. 2017). On preserved specimens (2 Golden Eagle eyes; 1 Bald Eagle eye), the distance between the central and temporal foveae was measured as 6.54 ± 0.25 mm for the Golden Eagle and 7.19 mm for the Bald Eagle. The central fovea projects into the lateral visual field and the temporal fovea projects into the visual field near the binocular overlap when the eyes are converged (Figure L1).

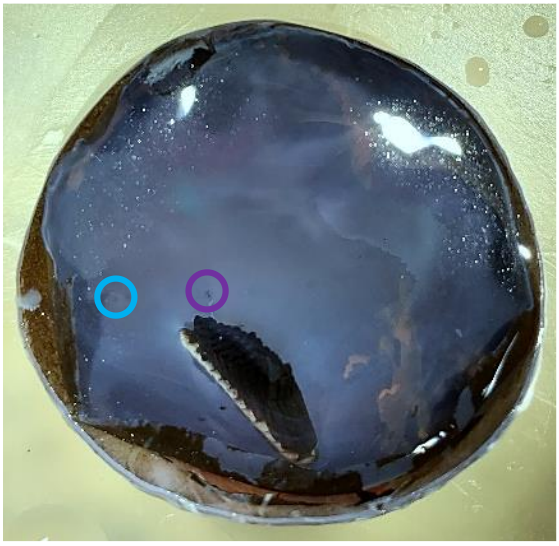

Figure L6. Hemisected right eye of a fresh Golden Eagle specimen. The central fovea is circled by the purple ring and the temporal fovea is circled by the blue ring.

The presence of the two foveae within each eye suggest that the frontal part of the eagle's head would have four areas with high acute vision. Additionally, in both species the binocular field extends above their beaks but not behind their heads when eyes are converged or diverged. If the eagles are flying with their bills parallel to the ground, they should be able visually to resolve a wind turbine from relatively far away, particularly when any of the four foveae are aligned with the wind turbine. However, if the eagles are flying but their beaks are perpendicular to the ground (i.e., beak facing towards the ground), they may have difficulty resolving the blades of a wind turbine. If the eagle is flying parallel to the wind turbine, peripheral vision may be of outmost importance. However, if the eagle is flying towards the wind turbine, our results indicate that it might not be able to see the turbine at all, increasing the chances of a collision.

Transmittance of the Ocular Media

We were able to measure the transmittance of the ocular media of 5 eyes (3 left, 2 right) from 3 Golden Eagles (3 Females). We did not have the opportunity to measure the ocular media on the Bald Eagle. We measured the wavelength at 50% transmittance ($\lambda_{T0.5}$) of the Golden Eagle cornea/lens as 383 ± 2.29 nm and the vitreous humor as 308 ± 1.03 nm (Figure L7). The cornea and lens could not be separated without destroying the sample, so they remained in their intact configuration during the measurements. The $\lambda_{T0.5}$ values from the measurements indicate that the vitreous humor can transmit light down to 308nm (75nm lower than the cornea/lens). However, because light first passes through the cornea and lens they are the primary structures that limit the wavelengths of light that reach the retina and not the vitreous humor. Therefore, we used the spectrum from the cornea/lens in the visual contrast perceptual modeling to account for the transmittance of the ocular media for the Golden Eagle.

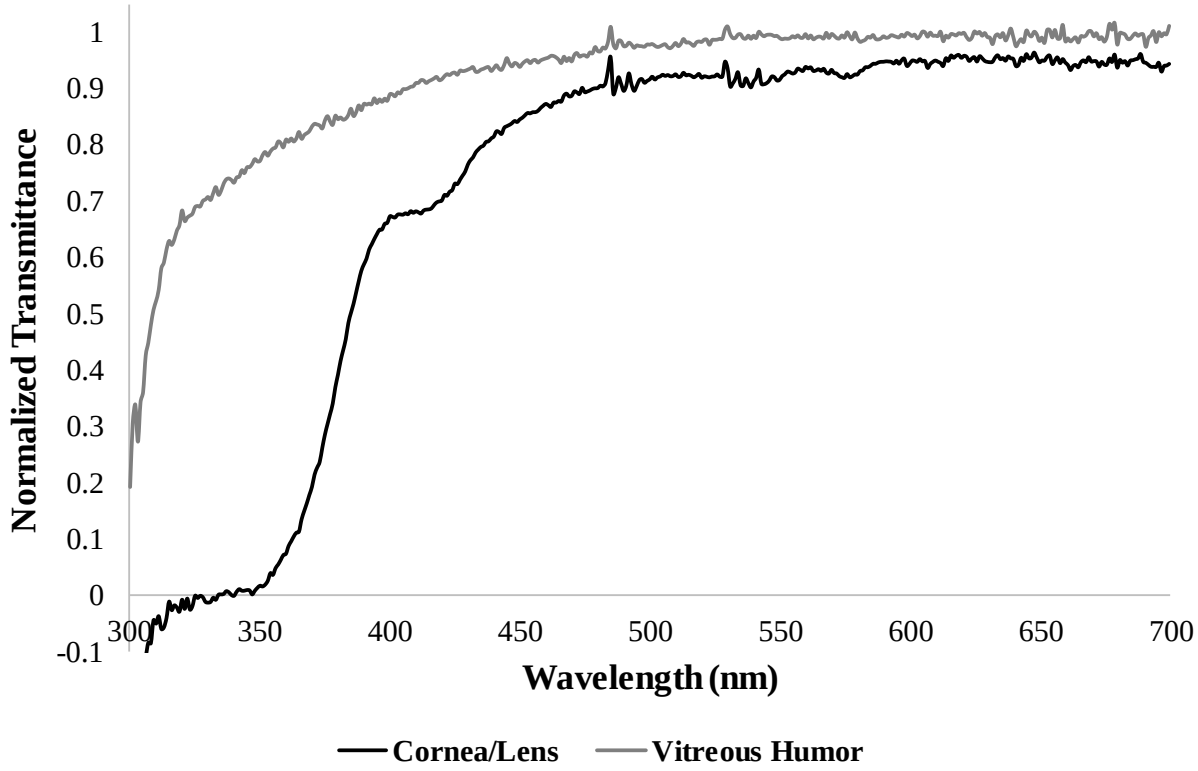

Figure L7. Average normalized transmittance of the ocular media of the Golden Eagle eye.

Photoreceptor Densities

We were able to calculate photoreceptor densities from 1 Golden Eagle retina (left eye; Female) and 2 Bald Eagle retinas (1 left, 1 right; 1 Male and 1 Unknown). We counted 40 sites from the Golden Eagle retina and 10 sites from the Bald Eagle retinal periphery, which are a small sub-sample of the countable sites. We were not able to count as many sites on the Bald Eagles due to a large amount of pigmented epithelium remaining attached in the center of each retina, and the quality of the epifluorescent images. In the images we were able to easily identify five types of oil droplets, the T-Type (violet-sensitive [VS] photoreceptor), the C-Type (short-wavelength sensitive [SWS] photoreceptor), the P-Type (double cone [DC] photoreceptor), the Y-Type (medium-wavelength sensitive [MWS] photoreceptor), and the R-Type (long-wavelength sensitive [LWS] photoreceptor) (Figure L8).

The mean density of all photoreceptor types was 17,930 ± 1,054 cells/mm^2 (peak density 33,200 cells/mm^2) for the Golden Eagle and 14,709 ± 738 cells/mm^2 (peak density 20,000 cells/mm^2) for the Bald Eagle. The mean density of the single cone photoreceptors (VS, SWS, MWS, and LWS) used for color vision was 9,410 ± 463 cells/mm^2 for the Golden Eagle and 9,073 ± 541 cells/mm^2 for the Bald Eagle. The mean density of the double cone photoreceptor (DC) used for achromatic vision was 8,520 ± 709 cells/mm^2 for the Golden Eagle and 5,636 ±

357 cells/mm^2 for the Bald Eagle. The mean density of the single cones by type for the Golden Eagle are: VS = 1,990 ± 165 cells/mm^2; SWS = 1,910 ± 138 cells/mm^2; MWS = 2,420 ± 183 cells/mm^2; LWS = 3,090 ± 191 cells/mm^2. The mean density of the single cones by type for the Bald Eagle are: VS = 1,227 ± 124 cells/mm^2 ^{2}; SWS = 2,304 ± 295 cells/mm^2; MWS = 2,291 ± 146 cells/mm^2; LWS = 3,251 ± 210 cells/mm^2. The relative densities of the photoreceptors for the Golden Eagle are: VS = 1, SWS = 0.96, MWS = 1.22, LWS = 1.55, DC = 4.28. The relative densities of the photoreceptors for the Bald Eagle are: VS = 1, SWS = 1.88, MWS = 1.87, LWS = 2.65, DC = 4.59. The spatial resolving power, a proxy for visual acuity, was 25.23 ± 0.75 cycles per degree (13.8-34.9 range) for the Golden Eagle and 19.52 ± 0.45 cycles per degree (18.0-22.7 range) for the Bald Eagle. Using a two-tailed T-test assuming equal variance, we found that the spatial resolving power was significantly different between the two species (t_{46} = 6.54, p <0.001). Please note that the spatial resolving power is likely to be much higher in and around the fovea of these eagles, however we were not able to count images from the fovea.

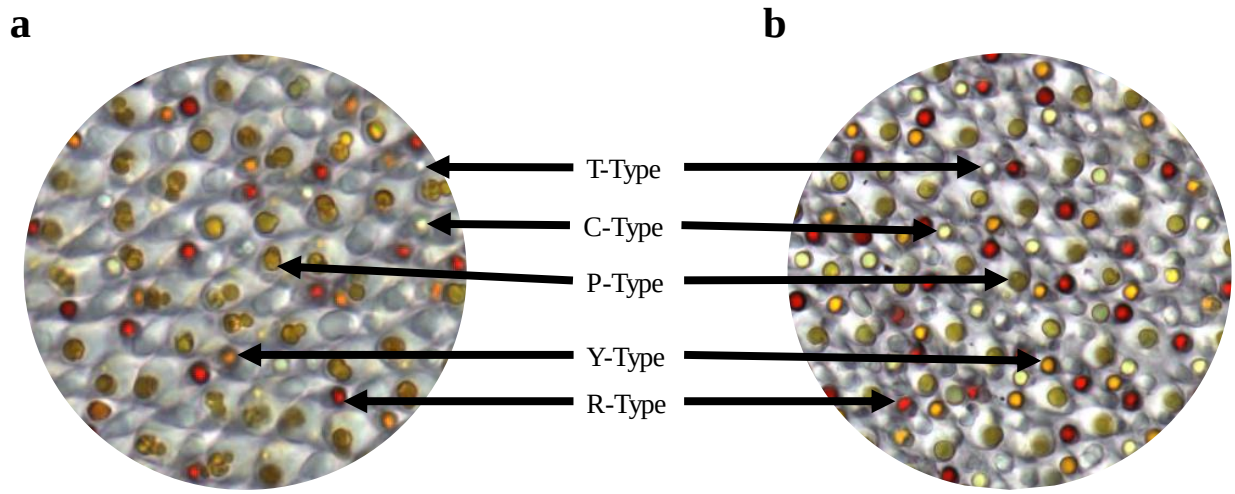

Figure L8. Brightfield image of the Golden (**a**) and Bald (**b**) Eagle retina, with black arrows indicating the type of oil droplet (T-, C-, P-, Y-, or R-Type).

We noticed that on gross examination of the Golden Eagle retina, and later confirmed on oil droplet images, that the central region of the retina and the area surrounding the central fovea contained more highly pigmented P-Type oil droplets (darker color) at higher densities (Figure L9a). These oil droplets were measured using microspectrophotometry (MSP) and were found to be P3-Type variants of the double cone oil droplet. Additional counting of sites across the retina will need to be performed to determine the actual density of these P3-Type variants and their distribution across the retina. From the small sub-sample of sites we counted, we found that in areas where the P3-Type variant was observed the density of the double cone increased from 6,117 ± 496 cells/mm^2 outside of this region (Figure L9b) to 12,125 ± 1,119 cells/mm^2 inside of this region (Figure L9c). We found P3-Type variants in the Bald Eagle retina (via MSP), but due to the large amount of pigmented epithelium in the central region of both retinas, we were unable to determine if this highly pigmented area was present. This area of higher density double cones,

containing more highly pigmented oil droplets, could improve the motion detection abilities of the Golden Eagle in the center of their retina and around their central fovea when flying.

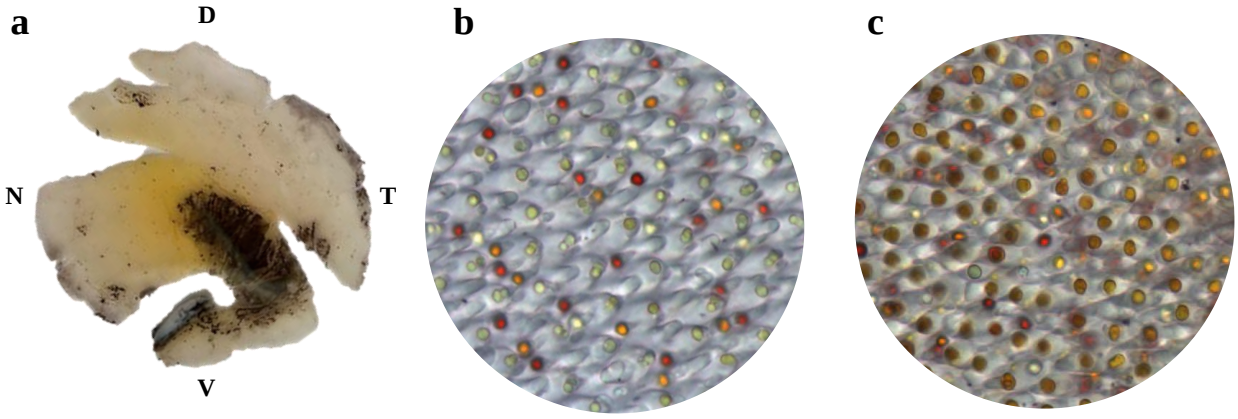

Figure L9. The Golden Eagle retina with observed highly pigmented (yellow-orange) region in the center of the retina (**a**), as well as the oil droplets outside (**b**) and within (**c**) this pigmented region. N = Nasal, T = Temporal, D = Dorsal, and V = Ventral.

Photoreceptor Sensitivities

We were able to measure the sensitivity of photoreceptor visual pigments and absorbance of oil droplets on fresh eagle tissue for 1 Golden Eagle (Female) and 1 Bald Eagle (Female). We were also given frozen eyes from 4 Golden Eagles (Sex Unknown) and 4 Bald Eagles (Sex Unknown), from which we measured additional oil droplets. We analyzed the absorbance curves of 38 cones and 15 rods from the Golden Eagle and 16 cones and 10 rods from the Bald Eagle.

We confirmed that both the Golden and Bald eagles have a violet-sensitive visual system containing four types of single cones, one type of double cone, and a rod photoreceptor. Template fitting revealed that the photoreceptor outer segments contained A1-rhodopsin pigments (Govardovskii et al. 2000). The mean λ_{max}, or peak absorbance, of the photoreceptors for the Golden Eagle were VS = 414 nm (violet-wavelength sensitive; Figure L10a), SWS = 477 nm (short-wavelength sensitive; Figure L11a), MWS = 496 nm (medium-wavelength sensitive; Figure L12a), LWS = 568 nm (long-wavelength sensitive; Figure L13a), DC = 565 nm (double cone; Figure L14a), and RH1 = 496 nm (rod; Figure L15a) (Table L2). The mean λ_{max}, or peak absorbance, of the photoreceptors for the Bald Eagle were VS = 413 nm (Figure L10b), SWS = 446 nm (Figure L11b), MWS = 507 nm (Figure L12b), LWS = 578 nm (Figure L13b), DC = 577 nm (Figure L14b), and RH1 = 504 nm (Figure L15b) (Table L3).

When viewing figures L10-15, please note that once a photoreceptor is measured (pre-bleach spectrum), the data are confirmed by bleaching it and re-measuring the same cell (post-bleach spectrum). Because the cell reacts with light, exposure to bright light for 60 seconds

degrades any light-absorbing visual pigments and therefore the absorbance peak disappears when measuring a photoreceptor cell's visual pigment after exposure to light. The pre-bleach and post-bleach curves are shown for the different cone types to confirm that the visual pigment did in fact bleach. In instances where it is difficult to see the difference between the pre-bleach and post-bleach spectra, a difference spectrum (grey markers) is shown. Pay particular attention to the peak of the pre-bleach spectra to see differences in the Golden and Bald Eagle peak sensitivities.

We analyzed the absorbance curves of 235 oil droplets from the Golden Eagle and 197 oil droplets from the Bald Eagle. We identified seven unique types of oil droplets across the Golden and Bald Eagle retinae, four types belonging to the four single cone photoreceptors and the remaining three types belonging to the double cone photoreceptor (Figure L16 for Golden Eagle; Figure L17 for Bald Eagle). The double cone oil droplet often varies in the concentration of the carotenoids across the retina (Hart 2001a ; Hart 2001b), producing these three variants of the P-Type oil droplet. For both species, the T-Type oil droplet corresponded to the VS single cone, the C-Type oil droplet corresponded to the SWS single cone, the Y-Type oil droplet corresponded to the MWS single cone, the R-Type oil droplet corresponded to the LWS single cone, and the P-Type oil droplet (P1-, P2-, and P3-Type variants) corresponded to the double cone.

All lambda parameters (λ_{cut}, λ_{mid}, λ_0, b, and β_{mid}) measured from the oil droplet spectra are provided in TableL2 (Golden Eagle) and TableL3 (Bald Eagle) for the sake of clarity. The T-Type oil droplet is transparent between 300-700 nm, so no lambda parameters could be measured. The P2-Type variant oil droplet, has two peaks within its spectra, so each were measured separately and labeled P2a (first, shorter wavelength peak) and P2b (second, longer wavelength peak). When viewing figures L16 and L17, we present the absorbance spectra for each type of oil droplet. It is from these spectra (specifically the right cut-off arm or the point where the absorbance drops from 1 to almost 0) we are able to calculate the lambda and shape parameters (Tables L2 and L3) that we use in the visual perceptual modeling.

Table L2. Peak absorbance (λ_{max}) ± SE of the single cone, double cone, and rod photoreceptors of the Golden Eagle. Lambda parameters (λ_{cut}, λ_{mid}, λ_0, b, and B_{mid}) ± SE measured from the oil droplet spectra of the Golden Eagle.

Golden Eagle	**Rod**	**Single Cones**				**Double Cone**			
		VS	**SWS**	**MWS**	**LWS**				
Visual Pigments									
Mean λ_{max} (nm)	496 ± 1.83	414 ± 2.85	477 ± 1.89	496 ± 1.88	568 ± 1.51	565			
N	15	8	4	8	17	1			
		T-Type	C-Type	Y-Type	R-Type	P1-Type	P2-Type a	P2-Type b	P3-Type
Oil Droplets									
Mean λ_{cut} (nm)			418 ± 4.17	520 ± 0.86	574 ± 0.84	442 ± 2.00	450 ± 1.61	495 ± 1.24	499 ± 1.09
Mean λ_{mid} (nm)			449 ± 1.21	537 ± 0.98	594 ± 0.79	460 ± 1.31	466 ± 1.27	510 ± 1.21	518 ± 1.16
Mean λ_0 (nm)			441 ± 1.52	532 ± 0.94	589 ± 0.80	455 ± 1.44	462 ± 1.33	506 ± 1.21	513 ± 1.10
Mean b			0.059 ± 0.0064	0.087 ± 0.0018	0.072 ± 0.0009	0.086 ± 0.0041	0.099 ± 0.0051	0.097 ± 0.0018	0.076 ± 0.0028
Mean B_{mid}			0.020 ± 0.0022	0.030 ± 0.0006	0.025 ± 0.0003	0.030 ± 0.0014	0.034 ± 0.0017	0.034 ± 0.0006	0.026 ± 0.0010
N		16	16	40	64	35	37	-	27

Table L3. Peak absorbance (λ_{max}) ± SE of the single cone, double cone, and rod photoreceptors of the Bald Eagle. Lambda parameters (λ_{cut}, λ_{mid}, λ_0, b, and B_{mid}) ± SE measured from the oil droplet spectra of the Bald Eagle.

Bald Eagle	**Rod**	**Single Cones**				**Double Cone**			
		VS	**SWS**	**MWS**	**LWS**				
Visual Pigments									
Mean λ_{max} (nm)	504 ± 0.81	413 ± 2.36	446	507 ± 1.70	578 ± 5.59	577			
N	10	4	1	6	4	1			
		T-Type	C-Type	Y-Type	R-Type	P1-Type	P2-Type a	P2-Type b	P3-Type
Oil Droplets									
Mean λ_{cut} (nm)			417 ± 1.78	523 ± 1.05	575 ± 0.63	442 ± 1.78	446 ± 2.71	493 ± 1.02	505 ± 1.16
Mean λ_{mid} (nm)			453 ± 1.16	541 ± 1.24	594 ± 0.71	461 ± 2.10	463 ± 1.25	509 ± 1.60	520 ± 1.27
Mean λ_0 (nm)			444 ± 1.11	536 ± 1.18	589 ± 0.63	456 ± 1.92	459 ± 1.58	505 ± 1.39	516 ± 1.23
Mean b			0.043 ± 0.0020	0.084 ± 0.0021	0.076 ± 0.0011	0.081 ± 0.0048	0.099 ± 0.0088	0.096 ± 0.0039	0.092 ± 0.0018
Mean B_{mid}			0.015 ± 0.0007	0.029 ± 0.0007	0.026 ± 0.0004	0.028 ± 0.0017	0.034 ± 0.0031	0.033 ± 0.0013	0.032 ± 0.0006
N		17	34	40	49	11	18	-	28

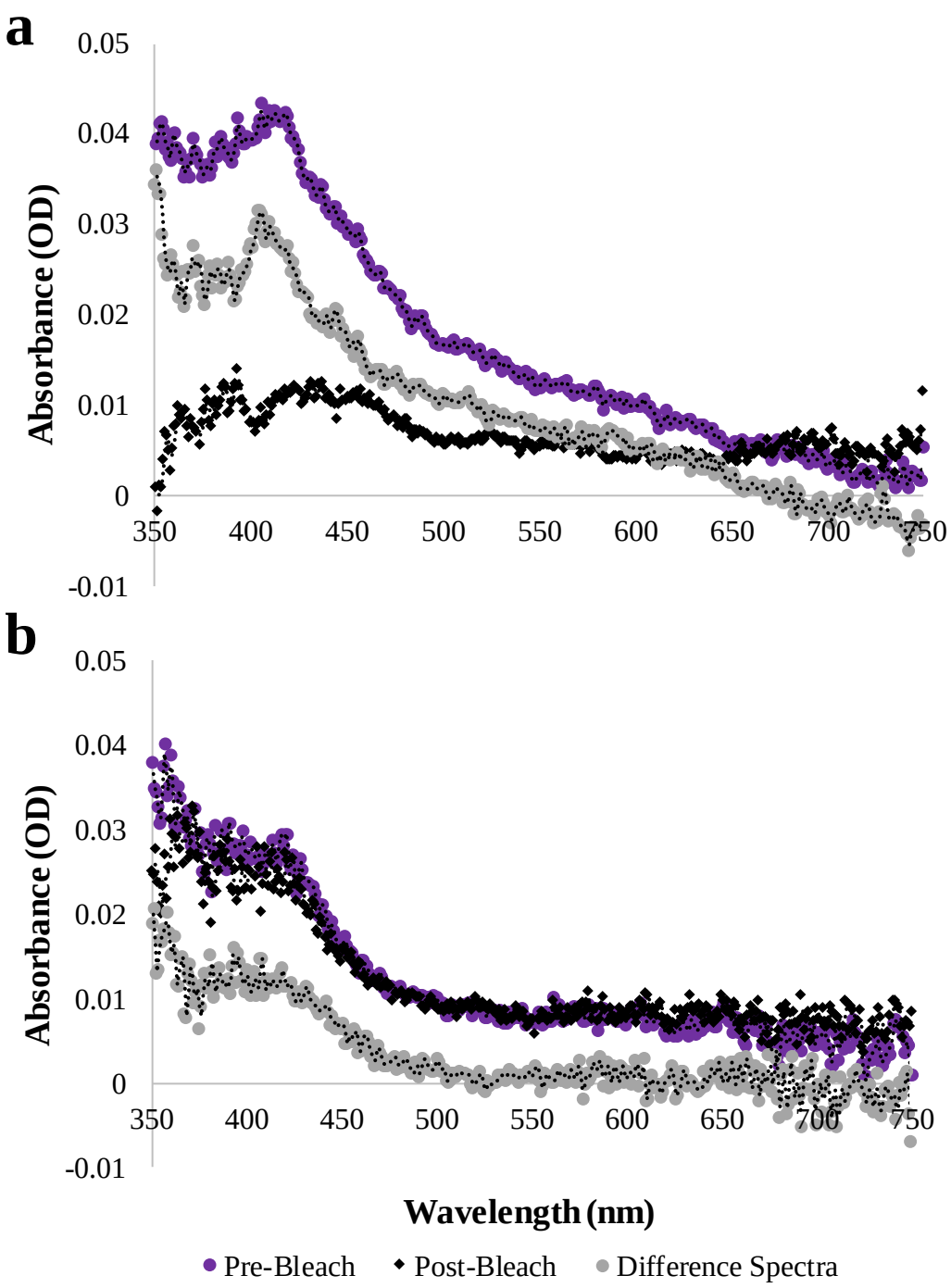

Figure L10. Pre- and Post-bleach absorbance spectra of the VS single cone from the Golden (**a**) and Bald (**b**) Eagle. Grey spectra show the difference between the pre- and post-bleach spectra. Moving averages are shown with black dashed lines.

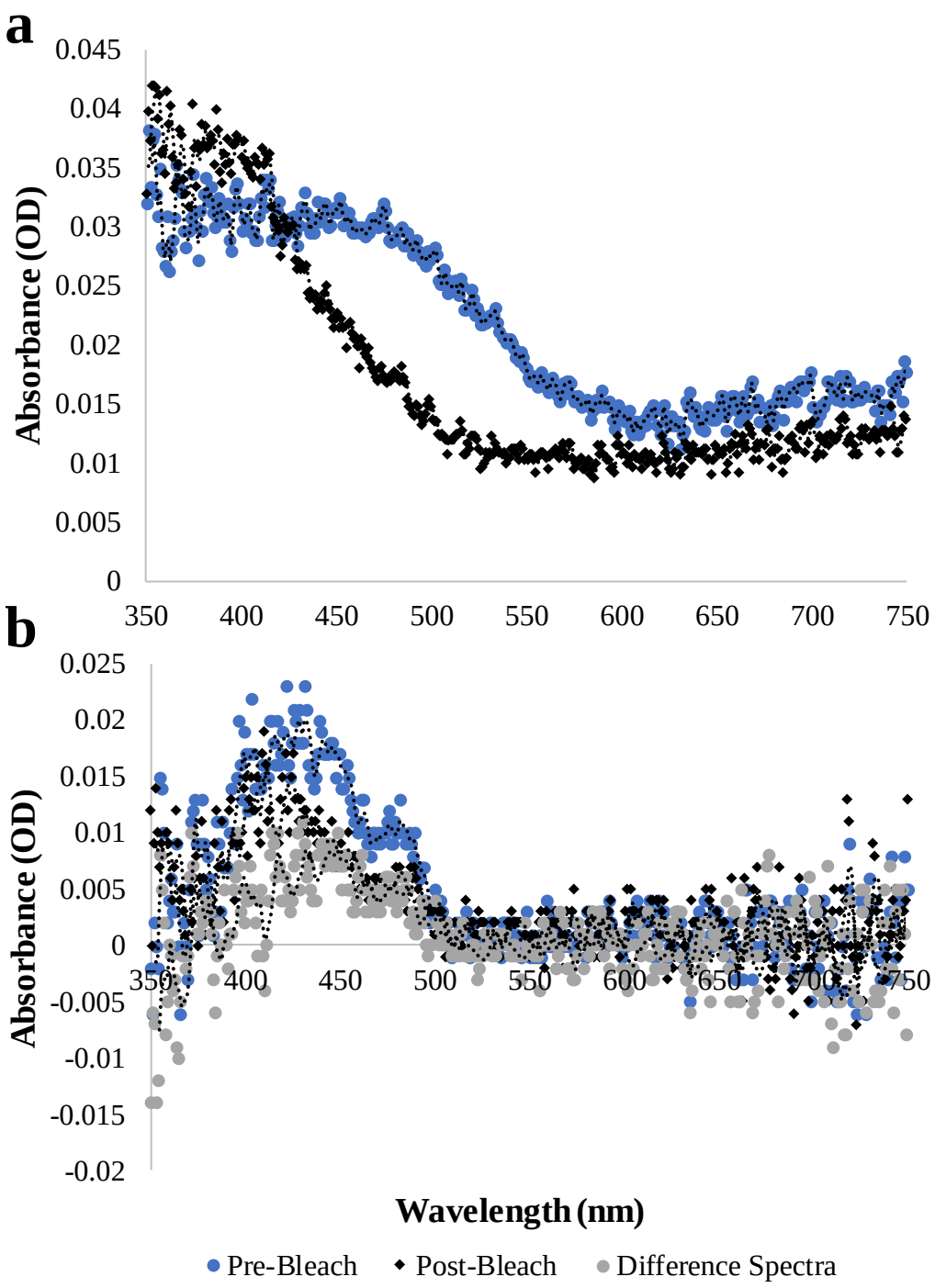

Figure L11. Pre- and Post-bleach absorbance spectra of the SWS single cone from the Golden (**a**) and Bald (**b**) Eagle. Grey spectrum shows the difference between the pre- and post-bleach spectra. Moving averages are shown with black dashed lines.

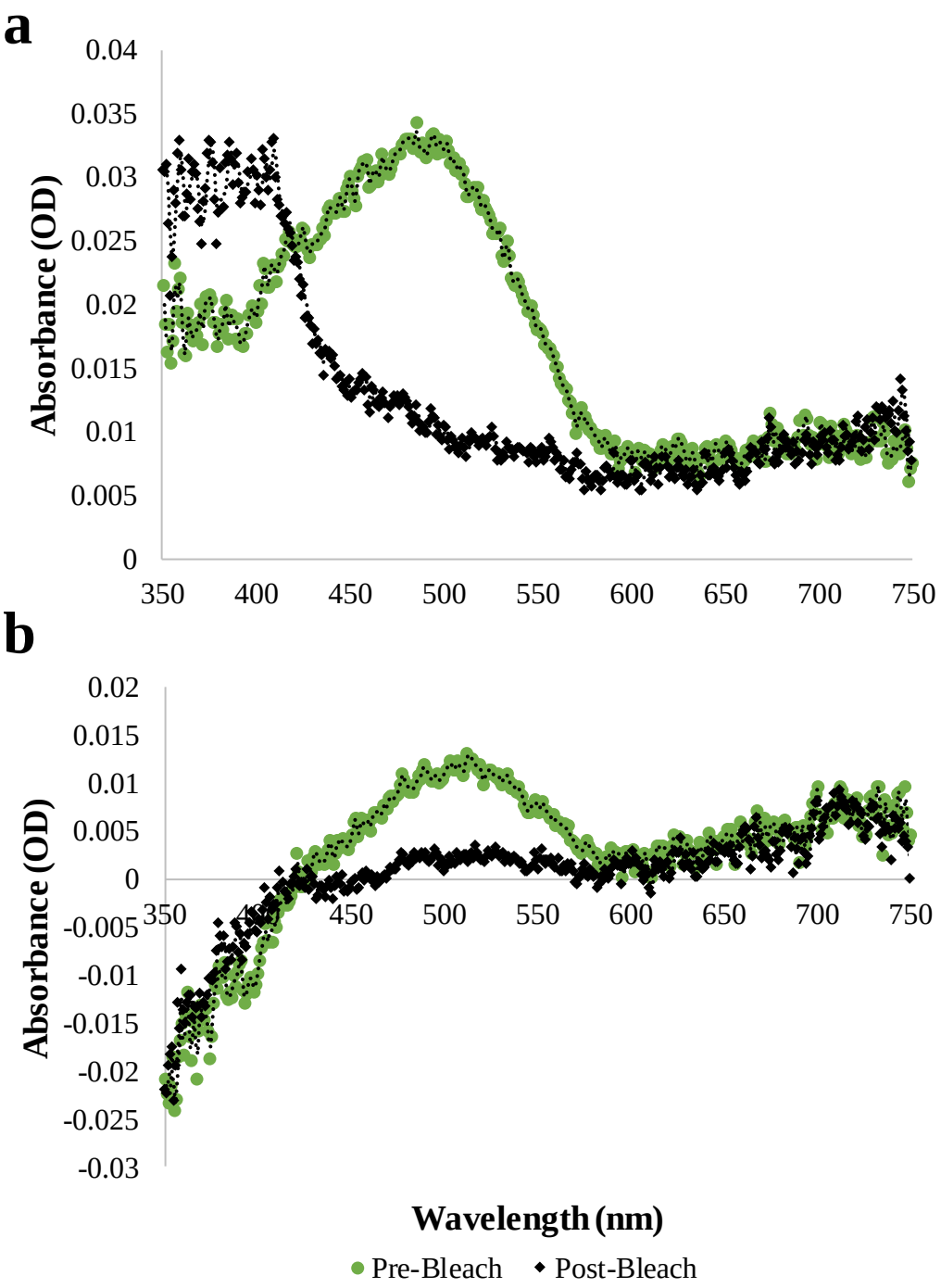

Figure L12. Pre- and Post-bleach absorbance spectra of the MWS single cone from the Golden (**a**) and Bald (**b**) Eagle. Moving averages are shown with black dashed lines.

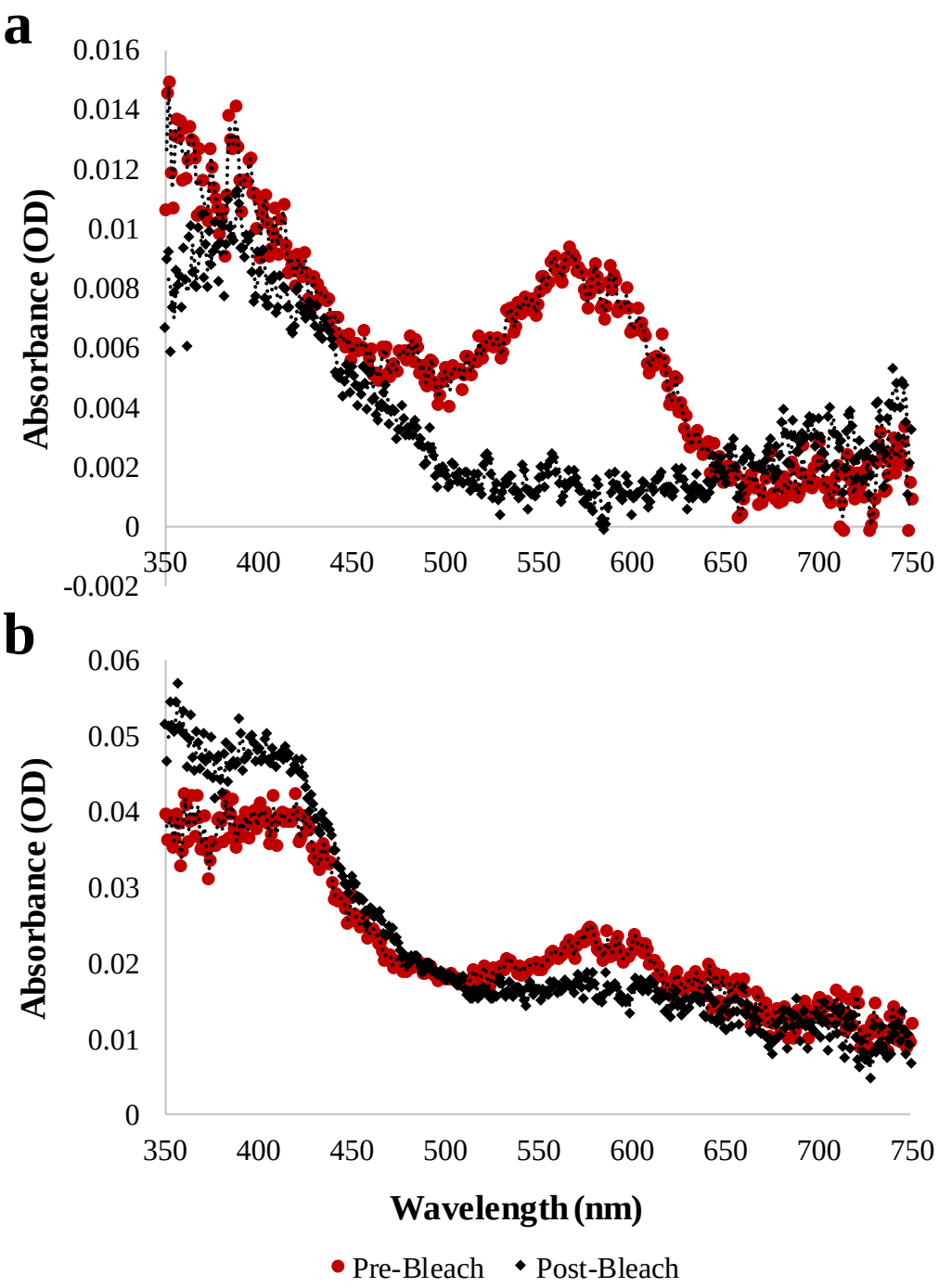

Figure L13. Pre- and Post-bleach absorbance spectra of the LWS single cone from the Golden (**a**) and Bald (**b**) Eagle. Moving averages are shown with black dashed lines.

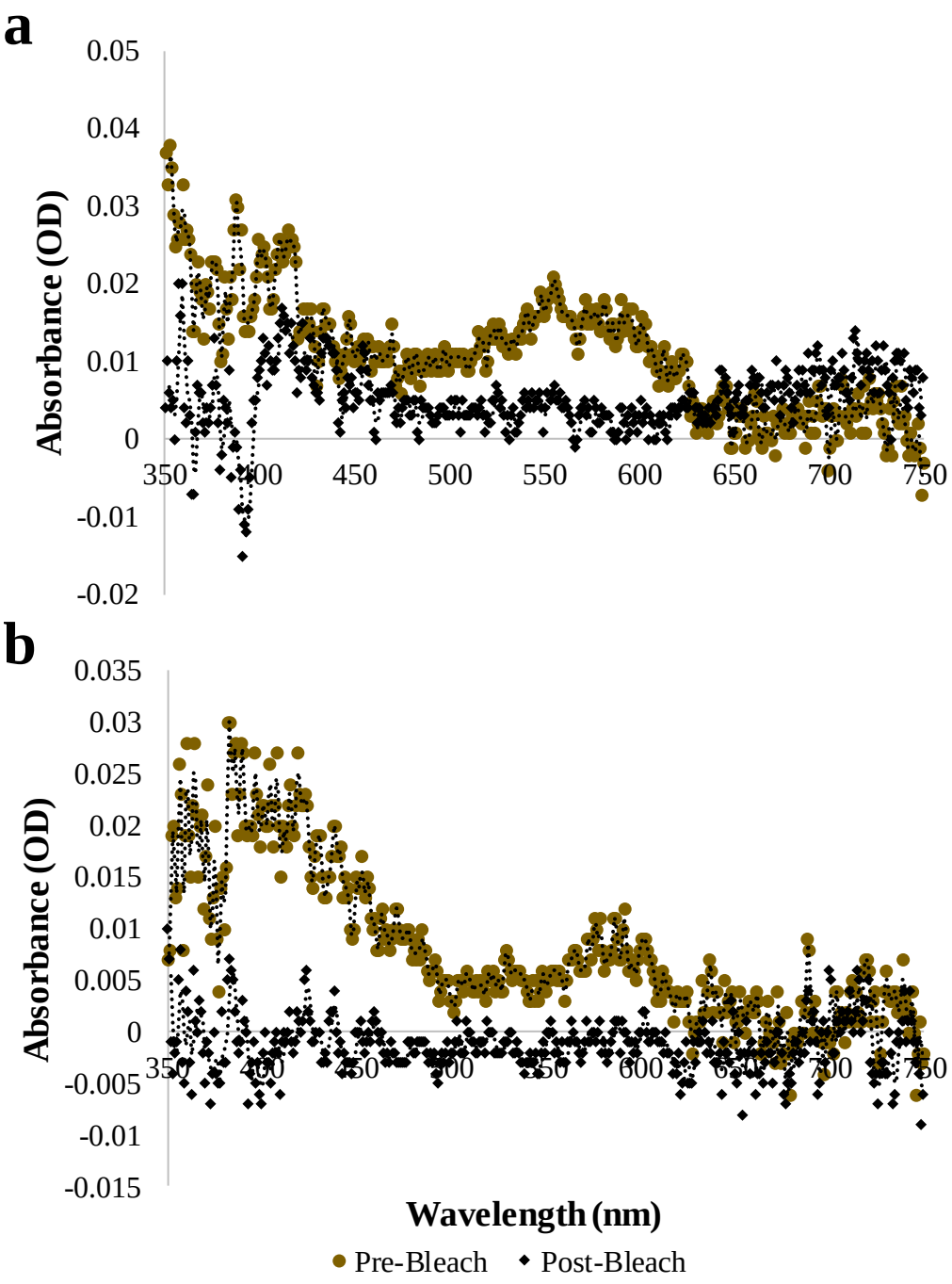

Figure L14. Pre- and Post-bleach absorbance spectra of the double cone from the Golden (**a**) and Bald (**b**) Eagle. Moving averages are shown with black dashed lines.

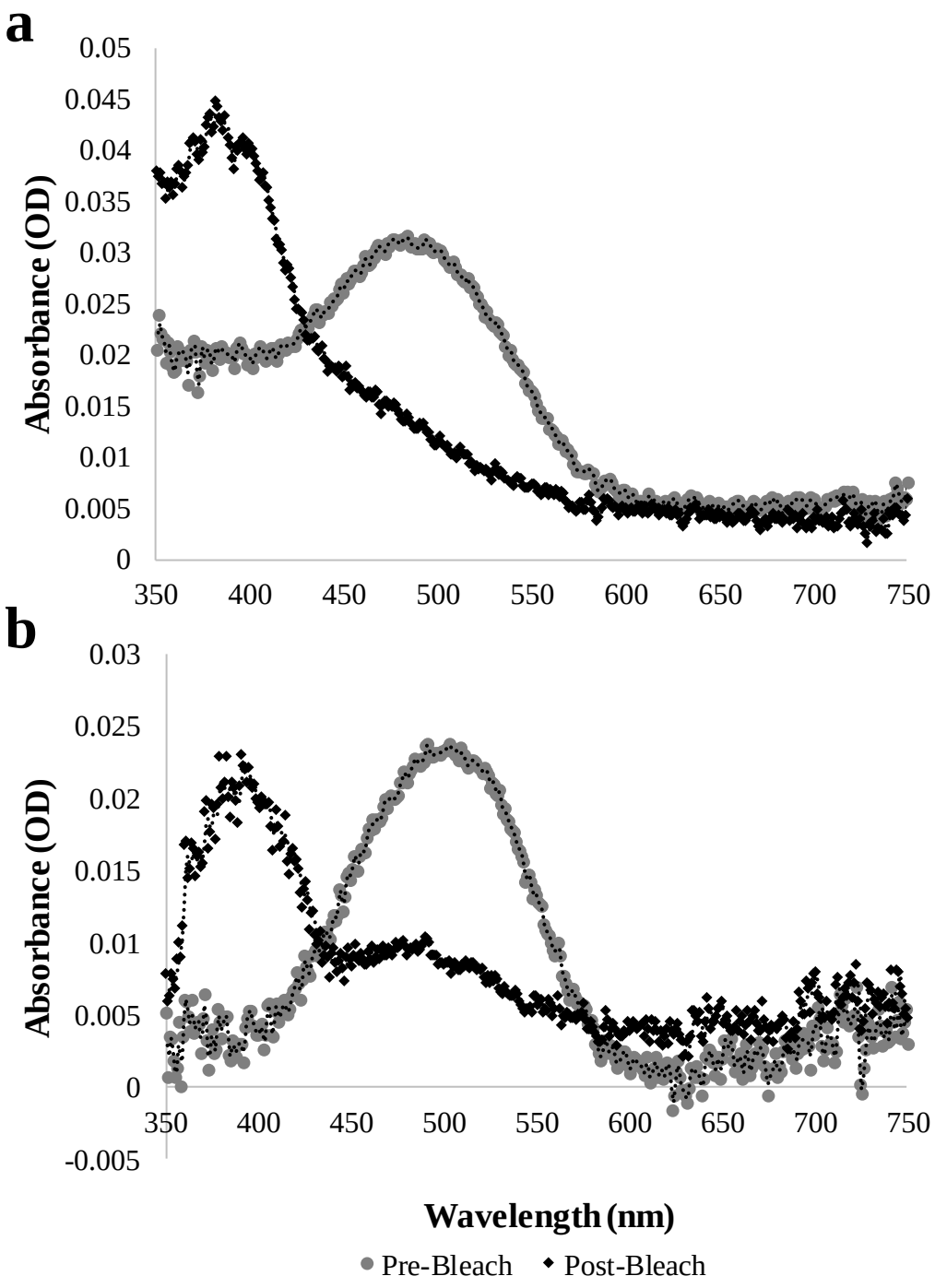

Figure L15. Pre- and Post-bleach absorbance spectra of the rod photoreceptor from the Golden (**a**) and Bald (**b**) Eagle. Moving averages are shown with black dashed lines.

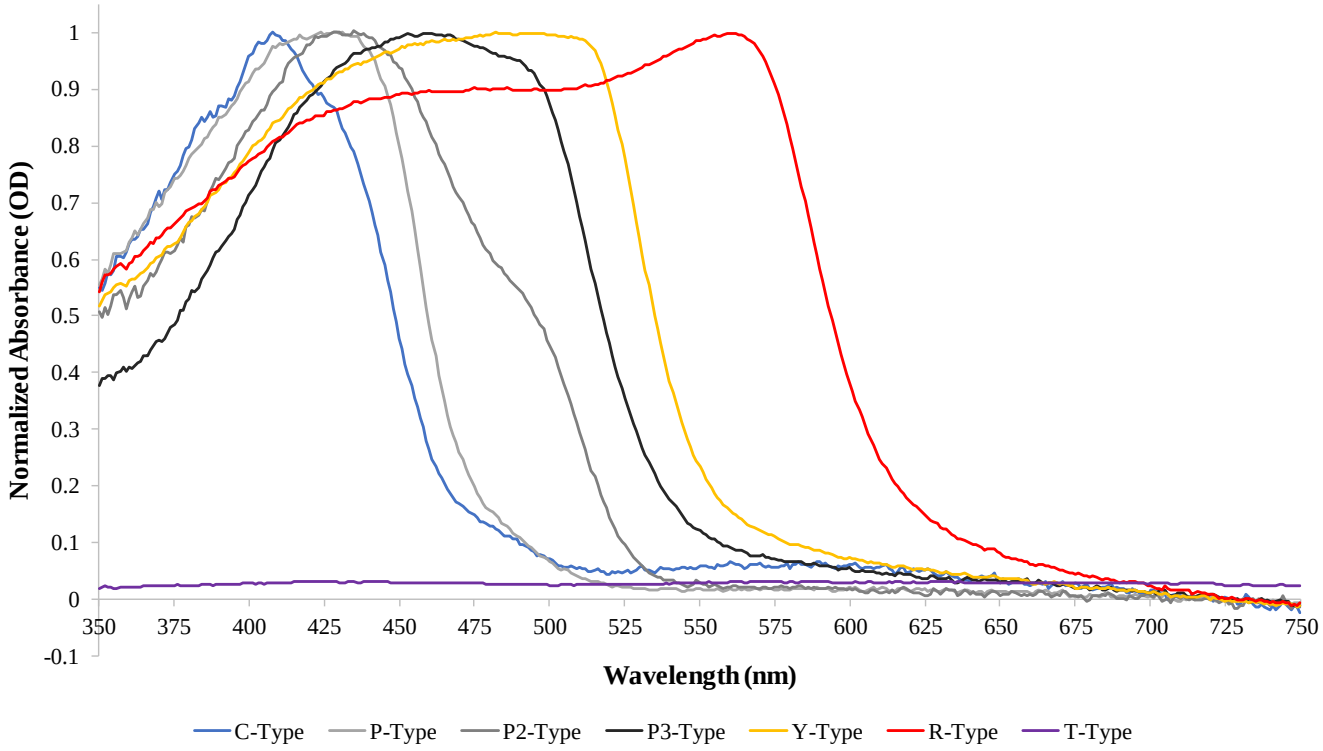

Figure L16. The average normalized oil droplet absorbance spectra from the Golden Eagle. The T-Type oil droplet (purple line) was not normalized for ease of viewing.

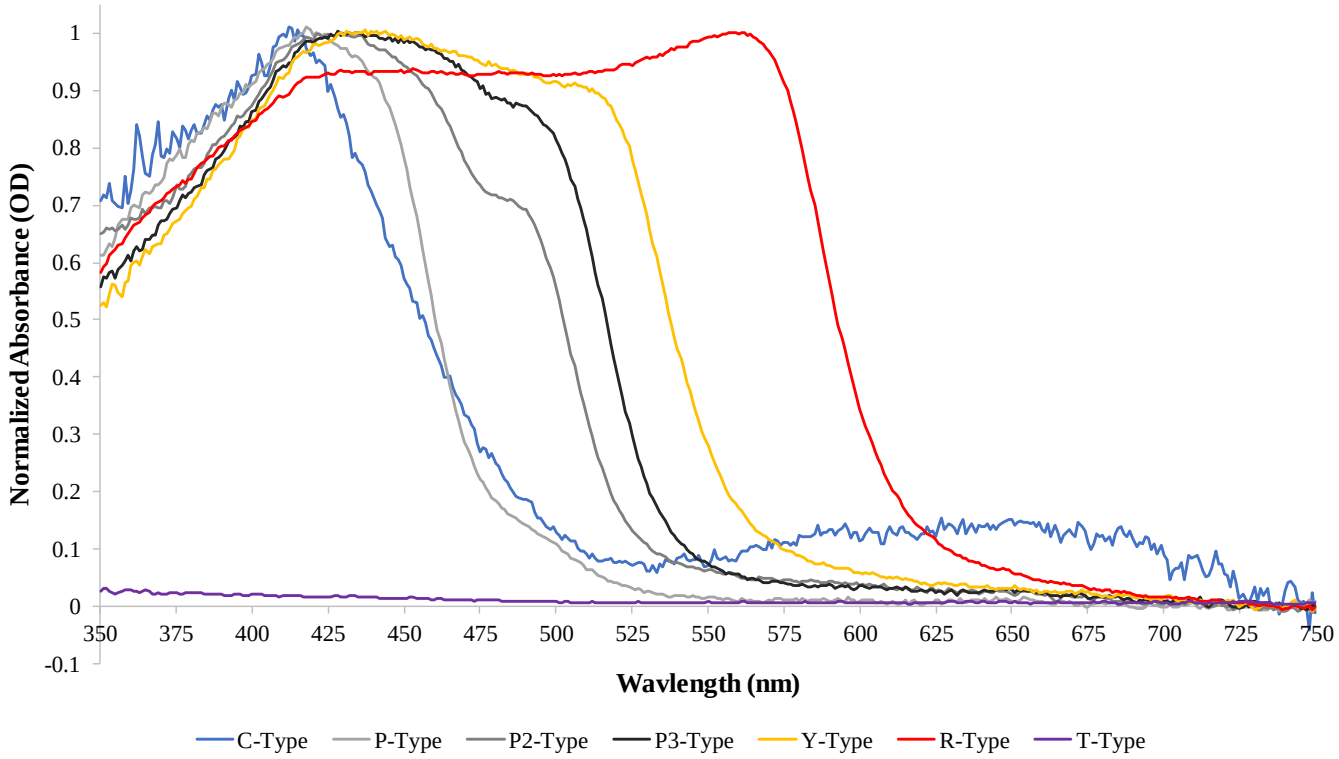

Figure L17. The average normalized oil droplet absorbance spectra from the Bald Eagle. The T-Type oil droplet (purple line) was not normalized for ease of viewing.

Visual Contrast Modeling

We calculated the chromatic contrast of the 201 LED spectra against various visual backgrounds using Pavo 2.3 in R. These calculations incorporated the transmittance of the ocular media properties of the Golden Eagle ($\lambda_{T0.5}$ = 383 nm; fitted curve from cornea-lens data in Figure L7), and the transmittance of the ocular media properties from other raptors in the literature, for the Bald Eagle ($\lambda_{T0.5}$ = 375 nm; Mitkus et al. 2018). These calculations also included the species-specific peak sensitivity of the visual pigments (λ_{max}), the cut-off absorbance values of the oil droplets (λ_{cut}), and the relative densities of the VS, SWS, MWS, and LWS oil droplets. We chose a weber fraction of 0.1 for the noise estimates from the photoreceptors (Olsson et al. 2018).

The results of the contrast calculations are presented as just noticeable differences (JND), the minimum threshold of stimulation that is required for the bird to see the stimulus 50% of the time. A JND of ≤4 is considered difficult to distinguish from the background and a JND >4 is considered easy to distinguish from the background. All LED lights had chromatic contrast results above 4 JND. Overall, the results from all visual backgrounds used in the calculations (Figures L18-L22) indicate that indigo/blue lights and orange/red light stimuli are good signal

candidate signals for the behavioral experiments. For Golden Eagles, an indigo/blue (peak wavelength from 410-460 nm; 436-442 nm maximum conspicuousness) and orange/red (peak wavelength from 590-655 nm; 616-626 nm maximum conspicuousness) light stimuli are good signal candidate signals (Figures L18-L22). For Bald Eagles, an indigo/blue (peak wavelength from 420-470 nm; 446-448 nm maximum conspicuousness) and orange/red (peak wavelength from 580-650 nm; 606-612 nm maximum conspicuousness) light stimuli are good signal candidate signals (Figures L18-L22).

The results of the LEDs against a blue sky background are as follows. For the Golden Eagle, an LED light with a peak wavelength ranging from 410-460 nm (indigo/blue; 436 nm maximum conspicuousness) or 590-655 nm (orange/red; 620 nm maximum conspicuousness) is the most conspicuous against a blue sky (Figure L18). For the Bald Eagle, an LED light with a peak wavelength ranging from 420-470 nm (indigo/blue; 446 nm maximum conspicuousness) or 580-650 nm (orange/red; 608 nm maximum conspicuousness) is the most conspicuous against a blue sky (Figure L18).

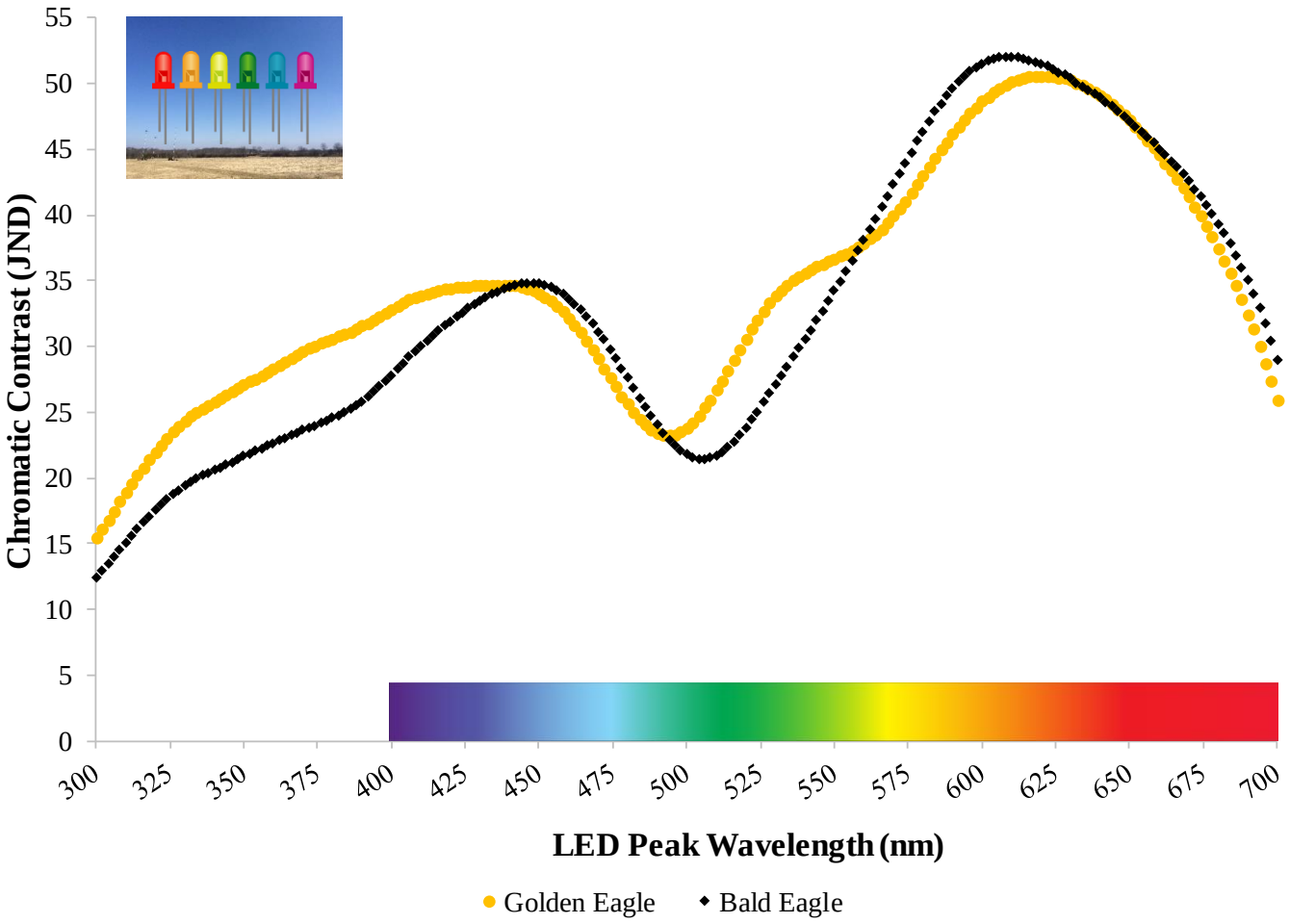

Figure L18. Chromatic contrast of LED lights against a clear blue sky for the Golden and Bald Eagle.

The results of the LEDs against a bare ground background are as follows. For the Golden Eagle, an LED light with a peak wavelength ranging from 410-460 nm (indigo/blue; 442 nm maximum conspicuousness) or 592-646 nm (orange/red; 618 nm maximum conspicuousness) is the most conspicuous against bare ground (Figure L19). For the Bald Eagle, an LED light with a peak wavelength ranging from 424-468 nm (indigo/blue; 448 nm maximum conspicuousness) or 580-644 nm (orange/red; 606 nm maximum conspicuousness) is the most conspicuous against bare ground (Figure L19). A third sweet spot is now clearly visible for the Golden Eagle with an LED light with a peak wavelength ranging from 530-546 nm (green/yellow; 538 nm maximum conspicuousness).

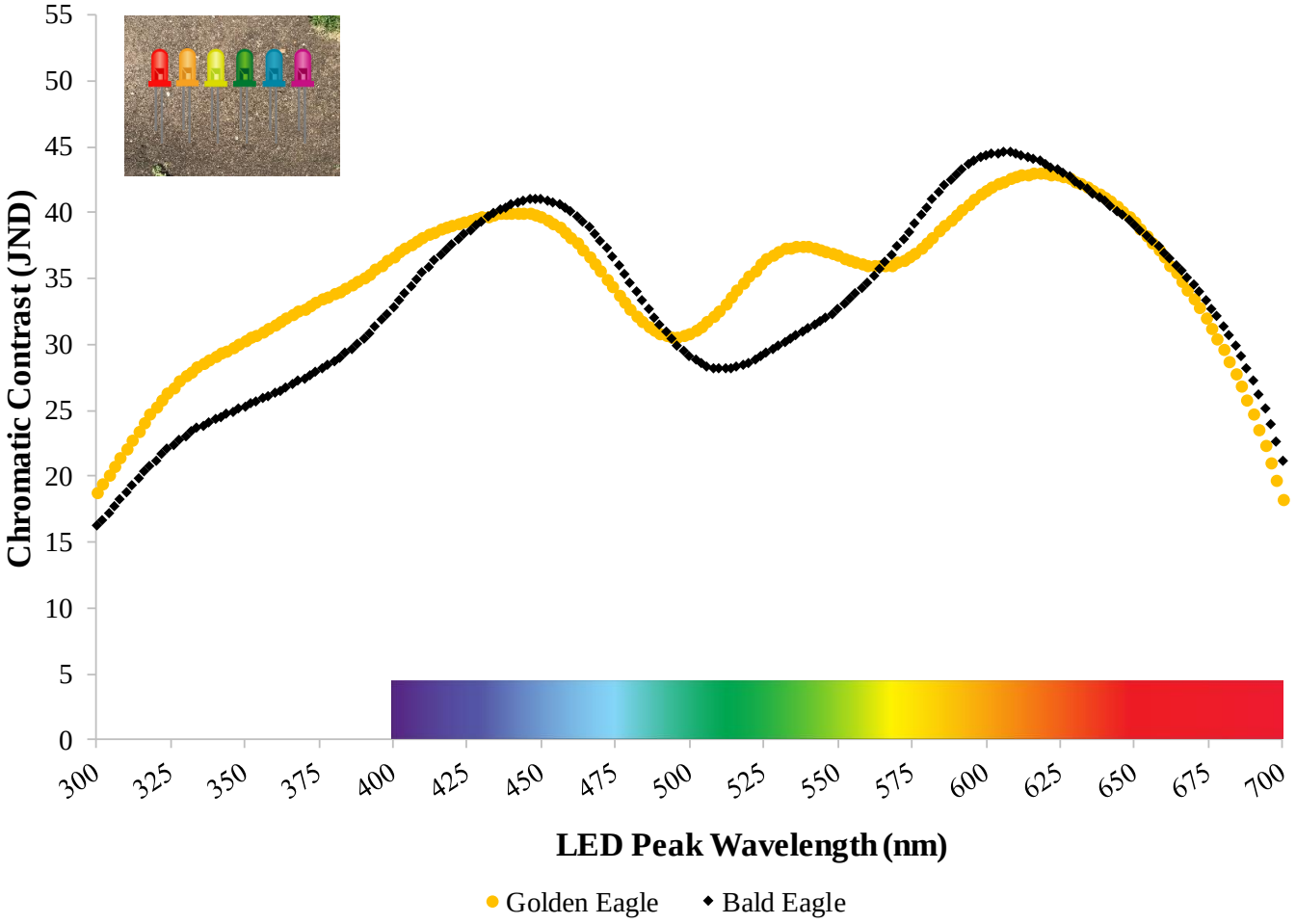

Figure L19. Chromatic contrast of LED lights against a bare ground background for the Golden and Bald Eagle.

The results of the LEDs against a dormant grass background are as follows. For the Golden Eagle, an LED light with a peak wavelength ranging from 416-456 nm (indigo/blue; 442 nm maximum conspicuousness) or 598-638 nm (orange/red; 616 nm maximum conspicuousness) is the most conspicuous against dormant grass (Figure L20). For the Bald Eagle, an LED light with a peak wavelength ranging from 422-468 nm (indigo/blue; 448 nm maximum conspicuousness) or 580-642 nm (orange/red; 606 nm maximum conspicuousness) is the most conspicuous against dormant grass (Figure L20). A third sweet spot is now clearly visible for the Golden Eagle with an LED light with a peak wavelength ranging from 536-552 nm (green/yellow; 542 nm maximum conspicuousness).

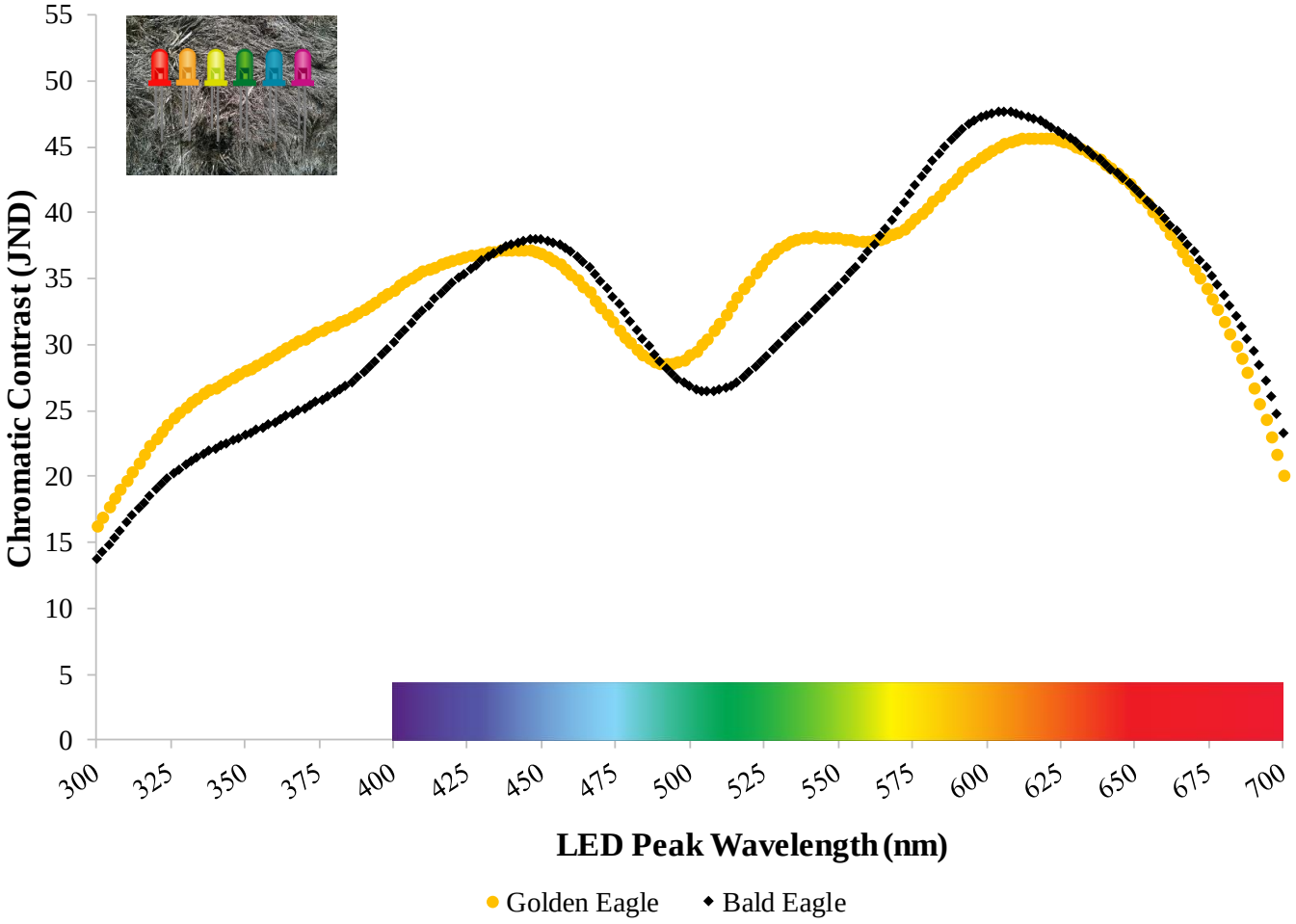

Figure L20. Chromatic contrast of LED lights against a dormant grass background for the Golden and Bald Eagle.

The results of the LEDs against a green grass background are as follows. For the Golden Eagle, an LED light with a peak wavelength ranging from 410-454 nm (indigo/blue; 438 nm maximum conspicuousness) or 606-648 nm (orange/red; 626 nm maximum conspicuousness) is the most conspicuous against green grass (Figure L21). For the Bald Eagle, an LED light with a peak wavelength ranging from 422-466 nm (indigo/blue; 446 nm maximum conspicuousness) or 590-642 nm (orange/red; 612 nm maximum conspicuousness) is the most conspicuous against green grass (Figure L21).

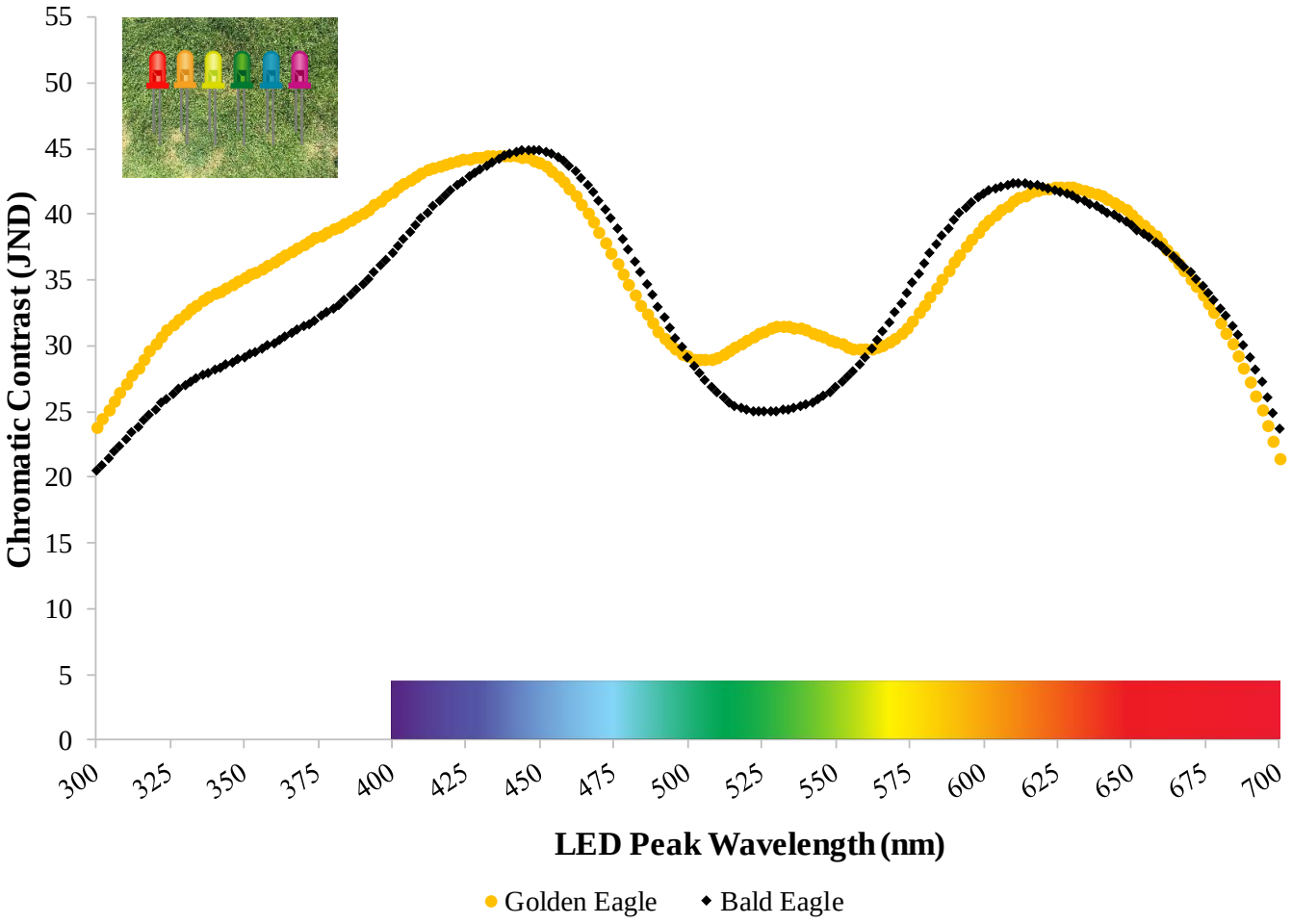

Figure L21. Chromatic contrast of LED lights against a green grass background for the Golden and Bald Eagle.

The results of the LEDs against a white paint background (proxy for color of wind turbines) are as follows. For the Golden Eagle, an LED light with a peak wavelength ranging from 416-456 nm (indigo/blue; 442 nm maximum conspicuousness) or 596-638 nm (orange/red; 618 nm maximum conspicuousness) is the most conspicuous against white paint (Figure L22). For the Bald Eagle, an LED light with a peak wavelength ranging from 424-468 nm (indigo/blue; 448 nm maximum conspicuousness) or 582-632 nm (orange/red; 606 nm maximum conspicuousness) is the most conspicuous against white paint (Figure L22). A third sweet spot is now clearly visible for the Golden Eagle with an LED light with a peak wavelength ranging from 538-556 nm (green/yellow; 550 nm maximum conspicuousness).

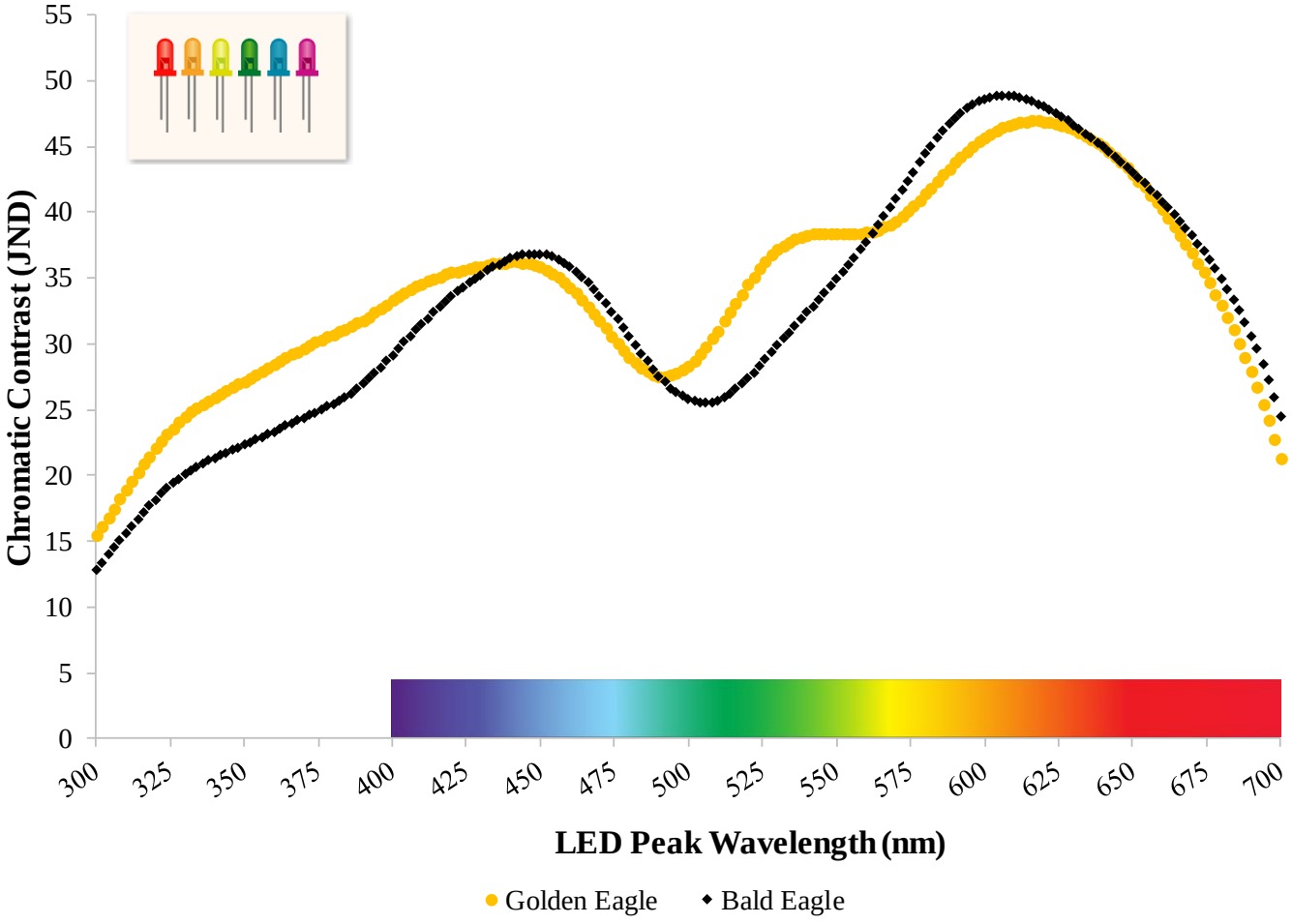

Figure L22. Chromatic contrast of LED lights against a white paint background for the Golden and Bald Eagle.

Summary of Results

We were able to attain visual system information from 15 Golden Eagles and 12 Bald Eagles over the course of this project. We found that both species of eagles have narrow binocular visual fields in front of the head and large blind areas above and behind the head when the eyes are converged forward. The Golden Eagle has wider blind areas above and behind the head than the Bald Eagle when the eyes are converged. This suggests their overall visual field coverage in space is reduced in size compared to the Bald Eagle. This is especially relevant when the Golden Eagle is looking down while flying during hunting bouts, as their visual fields and centers of high acuity (temporal fovea) are not projecting toward the path that the Golden Eagle is flying. Lack of high acuity vision and visual attention to their flight path may contribute to wind turbine collisions in this species. However, the Golden Eagle has larger eyes and higher peripheral visual acuity than the Bald Eagle. Which should lead to better visual capabilities in terms of resolution of an image of a wind turbine on the retina, potentially counteracting some of the drawbacks that the blind areas impose in their visual field space.

Investigation into the eyes of the Golden and Bald Eagle revealed species-specific differences and similarities that had impacts on candidate visual light stimuli that could be used in the behavioral experiments. We found that the ocular media of the Golden Eagle prevents this species from seeing ultraviolet (UV) light well below 383 nm, with no UV light below 350 nm reaching the retina. We were not able to measure the ocular media in the Bald Eagle. In Golden Eagles, and most likely Bald Eagles, this indicates that UV light signals are not good candidate stimuli for alerting eagles to or deterring them from wind turbines.

The sensitivities of the Golden and Bald Eagle retina were different for each species. The visual pigment peak sensitivity of the violet-sensitive photoreceptor was similar between the two species (414 nm for Golden Eagle and 413 nm for Bald Eagle). However, all other photoreceptors were different in visual pigment peak sensitivity, especially the short-wavelength sensitive visual pigment (31 nm difference), indicating that both species have different sensitivity ranges to light. When looking at the filtering properties of the oil droplets contained within the photoreceptors both species had very similar filtering cutoff values for all oil droplet types. This has an effect on the functional peak sensitivity of the photoreceptors. In the Bald Eagle, the short-wavelength visual pigment sensitivity range is severely restricted by the C-Type oil droplet when compared to the Golden Eagle. When this occurs in birds, it is thought to aid in increasing color discrimination. This indicates that the Bald Eagle has a region in their visual system that is more sensitive to variations in blue wavelengths of light than the Golden Eagle.

We found that each species have different relative densities of photoreceptors within the retina, which were incorporated into the visual contrast perceptual modeling. During visual contrast perceptual modeling, we found that the Bald Eagle has two visual “sweet spots” of increased visual conspicuousness of LEDs against a given background. The first sweet spot indicates that an indigo/blue light with a peak wavelength range from 420-470 nm (446-448 nm

peak chromatic contrast) is highly conspicuous for a variety of visual backgrounds. The second sweet spot indicates that an orange/red light with a peak wavelength range from 580-650 nm (606-612 nm peak chromatic contrast) is highly conspicuous for a variety of visual backgrounds. This means that LED lights that have a peak wavelength in the indigo/blue and orange/red portions of the electromagnetic spectrum are good candidate signals for the behavioral experiments with Bald Eagles.

We also found that the Golden Eagle had either two or three visual "sweet spots" depending on the visual background used in the contrast calculation. The first sweet spot indicates that an indigo/blue light with a peak wavelength range from 410-460 nm (436-442 nm peak chromatic contrast) is highly conspicuous for a variety of visual backgrounds. The second sweet spot indicates that an orange/red light with a peak wavelength range from 590-655 nm (616-626 nm peak chromatic contrast) is highly conspicuous for a variety of visual backgrounds. The third sweet spot indicates that a green/yellow light with a peak wavelength range from 530-556 nm (538-550 nm peak chromatic contrast) is highly conspicuous for the bare ground, dormant grass, and white visual backgrounds. This means that LED lights that have a peak wavelength in the indigo/blue, orange/red, and green/yellow portions of the electromagnetic spectrum are good candidate signals for the behavioral experiments with Golden Eagles.

The LED peak wavelength ranges for both species overlap for two of the visual system "sweet spots". The green/yellow light signal is only effective for the Golden Eagle and only on certain visual backgrounds, so we did not include its use in the behavioral experiment component of this project. We decided to focus on the use of indigo/blue and orange/red LED light stimuli in the finalized behavioral experiment protocol.

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ATTACHMENT M

Milestone 9.1

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the development of a prototype for the completion of Milestone 9.1.

> ***Milestone 9.1 – Develop an LED light + speaker system for playback of combinations of visual and acoustic stimuli***

<u>Task 9.0 – Developing prototype visual and acoustic stimuli for behavioral tests and behavioral assays</u> (Month 15-16)

Task Summary:

Based on the physiological information collected (Attachments I, J, K, L), we selected stimuli with different degrees of conspicuousness for the Golden and Bald Eagle sensory systems. We developed a prototype with LED lights and speakers to test the assumption that Golden and Bald Eagles would strongly respond to stimuli with higher levels of sensory saliency (Attachment M, Milestone 9.1). With the help of experts in raptor behavior in captivity, we developed a behavioral assay to measure the responses of eagles to these new stimuli (Attachment N, Milestone 9.2). We simplified this assay as much as possible to ensure it could be conducted in a reasonable period of time at rehabilitation centers where the aviaries that house the eagles differ in size and shape.

Milestone 9.1 – Develop an LED light + speaker system for playback of combinations of visual and acoustic stimuli (Month 15)

Milestone Summary: Using the information gathered about eagle auditory and visual physiology, we were able to develop a stimuli playback system prototype that played the lights and sounds used in the behavioral experiments. This prototype was tested with a falconry bird in Indiana and several Golden and Bald Eagles in Oregon before being fine-tuned and deployed at several raptor rehabilitation centers.

Objectives

To be able to perform the behavioral experiments at rehab centers, we first needed to develop a portable and wireless stimuli playback system that could accommodate a variety of

animal enclosures and environments. We designed a prototype stimuli playback system that we tested with a falconry bird in Indiana. After refining the stimuli system to eliminate several design issues we identified during the initial tests, we successfully tested our final version of the stimuli playback system with Golden and Bald Eagles at Blue Mountain Wildlife in Pendleton, Oregon.

Initial Prototype Development

To develop the initial prototype we knew we needed the system to: 1) play both light and sound stimuli, 2) be portable, 3) be wirelessly operated and battery powered, 4) be waterproof as the system will be used outdoors, 5) easily adaptable to a variety of animal enclosures, and 6) to minimize interference by the experimenter during the tests. We quickly determined that a Bluetooth operated, battery powered LED light panel and speaker inside of a compact housing would suit our needs.

We designed and constructed a pair of prototype systems that could be controlled by Bluetooth using a JBL Flip 3 Portable Bluetooth Speaker to play sounds. This speaker is waterproof and very loud, easily reaching the 80 dB volumes for all frequencies we tested in the anechoic chamber on the eagles. We also used an Arduino UNO R3 development board with an added DSD TECH HC-05 Classic Bluetooth 2.0 Serial Wireless BT Module to control the LED light panel. The Arduino system and LED light panel were powered by an EasyAcc 20000mAh Power Bank External Battery, which allowed us to power the Arduino system via USB cables. We have used an Arduino system to control LED light panels with previous experiments in our lab and have found it to be easy to both operate and modify for our needs. The whole system was controlled by an SLIDE Dual SIM Android 6.0 smart phone with Bluetooth capability.

The speaker and LED light panel were then mounted into a small plastic storage container painted black, with holes cut into it to allow the speaker and LEDs to be unobstructed (Figure M1). We used this setup because we thought it would be easy to transport and change out the LED light panel when needed during the experiment. To record the responses of the eagles to the stimuli, we put a GoPro HERO7 Black Camera on top of the container as we thought this would give us the best view to watch for head movement behaviors. The GoPro batteries only allowed for a 45-50 minute window of observation before they had to be replaced.

Figure M1. Stimuli playback system prototype in initial housing. This model was tested with a Red-tailed hawk falconry bird and is seen here within the hawk's indoor enclosure and when deployed outdoors.

Test of Initial Prototype with Falconry Bird

We tested these stimuli on a Red-tailed Hawk owned by a falconer in Indianapolis, Indiana. We conducted the first test with this animal outdoors as this would more closely simulate the conditions the eagles would be in at the rehab centers. We placed each stimulus prototype with camera on a tripod, equally spaced around the perch of the hawk in the front yard of the falconer's house. We hid behind a blind to watch the behavior of the animal and take additional video recordings.

The test of the equipment went well, but the hawk was unresponsive and would not perch. This was largely due to the number of visual distractions in the area including, a large amount of wind in the trees, cars passing by, pedestrians walking their dogs, etc. This was also a very different environment than the bird was used to, so we felt that moving indoors would provide a better location to perform the test. From this we learned that when collecting the videos for the eagles we should try to take the measurements in their home enclosure and remove as many visual distractors as possible.

Once indoors, the hawk was much more calm and was looking at the stimulus prototype, but not to a large degree. Our primary conclusion was that wild birds would be better subjects for these types of stimulus tests. The red-tailed hawk was primarily focused on the falconer, higher perches in the field of view, and other birds and showed little response to sudden onsets of lights or sounds.

The red-tailed hawk's focus on potential perches (like the cameras, electronics, and tripods) led us to design a canister-like housing for our stimuli to protect the electronics from potential raptor interactions like attempts to perch on the stimulus housing. Using a large piece of PVC pipe, we made holes for the speaker and LED light panel to be mounted, applied PVC endcaps, and then covered the entire canister with black matte gaffers tape (Figure M2). We found that this held up better during transport than the black spray paint and was easy to repair if needed.

Figure M2. Refined stimuli playback system used for the second round of behavioral tests with Golden and Bald Eagles. LED light panel is seen with the white LEDs in place and turned on.

Test of Refined Prototype with Eagles

We next took the stimuli playback systems to Blue Mountain Wildlife in Pendleton, OR, to test our stimuli on the four Golden Eagles and three Bald Eagles residing there at that time. At Blue Mountain Wildlife, the eagles were housed communally so that we had access to the four Golden Eagles and one juvenile Bald Eagle together on a perch and two adult Bald Eagles in a different enclosure, also on a single perch. We set up the stimuli and cameras to either side of these preferred (or "home") perches and started the video recordings (Figure M3).

Figure M3. Deployment of stimuli playback systems in the Golden Eagle enclosure. The canisters and cameras were suspended from the roof of the enclosure to reach the height of the perch. Perch can be seen covered with green astroturf.

The experimenter could then sit outside the enclosure (and out of view of the eagles) and control the stimuli (Figure M4). We presented a mix of lights (either steady or flashing), sounds, sound pairs, and light + sound pairs.

Four candidate lights: ‘ultraviolet’ (385 nm), ‘blue’ (460 nm), ‘red’ (615 nm), white (broad spectrum). Each light could be steady ‘ON’ or flashing ‘ON/OFF’ at 1 Hz with 50% duty cycle.

Four candidate sounds: mistuned harmonic stack, 0.4 kHz amplitude modulation (AM) with 2 kHz carrier, fast upward sweep (1-6 kHz in 30ms), and 70 Hz frequency modulation (FM) with 700 Hz depth (based on 2 kHz tone)

Time intervals between stimulus presentations were varied from 30 seconds to 5 minutes. The same stimuli were then tested on a pair of Bald Eagles later on the same day, using the same procedure. These experiments were repeated on two consecutive days. Overall, we collected 8, 45-minute videos of eagle behavior. These tests of the stimuli playback systems were very successful, so we felt no further refinement of the design was needed for our purposes.

Figure M4. Experimenter controlling the stimuli playback systems, while remaining out of view of the eagles. Eagle body outlines can be seen in the top quarter of the image while resting on their preferred perch. *Note* Cat was a resident to the center and approached the experimenter during the test. This cat was not visible to the eagles and did not affect our test or the eagle's behavior.

ATTACHMENT N

Milestone 9.2

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the development of a behavioral assay for the completion of Milestone 9.2.

> ***Milestone 9.2 – Develop a behavioral assay to test the responses of eagles to these visual and acoustic stimuli so that it can be conducted at different rehabilitation centers in the US***

Task 9.0 – Developing prototype visual and acoustic stimuli for behavioral tests and behavioral assays (Month 15-16)

Task Summary:

Based on the physiological information collected (Attachments I, J, K, L), we selected stimuli with different degrees of conspicuousness for the golden and bald eagle sensory system. We developed a prototype with LED lights and speakers to test the assumption that Golden and Bald Eagles would respond behaviorally to a larger degree to stimuli with higher levels of sensory saliency (Attachment M, Milestone 9.1). With the help of experts in raptor behavior in captivity, we developed a behavioral assay to measure the responses of eagles to these new stimuli. We simplified this assay as much as possible to ensure it could be conducted in rehabilitation centers where the aviaries that house the eagles differ in size and shape.

Milestone 9.2 – Develop a behavioral assay to test the responses of eagles to these visual and acoustic stimuli so that it can be conducted at different rehabilitation centers in the US (Month 15-16)

Milestone Summary: We analyzed test videos on eagles and were able to isolate eagle specific behaviors for the creation of a behavioral assay or ethogram. We consulted with several falconers and rehab center personnel and were able to compile a refined list of stress and non-stress related behaviors to identify in the behavioral experiment videos.

Objectives

Using the videos collected in Oregon, we created a behavioral assay or ethogram that was used to analyze future behavioral experiment videos. This ethogram is a list of eagle specific behaviors that focal eagles can exhibit during the behavioral experiment. After coding the videos for these behaviors, we were be able to develop and finalize a behavioral experiment protocol (Attachment O, Milestone 9.3) for use in several rehab centers.

Behavioral Assay Development

Using the videos collected from Blue Mountain Wildlife, as described in Attachment M, we were able to develop a list of behaviors that the eagles exhibited, both in response to the stimuli and while resting. We developed an ethogram which is a catalog of eagle specific behaviors that those animals exhibit that we could use as a framework for all future behavioral observations and video coding (Figure N1). Each observed behavior in the ethogram is noted along with any relevant variations that exist within that behavior. We were able to compile this list through years of observation of other bird species as well as consulting eagle behavior expert Erin Katzner, raptor handlers and falconers in the state of Indiana, and the scientific literature on raptor behavior.

Behavior	Description
Moves	Adjust feet (in place) + move on perch + stand on one
Flying	Airborne flight from or to perch
Maintenance	Preen, bill wipe, scratch, rousing, wing stretch, or tail shake
Gaping	Holding mouth open for prolonged time
Head movement	Head movement + looking around
Looking up + crouch	Beak above horizontal, legs crouched, wings partly open
Discharge	Mute or pellet
Resettles	Resettles wing + resettles feathers
Walk/run	Walking/running. Each stroke counts for step
Interact	Interaction with other eagle
Yawn	Beak opens wide then closes, oriented straight and above horizontal
Vocalization	Makes noise
Jump	Lifts both feet from perch or ground simultaneously
Stands on one leg	Stands on one leg and tucks other into feathers
Wing flap	Wings flap
Head swivel	Head moves without changing orientation

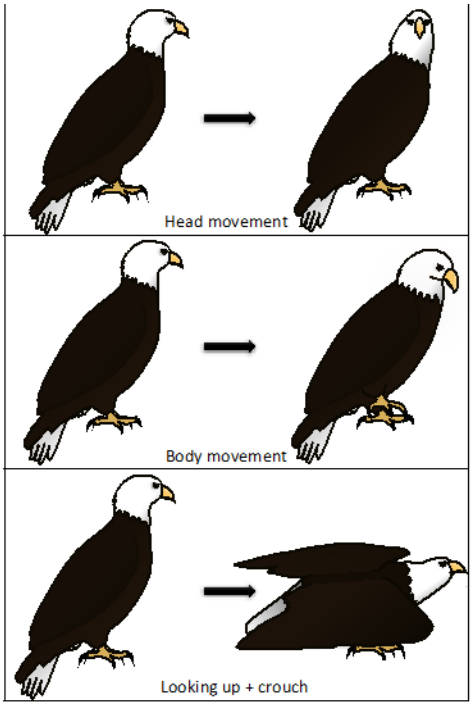

Figure N1. Ethogram of behaviors extracted from stimulus playback prototype test videos of Golden and Bald Eagles in Oregon. Also shown are visual examples of three common types of behaviors seen from the eagles in the videos; head movement, body movement, and looking up while crouching.

Once we were confident in our ethogram, we were then able to enter it into a software program called Behavioral Observation Research Interactive Software (BORIS). This event logging software allowed us to perform a detailed analysis of the Oregon test videos, including the ability to code behaviors using integrated playback at various speeds and frame-by-frame when necessary. Behaviors from the ethogram were assigned to individual keys, which were then pressed during video playback if that behavior was exhibited by the focal eagle. Upon each keystroke, the video pauses, enabling the user to add modifiers for each behavior before resuming playback. Modifiers were added to describe the body position of the subject during

behavior when body position was found to vary. This approach provided detailed descriptions of the behavior of individual focal Golden and Bald Eagles.

We performed frame-by-frame playback analysis of the videos from Blue Mountain in Pendleton, OR. This provided high-resolution details of what the focal eagles were doing both when the stimulus was off, and when the stimulus was presented. Over 300 minutes of footage were analyzed frame-by-frame. We compiled these results to see if rates or types of behavior would change with the onset or continued presence of the stimuli. As part of the analysis effort, we contacted multiple bird of prey handlers and raptor specialists to help create a list of stress and non-stress behaviors so that we could classify the coded frame-by-frame behaviors into categories that will gave us insight into the behavioral effects of the candidate stimuli. We identified gaping, discharge, looking up while crouching, flying, wing flapping and jumping as stress behaviors. Other behaviors in the ethogram were treated as non-stress behaviors.

Rough preliminary results of the frame-by-frame analysis indicated that the relative number of head movements of the focal eagle increased after a stimulus was played (Figure N2). Head movements in birds is an important indicator of visual exploratory behavior within the bird's environment (Fernández-Juricic 2012). However, the relative number of head movements would return to pre-stimulus levels after the stimulus was on for a minute. This preliminary information, coupled with constraints on the amount of time we could observe the eagle before the camera battery depleted, resulted in the decision to play the stimulus for one minute with a two to five minute rest period between each stimulus presentation. The random interval length between stimuli was used to prevent anticipation of future stimuli presentations. This allowed for the presentation of 9^{+} stimuli to the focal eagle and time to observe their subsequent behavior. We also noticed that the response to a stimulus appeared stronger when both a light and auditory stimulus were played at the same time, so we decided to include a few multimodal stimuli into the experimental protocol (see Attachment P for stimuli details).

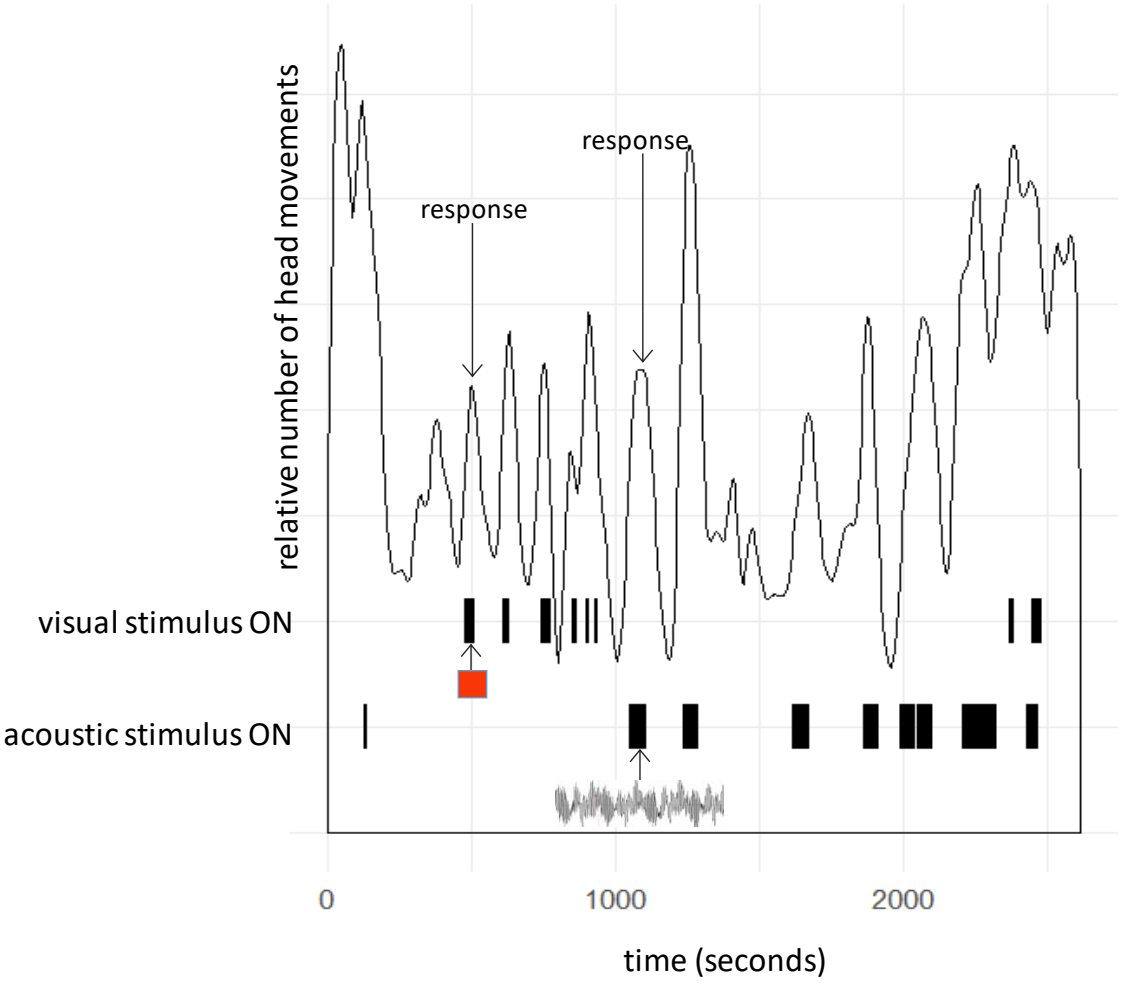

Figure N2. Rough preliminary visual schematic of focal eagles' response to a light, auditory, or multimodal stimulus. Black bars indicate the period over which a stimulus was played, and the single black line indicates the relative number of head movements of the focal eagle with time. Red box indicates that it is a colored LED light, and the grey "squiggle" is an example waveform of sound; these are purely illustrative to show which type of stimulus is ON in the figure. This preliminary look at the data allowed us to adjust the experimental protocol timing of stimuli and rest periods. All stimuli are pooled together in this figure, as we were interested in looking at the head movement rate with time.

Literature Cited

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ATTACHMENT O

Milestone 9.3

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the experimental protocol developed for the completion of Milestone 9.3.

Milestone 9.3 – Deliver experimental protocols to funding agencies

Task 9.0 – Developing prototype visual and acoustic stimuli for behavioral tests and behavioral assays (Month 15-16)

Task Summary:

Based on the physiological information collected (Attachments I, J, K, L), we selected stimuli with different degrees of conspicuousness for the golden and bald eagle sensory system. We developed a prototype with LED lights and speakers to test the assumption that Golden and Bald Eagles would respond behaviorally to a larger degree to stimuli with higher levels of sensory saliency (Attachment M, Milestone 9.1). With the help of experts in raptor behavior in captivity, we developed a behavioral assay to measure the responses of eagles to these new stimuli (Attachment N, Milestone 9.2). We simplified this assay as much as possible to ensure it could be conducted in rehabilitation centers where the aviaries that house the eagles differ in size and shape.

Milestone 9.3 – Deliver experimental protocols to funding agencies (Month 16)

Milestone Summary: Using the information gathered from and equipment built in milestones 9.1 and 9.2, we were able to develop a behavioral assay experimental protocol. This protocol was deployed at several rehabilitation centers across the country in order to collect recordings of the response of Golden and Bald Eagles to the acoustic and visual stimuli.

Objectives

We developed this behavioral assay experimental protocol to determine how Golden and Bald Eagles respond to stimuli. Using physiological data and modeling of the hearing and visual systems of these species, we were able to choose stimuli that should be maximally conspicuous to both species. By consulting and testing our stimuli prototype with falconry birds and rehab eagles, we were able to finalize the experimental design and protocol for these behavioral assays.

Behavioral Assay Experimental Protocol

First, we identified candidate Golden and Bald Eagles to take part in the behavioral experiment. These eagles were preferably individuals that had been recently rescued, were in relatively good health, did not have any obvious hearing or vision impairments, were solitarily housed, and exhibited behaviors typical of each species. Once a candidate focal eagle was identified, we went to their enclosure and inspected this area, looking for necessary items such as a preferred perch for the animal, areas to deploy the stimulus playback systems, and spots to mount cameras for observation of the focal eagle. We also identified if there were any visual distractors that could be hidden or removed from the enclosure or immediate vicinity. If substantial changes were needed to the area, we consulted with rehab center personnel on how to proceed.

We then deployed two stimuli playback systems inside of the enclosure. These stimuli playback systems were placed around the eagle's preferred perch directly opposite from each other and equidistant from the eagle, preferably at least 1 meter or more from each side of the eagle. We tried to place the stimuli playback systems at head level whenever possible. See Figure O1. Before deployment, speakers in the stimuli playback systems were calibrated so that the volume level at the head of the eagle is 60 decibels (dB) for each speaker. This is a moderate sound intensity similar to a normal conversation.

GoPro cameras were then deployed to record the responses of the focal eagle to the stimuli for later behavioral coding. The cameras were aligned in a manner that allowed for viewing of the focal eagle in its entirety and one or both of the stimuli playback systems (Figure O1). This allowed us to observe when a stimulus occurred in the video and determine if the focal eagle responded. Both cameras were shown a digital clapperboard (DigiSlate App) on the experimenter's phone, in order to synchronize the two cameras with each other when viewing the videos later.

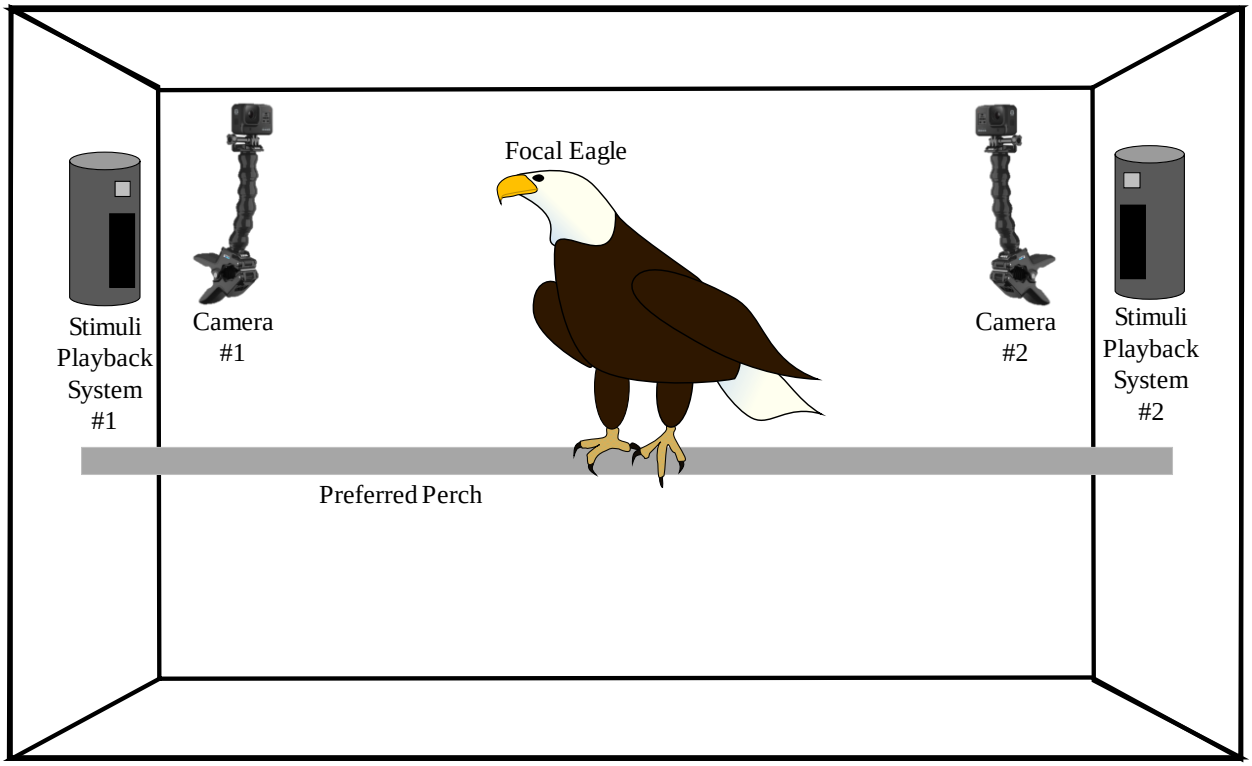

Figure O1. Example of experimental equipment setup in the enclosure of a focal eagle. Not drawn to scale.

The experimenter left the enclosure and allowed the focal eagle to adjust to the changes. This acclimation period lasted at least 10 minutes until the focal eagle was between the stimuli playback systems and exhibiting natural behaviors for that eagle. After the acclimation period, the experimenter began playing a series of physiologically conspicuous stimuli in a random order chosen before the experiment began. The stimuli were played for one minute, with a two to five minute rest period between each stimulus. This cycle of stimulus and rest are repeated until all stimuli were played. This marks the end of the behavioral experiment. We removed all equipment from the enclosure and immediately left the vicinity. If we were performing a second behavioral experiment at the same rehab center (on a different eagle), we took a break so that all eagles in the immediate vicinity had a chance to return to their normal behavior because they may have been disturbed by the previously played sound stimuli or changes in the subject eagle's behavior.

ATTACHMENT P

Milestone 10.0

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 10.0.

Milestone 10.0 – Gather behavioral responses from at least 6 Golden Eagles and 12 Bald Eagles

Task 10.0 – Measure behavioral responses of golden and bald eagles to prototype stimuli at rehabilitation centers (Month 16-26)

Task Summary:

We would identify rehabilitation centers that were housing golden and bald eagles, and visit them to conduct the behavioral experiment. The experiment will be conducted following the behavior experiment protocol developed in Attachment O, milestone 9.3. We would measure the responses to two stimuli playback systems that would play different combinations of light and sounds identified in Attachments K and L, task 8.0. Testing these stimuli was necessary as it would validate and inform the results obtained from the physiological information we gathered. This validation will be key to informing engineers as to the best combinations of lights and sounds to develop new eagle alert/deterrent technology for wind turbine farms.

Objectives

To test the effectiveness of the sound and light stimuli we developed, using information from the Golden and Bald Eagle sensory system, we would need to perform a behavioral experiment. We hoped that the behavioral experiment could be conducted on at least 6 Golden Eagles and at least 12 Bald Eagles. From these videos, we could then begin to perform behavioral analyses of the eagle's responses to the given stimuli.

Behavioral Experiment Participants

We were given the opportunity to perform behavioral experiments on 6 Golden Eagles (*Aquila chrysaetos*) and 6 Bald Eagles (*Haliaeetus leucocephalus*) sporadically from November 2018 to September 2019. Access to these eagles was provided to us by rehabilitation centers across the United States. Please review Table P1 for a breakdown of each eagle observed in the

behavioral experiment, location of the rehab center, and number of observations made. All work performed with these eagles was made with the consent of each rehabilitation center.

Table P1. Table of eagle individual identity, rehab center name and location, date and number of observations made. Sex and approximate age of eagle stated when known. The asterisk (*) in the Eagle ID column indicates whether the eagle was used in preliminary trials, or the updated experiment procedure (both described in the next section).

Species	Eagle ID	Sex	Age	Rehab Center	Location Of Center	First Observation	Second Observation	Third Observation
GOEA	201*		JV	BMW	Oregon	November 14, 2018	November 15, 2018	
GOEA	202*	F	JV	BMW	Oregon	November 14, 2018	November 15, 2018	
GOEA	203*		AD	BMW	Oregon	November 14, 2018	November 15, 2018	
GOEA	204*		JV	BMW	Oregon	November 14, 2018	November 15, 2018	
GOEA	205	F	AD	IRC	Indiana	May 10, 2019	May 24, 2019	July 11, 2019
GOEA	206			BMW	Oregon	September 22, 2019	September 23, 2019	
BAEA	201*	M	AD	BMW	Oregon	November 14, 2018	November 15, 2018	
BAEA	202*	F	AD	BMW	Oregon	November 14, 2018	November 15, 2018	
BAEA	203*		JV	BMW	Oregon	November 14, 2018	November 15, 2018	
BAEA	204	F	AD	IRC	Indiana	April 16, 2019	August 1, 2019	
BAEA	205			BMW	Oregon	September 22, 2019	September 23, 2019	
BAEA	206	M	AD	BMW	Oregon	September 22, 2019		

GOEA = Golden Eagle, BAEA = Bald Eagle, F = Female, M = Male, AD = Adult, JV = Juvenile, BMW = Blue Mountain Wildlife, IRC = Indiana Raptor Center.

Conducting the Behavioral Experiments

Following the behavioral experiment protocol we developed in Task 9 (Attachment O, Milestone 9.3), we performed behavioral experiments on the Golden and Bald Eagles listed above. Briefly, the behavioral experiment consisted of the deployment of two stimulus playback systems inside of the enclosure of the focal eagle(s), an acclimation period lasting at least 10 minutes, and then presentation of a series of audio and/or visual stimuli, in a random order, to the focal eagle(s). Eagle responses were recorded with video. The stimuli were presented with a variable rest period between each stimulus to prevent the focal eagle(s) from anticipating the next signal. The cycle of stimulus and rest was repeated until all stimuli were played.

For the preliminary study of behavioral responses to light and sounds and test of the stimulus playback system, we travelled to Blue Mountain Wildlife in Pendleton, OR in November 2018 and worked with four Golden Eagles and one Bald Eagle in a flight pen, and two Bald Eagles housed in a smaller aviary (Table P1, Eagle IDs with asterisk). Using partial data from the visual and auditory measurements we had at the time, we selected the following preliminary stimuli for testing:

Four candidate lights: ‘ultraviolet’ (385 nm), ‘blue’ (460 nm), ‘red’ (615 nm), white (broad spectrum). Each light could be steady ‘ON’ or flashing ‘ON/OFF’ at 1 Hz.

Four candidate sounds: mistuned harmonic stack, 0.4 kHz amplitude modulation (AM) with 2 kHz carrier, fast upward sweep (1-6 kHz in 30ms), and 70 Hz frequency modulation (FM) with 700 Hz depth (based on 2 kHz tone)

Stimuli were presented to the four Golden Eagles and one Bald Eagle first, on the preferred perch where all five individuals could sit simultaneously. Because of the aviary design, we could only attach the stimuli playback systems to the roof at perch height, not eagle head height, one on either side of the perch. Each day we tested a pair of lights (day 1: blue & red; day 2: white & ultraviolet) with steady and flashing randomly assigned (duration 30s), followed by the four candidate sounds (duration 1 min) in random order. We finished by testing paired sounds (either a sweep followed by FM or AM followed by a mistuned stack) and random combinations of a sound and a light presented together (multimodal stimuli). A ‘rest’ period was included between subsequent stimuli and randomly lasted between 30 sec and 5 min. Total experiment duration was capped at 45 min because battery power for cameras and stimuli was limited. After changing to fresh batteries, we repeated the experiment with a pair of Bald Eagles. In total, we collected two sets of paired preliminary experiments, one performed with Bald Eagles and one with Golden Eagles.

After the preliminary tests, we were able to acquire and analyze additional sensory data for both hearing and vision to help refine the selection of the stimuli used in the behavioral experiments. We were also able to update the experimental procedure. The updates were as follows:

1. All stimulus durations = 1 min.

2. Minimum of 2 min and maximum of 5 min 'rest' period between stimuli.

3. Focus on 'red' and 'blue' LEDs for light stimuli (still flashing and steady).

    a. Through visual modeling using Golden and Bald Eagle visual system information (Attachment L), we identified two regions with increased contrast against various backgrounds; indigo/blue and orange/red. We therefore eliminated the 'ultraviolet' (385 nm) and white (broad spectrum) stimuli from the behavioral experiments.
        i. The 'ultraviolet' (385 nm) signal was also eliminated because the eagles would not be able to see it very well, based on ocular media measurements.
    b. Flashing lights were used because they have been found to elicit alert responses in birds (Blackwell et al. 2012).

4. Auditory data showed that Golden Eagles do not hear rapid frequency changes as well as Bald Eagles, therefore slower sounds are better: we selected a slower downward sweep (6-1 kHz in 50ms) and slower 70 Hz FM with 400 Hz depth stimulus and kept the mistuned stack.

5. Use eagles in individual enclosures.

For subsequent experiments at the Indiana Raptor Center (May-August 2019) and additional experiments at Blue Mountain Wildlife (September 2019) we used the revised protocol. We presented nine randomly selected stimuli (3 light stimuli, 3 sound stimuli, 3 multimodal light + sound stimuli) with random 2 to 5min 'rest' periods between subsequent stimuli. Each stimulus was only presented from one canister (1 or 2, exact location depends on cage configuration). A list of the stimuli we used are as follows:

Two candidate lights: 'blue' (460 nm) and 'red' (615 nm). Each light could be steady 'ON' or flashing 'ON/OFF' at 1 Hz with 50% duty cycle.

Four candidate sounds: mistuned harmonic stack, 0.4 kHz amplitude modulation (AM) with 2 kHz carrier, downward sweep (6-1 kHz in 50ms), and 70 Hz frequency modulation (FM) with 400 Hz depth (based on 2 kHz tone).

For example, on April 16, 2019 we presented the following stimuli to a Bald Eagle at the Indiana Raptor Center:

10 minute rest after setup and before stimulus presentation begins

1. [Light-1] Blue steady followed by 3:00 min break
2. [Sound-2] FM followed by 4:40 min break
3. [Sound-2] Mistuned stack followed by 3:20 min break

4. [Combo-1] Blue flashing + mistuned followed by 4:05 min break
5. [Combo-2] Red flashing + sweep followed by 2:45 min break
6. [Light-1] Blue flashing followed by 3:10 min break
7. [Sound-1] Sweep followed by 2:00 min break
8. [Combo-1] Blue steady + FM followed by approx. 2 min break (stopwatch stopped)
9. [Light-2] Red flashing followed by at least 1:00 min break and clean-up

Total time: approx. 46-47 min

The experiments were repeated on multiple days to ensure all 'light', 'sound', and 'light + sound' stimuli were tested. Several additional difficulties arose during the behavior experiments:

1. Birds would occasionally avoid or leave their preferred perches (stimuli playback systems were set up relative to this perch) and sit on the ground. If the animal was clearly visible, we would sometimes continue the experiment but animal movement in the enclosure could also disrupt the experiment.
2. We were working in different aviaries at two different rehab centers and could not control camera and stimuli playback system placement limitations imposed by enclosure design. Furthermore, the aviaries were outdoors so other animals, anthropogenic noises, weather conditions, and light conditions (for example sunshine vs. overcast) were not in our control.
3. Elevated summer temperatures (>80°F) and humidity disrupted normal function of the electronics. Go-Pro cameras would automatically turn off, and phones used to control light and sound stimuli would lose Bluetooth connection frequently, or overheat and turn off like the cameras. Camera shutdown was noticed only after the experiment was completed and we entered the aviary to recover equipment.
    a. In instances of equipment failure, the behavior experiment was repeated at a later date to ensure that all stimuli and combinations were presented to the eagle.

Literature Cited

Blackwell, B.F, T. L. DeVault, T. W. Seamans, S. L. Lima, P. Baumhardt, and E. Fernández-Juricic (2012). Exploiting avian vision with aircraft lighting to reduce bird strikes. Journal or Applied Ecology 49:758-766.

ATTACHMENT Q

Milestone 11.0

DE-EE0007882

Purdue University

Understanding the Golden Eagle and Bald Eagle Sensory Worlds to Enhance Detection and Response to Wind Turbines

This document provides the steps undertaken for the completion of Milestone 11.0.

> ***Milestone 11.0 – Estimate whether Golden and Bald Eagles are attracted/repelled by combinations of visual and acoustic stimuli with different degrees of sensory conspicuousness***

Task 11.0 – Process and analyze the results of the behavioral experiment (Month 21-30)

Task Summary:

We processed the videos collected from the behavioral experiment in the rehabilitation facilities, and measured different behavioral responses of Golden and Bald Eagles. We found that head movement rates were higher for blue vs. red lights, with blue flashing lights the most alerting stimulus. However, there were no significant differences in the response to the different sound stimuli used in the experiment, indicating they are all good sound stimuli candidates. The blue flashing light with the mistuned harmonics seemed to be a good light + sound stimulus, although this part of the data set was limited. In addition, Golden Eagles showed a higher proportion of stress-related behaviors (compared to non-stress behaviors). Finally, this ratio was higher for light + sound stimuli in Bald Eagles, and higher for light-only stimuli in Golden Eagles.

Objectives

To determine the effectiveness of the sound and light stimuli as deterrents or attractants to the Golden and Bald Eagles, we analyzed the videos from the behavioral experiment. Data collected from the video coding were processed in SAS v9 using repeated measures analysis of co-variance (PROC MIXED) for head movement rates and using repeated measures Poisson regression (PROC GLIMMIX with a log link function) for stress vs. non-stress behaviors. We had only a limited amount of time to test each eagle, so we limited the test stimuli to stimuli that we anticipated would be alerting based on our physiological measurements. These included two colors of light (red and blue light, both flashing and steady), 4 different sound stimuli (sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack), and several bimodal stimuli (e.g. blue flashing light with mistuned harmonic stack sound, blue flashing light with sinusoidal FM, red flashing light with sinusoidal FM, and red steady light with a linear frequency sweep).

Methods

All videos collected from the behavioral experiments were either 60 frames per second (fps) or 30 fps in 1080p resolution or higher. All videos that were 60 frames per second were converted to 30 fps for analysis. The GoPro videos were encoded in a unique format that could not be read by the analysis software we used, so all videos were converted to mp4 files. We coded the behaviors of the eagles in the videos collected using Behavioral Observation Research Interactive Software (BORIS). This event logging software allowed us to perform a detailed analysis of the videos during each stimulus presentation. Individual behaviors from the ethogram developed in Attachment N were coded frame-by-frame.

We focused the main analysis on head movement rates during each stimulus for two reasons. First, differences in movement rates in response to different stimulus types are a direct measure of the efficacy of each stimulus type to draw the attention of the eagle. Second, there will be some carryover of stimulus effect into the non-stimulus intervals. As such, a direct comparison of behavior rates during the stimulus presentation itself is likely to be the most robust descriptor of stimulus effects, as opposed to comparing pre-stimulus vs during-stimulus rates. Numbers of behaviors exhibited by the eagles (e.g. head movement) were extracted from the dataset and converted to rates, by dividing the number of behavioral observations by the duration of each separate stimulus presentation. All head-movement rates were square-root transformed to normalize the residuals of the model. Our figures present least-squares means and standard errors of the square-root transformed data generated by the statistical models. These means allow us to factor out variables (such as changes in behavior over the course of the experiment) and thereby provide an average response by each eagle species during an experiment. Note that the duration of stimulus presentation was varied in a few early behavioral trials. Theoretically, the duration of a stimulus presentation might affect behavioral rates if the rate changed over the course of the stimulus. For example, if the head turning rate was higher at the start of a stimulus than at the end of the stimulus. We tested whether the difference in duration would affect the behavioral rates by adding stimulus duration to the statistical analysis of head turning rates – this is the most robust behavior to test this with given the high rates of head turning while stimuli were presented. The effect was not close to significant ($F_{1,124} = 0.30$, P=0.587), suggesting that the specific duration of any given stimulus had no effect on our estimate of rate.

Our analysis of stress-related vs. non-stress related behaviors included the number of each type of behavior pattern exhibited during a stimulus presentation. See page 159 for a list of stress-related and non-stress related behaviors. The duration of the stimuli was somewhat shorter than 60 sec for some of the presentations. We therefore standardized all numbers to a 60 sec interval. For example, if the stimulus presentation was 45 sec, we multiplied the number of stress behaviors by 60/45.

Results

Head movement rates during sensory stimuli presentation

Several patterns are evident in the head movement data:

(1) Golden Eagles move their heads more than Bald Eagles (Table Q1). Note that there were no significant interactions between species and any of the other factors in the model. Therefore, even though Golden Eagles moved their heads more than Bald Eagles, the pattern of movement in response to stimulus type or the relative change in head movement over the course of the experiment was not different between the two species.

Table Q1. Results of repeated measures analysis of co-variance relating head movement rates to eagles species, sensory modality (light, sound, or light + sound), stimulus type nested within modality (i.e. variation in head movement rates between specific types of stimuli within each sensory modality category), time-during-trial, time-during-trial^2 (i.e. testing for a non-linear change in movement rates over the duration of the experiment), and the interaction between time-during-trial × stimulus type nested within sensory modality (i.e. how the head movement rate in response to a specific stimulus changes over the course of the experiment).

Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Eagle species	1	8	8.80	0.0180
Sensory modality	2	11	0.40	0.6780
Stimulus type nested in modality	10	48	2.52	0.0158
Time-during-trial	1	125	5.20	0.0243
Time-during-trial squared	1	125	6.76	0.0104
Time-during-trial × stimulus type	12	125	4.23	<.0001

(2) There was no overall difference in how the birds responded to the different modalities of stimuli (light vs. sound vs. light + sound; Table Q1 as Sensory modality).

(3) We found that the specific type of stimulus presented (e.g. red flashing light, sweep, etc.) accounted for a significant amount of variation in head movement rates (Table Q1). Specifically, blue flashing light seemed to be particularly alerting, especially for Golden Eagles (Figure Q1). In contrast, there were no substantial differences in the response to the different sounds (Figure Q2). This indicates all are equally good candidate sounds to deploy on wind turbine deterrent systems. Please note that the sounds seem to be particularly alerting for Golden Eagles compared to Bald Eagles (Figure Q2). We have relatively few trials with light + sound stimuli, and fewer trials of specific light + sound stimuli to generate a least-squares mean estimate of head movement rate. The means are plotted in Figure Q3.

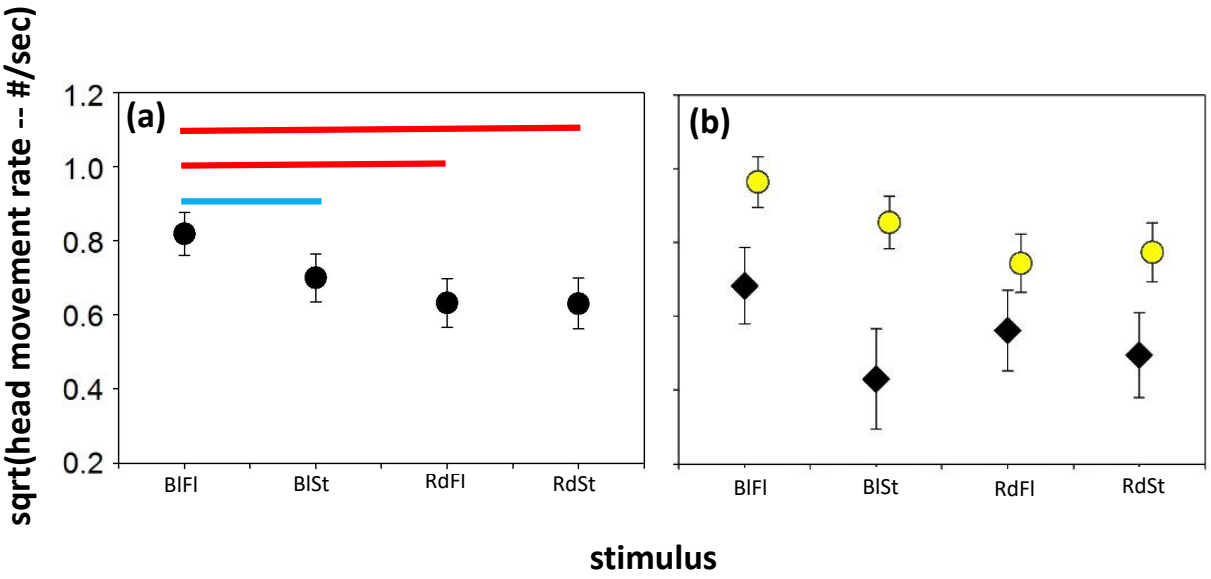

Figure Q1. Least squares means ± SE head movement rates of eagles (square root transformed) in response to 4 light stimuli: BlFl = blue flashing, BlSt = blue steady, RdFl = red flashing, RdSt = red steady. (a) Means of main effects of light stimuli on head movement rates. Red lines indicate means that differ at $P<0.005$; blue line indicates means that differ at $P<0.06$. Means not connected with lines are not significantly different. (b) Means from the non-significant eagle species × light stimulus type illustrating the patterns across species. Golden Eagle: yellow circle. Bald Eagle: black diamond. Note that this n.s. interaction is not in the final statistical model – these data are shown for illustrative purposes.

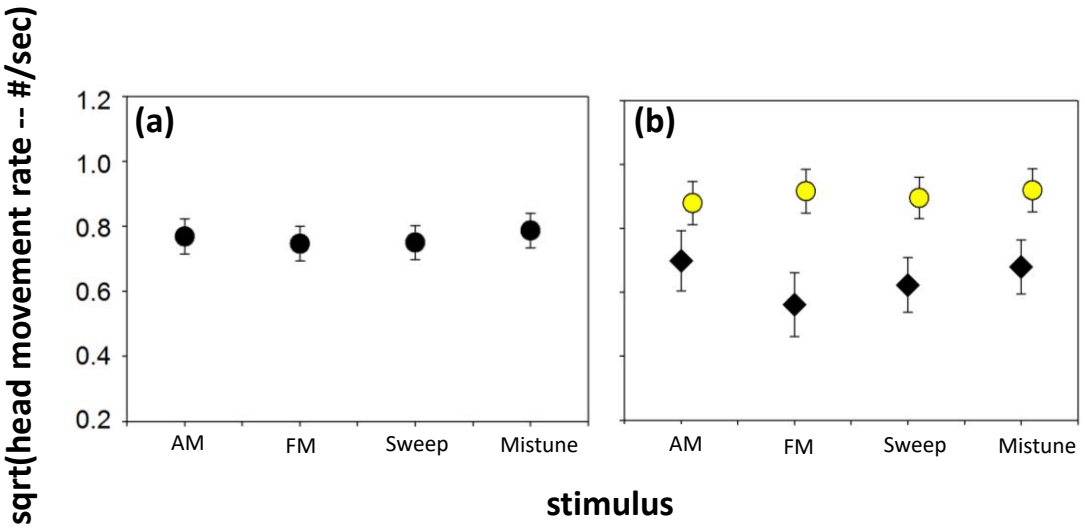

Figure Q2. Least squares means ± SE head movement rates of eagles (square root transformed) in response to 4 sound stimuli: AM = amplitude modulation (400 Hz AM with 2000 Hz carrier), FM = sinusoidal frequency modulation (70 Hz with 400 Hz depth), Sweep = linear downsweep from 1-6 kHz in 60 msec, Mistune = mistuned harmonic stack (1.0, 2.2, 3.3, 3.6 and 4.7 kHz). (a) Means of main effects of sound stimuli on head movement rates. Means not connected with lines are not significantly different. (b) Means from the non-significant eagle species × sound stimulus type illustrating the patterns across species. Golden Eagle: yellow circle. Bald Eagle: black diamond. Note that this n.s. interaction is not in the final statistical model – these data are shown for illustrative purposes.

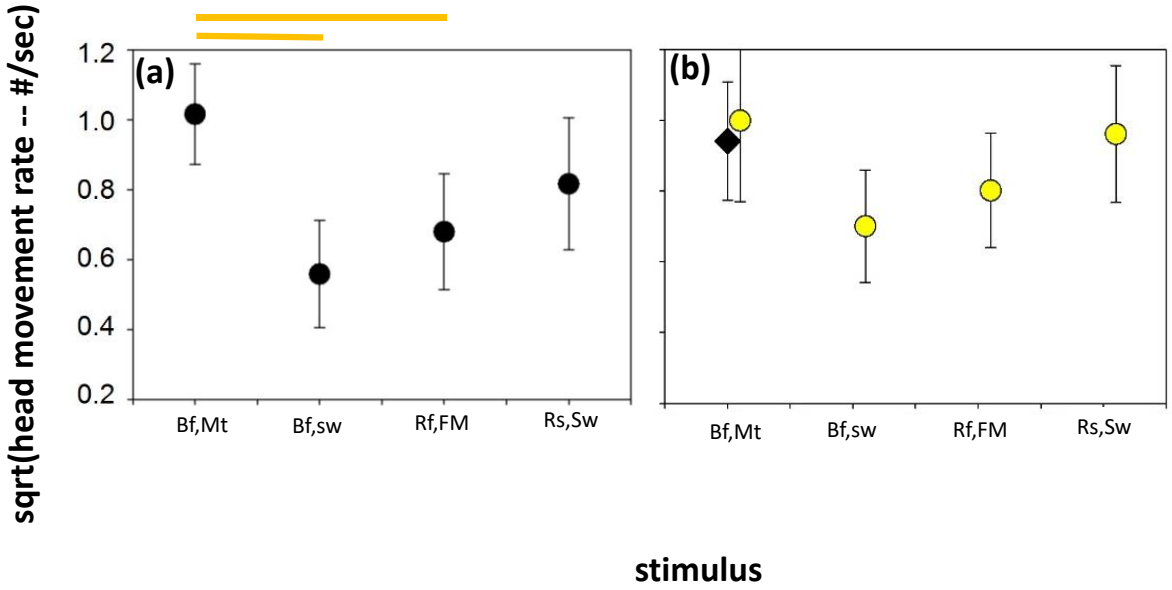

Figure Q3. Least squares means ± SE head movement rates of eagles (square root transformed) in response to 4 light + sound stimuli: Bf,Mt = blue flashing light with mistuned harmonics,

Bf,sw = blue flashing light with linear frequency sweep, Rf,FM = red flashing light with sinusoidal frequency modulation, Rs,Sw = red steady light with a linear frequency sweep. (a) Means of main effects of sound stimuli on head movement rates. Means connected with orange lines are significantly different at P<0.02. Means not connected with lines are not significantly different. (b) Means from the non-significant eagle species × light + sound stimulus type illustrating the patterns across species. Golden Eagle: yellow circle. Bald Eagle: black diamond. Note that this n.s. interaction is not in the final statistical model – these data are shown for illustrative purposes.

(4) The head movement rates peaked early in the trial then decreased non-linearly (as indicated by the significant time-during-trial^2 effect; Table Q1).

(5) Finally, there is some evidence that the change in head movement rate resulting from specific stimuli changes over the course of the trial (Table Q1); however, these changes are due only to changes in the rate of response to light + sound stimuli (Figure Q4).

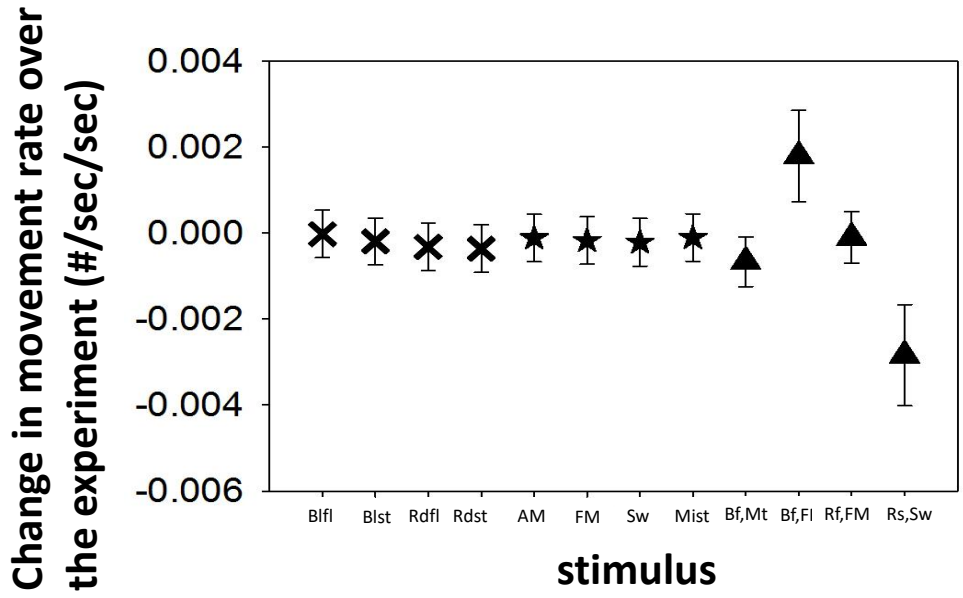

Figure Q4. Rate of change of head movement rates of eagles (square root transformed) over the course of an experiment in response to light (Blfl = blue flashing, Blst = blue steady, Rdfl = red flashing, Rdst = red steady), sound (AM = amplitude modulation, FM = sinusoidal frequency modulation, Sw = sweep/linear frequency modulation, Mist = mistuned harmonic stack) and light+sound stimuli (Bf,Mt = blue flashing light with mistuned harmonic stack, BfFM = blue flashing light with sinusoidal frequency modulation, RfFM = red flashing light with sinusoidal

frequency modulation, RsSw = red steady light with linear frequency modulation). The different symbols represent different modalities: X=light, ★=sound, ▲=light + sound.

Number of stress vs. non-stress behaviors during sensory stimuli presentation

The most common stressful behaviors were move, looking up and crouch, and wing flapping. Several patterns are evident from the analysis (see Table Q2):

Table Q2. Poisson regression analysis relating the number of behavioral events during a stimulus presentation to eagles species, sensory modality (light, sound, or light + sound), stimulus type nested within sensory modality, time-during-trial, time-during-trial^2 (i.e. testing for a non-linear change in movement rates over the during of the experiment), the type of behavior (stress-related or non-stress-related), the interaction between eagle species and the type of behavior (i.e. did the species differ in the relative number of stress vs. non-stress behaviors?), and the interaction between the sensory modality of the stimulus and the relative number of stress vs non-stress behaviors.

Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Eagle species	1	8	38.31	0.0003
Sensory modality	2	11	0.00	1.0000
Stimulus type nested in sensory modality	32	56	0.83	0.7103
Time-during-trial	1	280	9.75	0.0020
Time-during-trial squared	1	280	9.25	0.0026
Stress vs non-stress/stress behavior category	1	8	89.59	<.0001
Eagle species × (stress vs. non stress behavior category)	1	8	9.92	0.0136
Sensory modality × non-stress/stress category	2	11	3.99	0.0499

(1) As above, Bald Eagles showed fewer behaviors overall than the Golden Eagles (see Figure Q5).

(2) There was no differential behavioral response to the types of stimuli (i.e. sensory modality, e.g. sound vs light, light vs light + sound, etc.) or to the specific type of stimulus (e.g. red flashing light).

(3) As above, the overall rate of behavior changed non-linearly over the course of the trial – higher behavior rates earlier and fewer later.

(4) The number of stress-related behaviors was less than the number of non-stress-related behaviors (Figure Q5).

(5) The ratio of stress-related to non-stress related behaviors was higher in Golden Eagles than in Bald Eagles (as indicated by the eagle species × (stress vs. non stress behavior category) interaction term; see Figure Q5). These ratios are 0.042 for Bald Eagles and 0.162 for Golden Eagles. In other words: 4 percent of Bald Eagle behaviors were stress related and 16 percent of Golden Eagle behaviors were stress related.

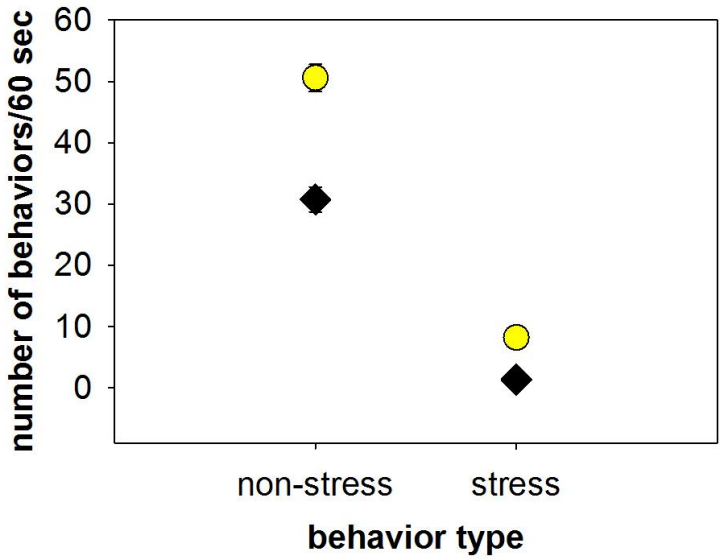

Figure Q5. Average ± SE number of behaviors in the non-stress and stress-categories over the course of an experiment in response to any of the stimuli. Golden Eagle: yellow circle. Bald Eagle: black diamond.

(6) The ratio of stress-related to non-stress related behaviors differed with the modality of the signal (Figure Q6). These ratios for Bald Eagles are 0.044 for light, 0.038 for sound, and 0.067 for light + sound. For Golden Eagles the ratios are 0.210 for light, 0.134 for sound and 0.173 for light + sound.

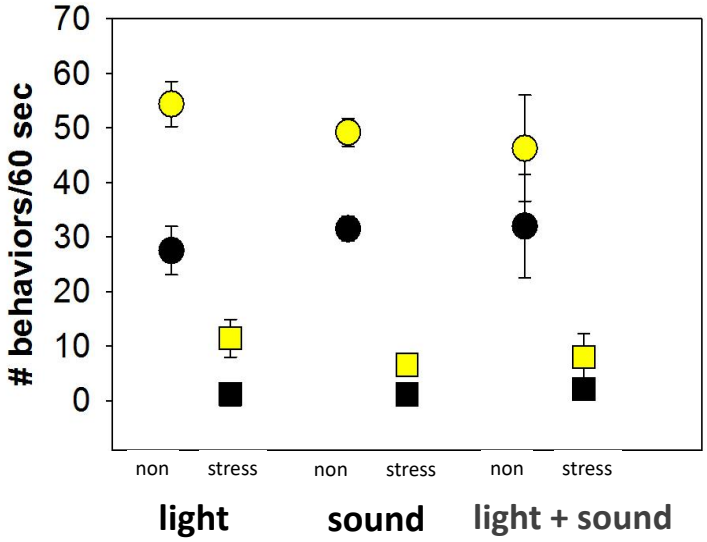

Figure Q6. Average ± SE number of behaviors in the non-stress (non) and stress-related (stress) categories over the course of an experiment in response to the three types of stimuli sensory modalities. Golden Eagle: yellow markers. Bald Eagle: black markers. Non-stress related behavior: circles. Stress-related behavior: squares.

Summary of Results

Golden Eagles exhibited a higher rate of visual exploratory behavior (head movement) and stress behaviors in response to the stimuli than Bald Eagles during the behavioral experiments. Although there were no statistically significant differences between light, sound, and light + sound stimuli in general, we were able to identify specific stimuli that increased the visual exploratory behavior of the eagles. Please keep in mind that all stimuli used in the experiments elicited visual exploratory behavior, but some were more effective than others. The blue flashing stimulus (460 nm LED) was particularly alerting to the eagles, especially to the Golden Eagles. This stimulus falls within the 410-470 nm visual "sweet spot" for both eagle species against a variety of backgrounds, so we can recommend blue light with a peak wavelength in this range as a stimulus for wind turbine eagle deterrent systems.

All sound stimuli (sinusoidal frequency modulated sound, linear frequency sweeps, amplitude modulated sound, and a mistuned harmonic stack) were equally alerting to the eagles, indicating they are all good candidate sounds to deploy on wind turbine deterrent systems. We had relatively few trials with light + sound stimuli so we couldn't find significant differences in

behavior for these stimuli, but the blue flashing light with the mistuned harmonic stack stimulus seemed to be a good light + sound stimulus for the eagles. This stimulus would be the first light + sound stimuli we would recommend for use in wind turbine deterrent systems.

The most common stressful behaviors exhibited during the behavioral experiments were move, looking up and crouch, and wing flap. There were no significantly different stress-related behavioral responses between light, sound, and light + sound stimuli in general. However, the ratio of stress to non-stress related behaviors differed with stimuli sensory modalities (i.e. light, sound, and light + sound stimuli). The highest ratio of stress to non-stress behaviors for the Golden Eagle was in response to light stimuli, and the highest ratio for the Bald Eagle was in response to light + sound stimuli. We did notice an effect of time on the rates of behaviors exhibited during the behavioral experiments (rates decreased with time) suggesting there might be some habituation to the stimuli playback system, or result from an initial enhanced stress level caused by the setup and beginning of the experiment. However, further behavioral experiments would need to be performed to confirm that this is the case.

Overall, the stimuli we would recommend for use in field-testing on eagle specific wind turbine deterrent systems are as follows:

Visual Stimuli (ranked in order of effectiveness of eliciting a response in the behavioral experiment):

5) Blue flashing LED light at 1 Hz
6) Blue steady LED light
7) Red flashing LED light at 1 Hz
8) Red steady LED light

Auditory Stimuli (ranked in no particular order):

5) Mistuned harmonic stack (1.0, 2.2, 3.3, 3.6, and 4.7 kHz)
6) 0.4 kHz amplitude modulation (AM) with 2 kHz carrier
7) Downward sweep (6-1 kHz in 50ms)
8) 70 Hz frequency modulation (FM) with 400 Hz depth (based on 2 kHz tone).

As we have discussed in the past, a random assortment of stimuli is likely to be best in alerting eagles to the wind turbine. Our results suggest that any of the four sound stimuli are equally likely to be alerting. This fact implies that a useful presentation scheme would be to rotate randomly through those sounds with each broadcast for one minute or so. Any field tests would have to address the problem of habituation. Varying both the stimulus duration and relative pattern of stimulus presentations would help provide a measure of habituation effects.