Title: Functional visual sensitivity to ultraviolet wavelengths in the Pileated Woodpecker (*Dryocopus pileatus*), and its influence on foraging substrate selection.

Authors:

Sean T. O'Daniels^{a,b,1,*}

a: Missouri Cooperative Fish and Wildlife Research Unit, 302 ABNR, University of Missouri, Columbia, MO, USA 65211

b: Department of Fisheries and Wildlife Sciences, 302 ABNR, University of Missouri, Columbia, MO, USA 65211

1: USDA/APHIS/Wildlife Services, 10 NW Richards Rd., Kansas City, MO 64116

Dylan C. Kesler^{b,2}

2: Institute for Bird Populations, 11435 CA-1 Suite 23, Point Reyes Station, CA 94956

Jeanne D. Mihail^c

c: Division of Plant Sciences, 110 Waters Hall, University of Missouri, Columbia, MO, USA 65211

Elisabeth B. Webb^{d,b}

d: U.S. Geological Survey, Missouri Cooperative Fish and Wildlife Research Unit, 302 ABNR, University of Missouri, Columbia, MO, USA 65211

Scott J. Werner^e

e: USDA/APHIS/Wildlife Services, National Wildlife Research Center, 4101 Laporte Ave, Ft. Collins, CO, USA 80521

Note: Secondary authors listed alphabetically.

*Corresponding Author: email odaniels.sean@gmail.com

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Abstract

Most diurnal birds are presumed visually sensitive to near ultraviolet (UV) wavelengths, however, controlled behavioral studies investigating UV sensitivity remain few. Although woodpeckers are important as primary cavity excavators and nuisance animals, published work on their visual systems is limited. We developed a novel foraging-based behavioral assay designed to test UV sensitivity in the Pileated Woodpecker (*Dryocopus pileatus*). We acclimated 21 wild-caught woodpeckers to foraging for frozen mealworms within 1.2 m sections of peeled cedar (*Thuja* spp.) poles. We then tested the functional significance of UV cues by placing frozen mealworms behind UV-reflective covers, UV-absorptive covers, or decayed red pine substrates within the same 1.2 m poles in independent experiments. Behavioral responses were greater towards both UV-reflective and UV-absorptive substrates in three experiments. Study subjects therefore reliably differentiated and attended to two distinct UV conditions of a foraging substrate. Cue-naïve subjects showed a preference for UV-absorptive substrates, suggesting that woodpeckers may be pre-disposed to foraging from such substrates. Behavioral responses were greater towards decayed pine substrates (UV-reflective) than sound pine substrates suggesting that decayed pine can be a useful foraging cue. The finding that cue-naïve subjects selected UV-absorbing foraging substrates has implications for ecological interactions of woodpeckers with fungi. Woodpeckers transport fungal spores, and communication methods analogous to those of plant-pollinator mutualisms (i.e. UV-absorbing patterns) may have evolved to support woodpecker-fungus mutualisms.

Keywords *Dryocopus pileatus*, feeding cue, foraging behavior, fungi, mutualisms, wildlife damage control

Ethical Note

The capture, care, and use of study subjects were approved by the National Wildlife Research Center's Institutional Animal Care and Use Committee (NWRC study protocols QA2242, QA2398) and the following collecting permits: U.S. Fish & Wildlife Service Scientific Collection Permit # MB019065-2; USDA Forest Service Scientific Research Permits SO-FW-FY14-15 and SO-FW-FY15-04; Arkansas Game & Fish Commission Scientific Collection Permit # 011520141; and Missouri Department of Conservation Wildlife Collector Permit # 15961. See Appendix 1 for details regarding the capture, care and use of study subjects, and a description of the testing facilities.

1. Introduction

Birds rely on sight for many aspects of their life history and thus have evolved complex visual systems. Diurnal avifauna possess perhaps the most sophisticated color vision system among vertebrates [1], and most, if not all, are sensitive to near ultraviolet (UV, 300-400 nm) wavelengths [2] and [3]. Avian visual systems are typically categorized as either UV-sensitive (UVS) or violet-sensitive (VS), depending on the wavelength of maximum absorbance ($\lambda_{\max}$) for the first of two short wave-sensitive visual pigments (SWS1) [4]. The UVS system is characterized by an SWS1 $\lambda_{\max}$ from 355nm – 373nm [5], and is found in Passeriformes (Passerida only, not Corvida or Tyrannida), Paleognathae, Psittaciformes, and Laridae [6]. All other avian taxa are presently presumed to possess a VS system [2], characterized by an SWS1 $\lambda_{\max}$ from 402nm – 426nm [5].

Measurements of $\lambda_{\max}$ values for visual pigments can be obtained by microspectrophotometry (MSP) [7], or estimated from total DNA [8], giving a sense of a species' visual system. These data then can be incorporated into models to estimate the saliency of wavelengths, or the discriminability of colors, in species of interest [4] and [9]. Kemp et al.

[10] recognized such modeling as a valuable first step to investigating visual systems, but stressed the importance of behavioral studies in vision research. Behavioral studies may not categorize a species' visual system as UVS or VS, but they can demonstrate the ability to detect and respond to UV cues, and thus provide potentially useful insights into visual systems and the functional significance of UV sensitivity (i.e. the visualization of UV wavelengths).

There is ample behavioral evidence that reflectance (the amount of light reflected by substrates) of UV wavelengths informs mate choice and foraging decisions for both UVS and VS species. Female preference for UV-reflective males over UV-blocked or UV-reduced males has been demonstrated in several species [11], [12], and [13]. As a plumage or integument characteristic, UV reflectance may signal male fitness in terms of resource acquisition or overall health [13] and [14]. Behavioral studies also have demonstrated the potential importance of UV reflectance for foraging activities in frugivores [15] and insectivores [16].

Behavioral studies of avian UV sensitivity have been conducted largely with passerine species, and the species evaluated thus far primarily represent those of ecologic, economic, or recreational interest. Woodpeckers (Piciformes: Picidae: Picinae, Leach 1820) are a widely distributed, ecologically and economically important group, yet little work on their visual systems has been published. To our knowledge the Great Spotted Woodpecker (*Dendrocopos major*; GSWO) is the only woodpecker species to have had its visual system categorized, with an estimated SWS1 λ_{max} of 405 nm [6], suggesting a VS system.

As primary cavity excavators, woodpeckers are foundational links in nest web communities [17] because the cavities they create are used by dozens of other vertebrate species, and their cavities are resources from which those secondary users would otherwise be excluded

[18]. Woodpeckers are known to carry fungal spores on their bills and feathers [19], and can facilitate fungal colonization of wood substrates as well [20]. Through their excavating behaviors, woodpeckers often fill the role of keystone species in forest ecosystems [21]. However, when directed towards anthropogenic structures, these excavating behaviors can cause structural damage and impose significant financial costs [22].

The Pileated Woodpecker (PIWO; *Dryocopus pileatus*) is the largest extant woodpecker in North America, with a range that spans much of the north and east of the continent. Conner et al. [23] reported that several woodpecker species, including PIWO, foraged by excavation within wood substrates that were softer (decayed) than adjacent unselected substrates, and these selected substrates contained a higher arthropod biomass than the unselected substrates. It is not known how woodpeckers identify decayed substrates or select specific sites on a given substrate for foraging [24], but they may use cues outside of human perception [25]. One potential cue is UV reflectance, as wood that has been decayed by fungi can exhibit a UV reflectance pattern that differs from uninfected wood [26]. We hypothesized that some woodpecker species possess UV sensitivity, and that they use UV reflectance characteristics of wood to identify decayed substrates for foraging and possibly cavity excavation.

Herein, we present a study that tested these hypotheses using controlled behavioral experiments. We developed a novel foraging-based behavioral assay designed to determine whether altering the UV reflectance of a wood substrate influences selection of foraging substrates by woodpeckers. We conducted five, two-choice experiments with captive, wild-caught PIWO. We artificially increased (Experiment 1) and decreased (Experiments 2 and 3) the UV reflectance of experimental substrates relative to control substrates. We predicted that study subjects would respond differently to control and UV-altered treatment substrates. Additionally,

we tested whether treating increased UV substrates with a UV-absorbing substance affected study subjects' ability to discriminate these treatments from unaltered control substrates (Experiment 2). Finally, we tested whether decayed wood is a useful foraging cue for woodpeckers (however, not specifically a visual cue), and if decreasing UV reflectance of both decayed and control substrates diminishes woodpeckers' ability to discriminate between those substrates (Experiments 4 and 5). We predicted that study subjects would respond differently to decayed and control substrates, and that decreasing the UV reflectance of decayed and control substrates would have little to no effect on substrate discrimination. This last prediction was based on Experiments 1 and 2 which occurred prior to Experiment 5, on work with other avian species [27] and [28], and in part on our inability to completely control potential confounding influences (see 7.2).

2. General Methods

2.1 Experimental Design

2.1.1 Test Apparatus

A test pole was prepared for each study subject, and the same test pole was presented to each subject throughout the duration of experiments in which each subject participated. Human sebaceous oils are UV-absorbing [29], therefore poles were handled with nitrile gloves for the duration of all experiments. The test pole was a 20 cm diameter x 120 cm section of cedar (*Thuja* spp.). Each test pole contained a total of 12 pre-drilled holes (2.2 cm diameter x ~ 7.5 cm depth) at 45° from vertical. Four holes were evenly spaced around the test pole at each of three heights (30, 60, 90 cm from the base; Fig. 1). At each height, holes were randomly assigned to either the treatment or control group (two each per height), and assignments were re-randomized for each trial. Treatment holes received ~ 3 g frozen mealworms and were sealed with a

treatment substrate. Control holes received ~ 3 g of mealworm bedding material (sawdust, frass, etc.) to control for olfactory and resonance cues, and were sealed with a control substrate. For each of Experiments 1 - 5, experimental substrates were randomly assigned a position in a test pole after the appropriate preparation (see 2.1.2).

Figure 1. Photograph of a test pole used in behavioral experiments with Pileated Woodpeckers (*Dryocopus pileatus*). Note that experimental cork substrates are already in position at each height.

2.1.2 Corks

Since woodpeckers may preferentially forage from softer wood substrates [23], we used natural corks (Size #10, Carolina Biological, Burlington, NC, USA) as a surrogate for soft wood. All corks for Experiments 1 & 2 were soaked in demineralized water for a period of 24 hours, after which each was randomly assigned to either the treatment or control group. We previously had determined that soaking was necessary to ensure retention of the UV-reflective treatment by

the corks. Treatment corks were further prepared as either UV-reflective (Experiment 1) or UV-absorptive (Experiments 2 & 3), while control corks received no additional preparation. As with the test poles, all corks were handled only with nitrile gloves for the duration of experiments.

The UV-reflective treatment corks were created by submersion in a warmed 0.07% magnesium carbonate (MgCO_3 ; Sigma-Aldrich, St. Louis, MO, USA) suspension (by weight) [28] for 20 s, with constant agitation by magnetic stir bar. MgCO_3 is a naturally occurring compound that is odorless and tasteless to humans and exhibits its peak reflectance below 300 nm. The suspension was warmed on the lowest setting of a laboratory hot plate for at least 5 min prior to application with a stirring speed of 6 (of 10). Treatment and control corks were then dried for a minimum of 2 h at 200° C in a laboratory drying oven, or a minimum of 24 h under a fume hood at room temperature. Any corks that exhibited a human visible residue were excluded from further use, and for consistency the same person (STO) prepared and selected all of the corks. The UV-absorptive treatment corks (Experiments 2 and 3) were prepared by submerging corks in a bath of UV Killer[®] (Atsko, Inc., Orangeburg, SC, USA; henceforth, UVK), a UV absorbing liquid, for 20 s with constant agitation. Each cork was prepared at least 24 h in advance of the trial in which it was used.

UVK is commercially marketed as a clothing treatment for hunting garments that is purported to reduce visibility to game animals. We measured the absorbance spectrum of UVK over 190 - 400 nm via high performance liquid chromatography to verify that it is a UV-absorbing liquid. An aliquot of the solution was filtered through a 0.45 μm PTFE filter into an LC vial and capped, and 20 μL was injected on an Agilent LC 1100 with a mobile phase of 100% H_2O pumping at 0.5 mL/min, isocratically. The pump was connected directly to the detector, and the analyte was not chromatographed on an LC column. The detector was an

Agilent 1100 UV/Vis detector with a 10 μ L sample cell, set to scan from 190 - 400 nm using Agilent ChemStation spectrum analysis software.

Prior to each trial, the surface reflectance of each treatment and control cork was measured using an Ocean Optics USB2000+ microspectrophotometer calibrated for 200-850 nm with a QR400-7-UV-BX reflectance probe and a PX-2 pulsed xenon light source (Ocean Optics; Dunedin, FL, USA). The probe was calibrated against white (WS-1 Spectralon) and black (i.e. dark) standards, and was re-calibrated after groups of six corks. Measurements were recorded at three points on the widest end of the cork. We used a modified black rubber stopper to hold the probe at a fixed distance (5 mm) and angle (90°), and to eliminate ambient light during reflectance measurements. Due to the rugose nature of the cork surface, we only collected measurements from the flattest, smoothest portions of the cork surface to ensure a constant, fixed distance from the probe.

2.1.3 Wood Wafers atop Corks

For Experiments 4 and 5, we inoculated red pine (*Pinus resinosa*) wafers with *Porodaedalea pini*, a wood decay fungus that is thought to be associated with Red-cockaded Woodpecker (*Picoides borealis*) cavity sites [30, 31]. Wafers were 20 x 20 x 3 mm, and had been cut perpendicular to the transverse plane (across the grain) to facilitate fungal colonization of the entire wafer. All wafers were autoclaved in a 2% malt extract (2M) broth and placed in petri dishes (plates) on 2M agar, seven wafers per plate. Plates sat for seven days in the dark at indoor, ambient conditions to monitor for contamination. We then assigned plates to either control or treatment conditions. All treatment plates were inoculated on the same day by agar block transfer from pure cultures of *P. pini* (strain AZ-10-T; Center for Forest Mycology Research Culture Collection, US Forest Service, Madison, WI), while control plates remained

unmanipulated. All plates were then returned to dark storage, and monitored periodically for contamination.

After ~5 months, treatment wafers were extracted from plates and fungal mycelia were manually scraped from wafer surfaces. Control wafers also were manually scraped such that any tool marks left on wafers would be similar between treatments and controls. Scraped wafers were placed on paper towels and allowed to desiccate for at least 72 h before further use. Upon drying, each wafer was attached to the 25-mm side of a Size #10 cork (Carolina Biological) with WeldBond adhesive (F.T. Ross & Sons, Ltd., Markham, Ontario, CAN). We maintained the same orientation of wafers throughout this process, such that the surface initially in contact with agar was the bottom surface while drying and the bottom surface was adhered to corks.

Surface reflectance of each control and treatment wafer was measured in the manner previously described, except that reflectance was collected at six points on the top surface. Additionally, wafer reflectance was collected prior to the first trial in which each was used, but not before each trial. Undamaged wafers were re-used because we did not have enough control or treatment wafers to conduct 10 trials with each study subject without reuse. After each trial, wafers with visible damage were excluded from further use.

2.2 Training

For each of Experiments 1 - 5, all study subjects were trained to forage for frozen mealworms (*Tenebrio* spp. larvae) within a test pole prior to participating in experiments. This step was added after study subjects for Experiment 1 failed to participate in five consecutive trials. Each subject participated in two training periods each day from ~ 0800 - 0900 h MDT and from ~1600 - 1700 h for three consecutive days (six sessions), immediately prior to the experiments in which they participated. All subjects were food-deprived prior to the start of each

training session to ensure adequate levels of food motivation [32]. Maintenance diets were removed at ~1900 h the evening before each morning session and ~1300 h prior to each afternoon session. Additionally, the daily ration of mealworms was removed from the maintenance diet, and mealworms were offered only during subsequent training periods. Water was provided *ad libitum* throughout training and experiments. See Appendix 1 for details regarding the capture, care, and use of study subjects, and a description of the testing facilities.

Each training session consisted of preparing the test poles by placing ~ 1.5 g frozen mealworms in each of the 12 holes with no obstructions. We accurately weighed 50% of the daily ration (18.0 ± 0.1 g (2014); 20.0 ± 0.1 g (2015)) for each bird prior to the start of each session, but no effort was made to ensure that each hole received equal amounts. The enrichment poles were then removed from the aviaries and replaced with the test poles. Each subject was allowed to explore and forage from the test pole for ~60 min during each training session, and each session was recorded with digital video. At the end of each training session, we removed the test poles and replaced the enrichment poles. To verify that establishing conditions (i.e. mealworm consumption) were met, we collected and weighed any remaining mealworms from each test pole after each training session (not reported).

2.3 Statistical Analyses

2.3.1 Behavioral Analyses

We examined the fate of each cork presented to study subjects during each experiment with generalized linear mixed models (GLMM). The dependent measures for detection of UV cues paired with food rewards were contact with corks, removal of corks from test poles, cork handling time (CHT), and the order in which corks were removed. Data for all response variables were collected after reviewing video recordings of trials. Values for CHT were

calculated by summing the amount of time (± 1 s) that a subject physically interacted with a cork prior to removing it from the test pole. Values of CHT that were two or more orders of magnitude greater than the median value were considered outliers and excluded from analyses (3 of 3,745; 0 values not included).

We created GLMMs using the *lme4* package [33] within R (version 3.2.3) [34] to analyze each dependent variable, and we considered differences significant at $\alpha < 0.05$. The GLMMs included cork type (treatment/control) as a fixed variable and random variables for both Subject ID and trial number to account for non-independence due to repeated testing of the same subjects (Table 1). We assumed a binomial distribution for contact and removal variables and a Poisson distribution for handling time and ranked variables. We used logistic regression for the binomial response variables to generate probability estimates for each substrate condition.

Experiment (# of Trials)	Bird ID	Sex Ratio	Date
1 (10)	103, 104, 108, 110, 112, 114	5 M:1 F	31 May – 4 June, 2014
2 (10)	103, 104, 110, 112, 114	4 M:1 F	8 – 12 June, 2014
3 (10)	708, 709, 712, 730, 733	5 M	20 – 25 May, 2015
4 (10)	705, 706, 707, 711, 731, 732, 734, 735, 736, 737	8 M:2 F	4 – 8 May, 2015
5 (10)	705, 706, 707, 711,	8 M:2 F	12 – 16 May, 2015

731, 732, 734, 735,
736, 737

Table 1. List of Subject ID by experiment, including sex ratios and dates of experiments.

For most variables analyzed, the null hypothesis was no difference between control and treatment values and the alternative hypothesis was treatment values greater than control values. For the order removed variable, we expected treatments would be removed earlier than controls resulting in an alternative hypothesis of treatment value less than control value. Any woodpecker-substrate interactions that occurred after 60 min were excluded from analysis.

2.3.2 Spectral Analyses

Mean reflectance spectra of each substrate were analyzed for their departure from the overall mean reflectance spectrum of the opposite substrate condition, over 300 - 700 nm (i.e. each treatment substrate spectrum vs. mean control spectrum). We then conducted a z-test on the average departure between treatments and controls for a UV bin (< 390 nm) and a visible bin (> 410 nm) using a generalized linear model, and we considered differences significant at $\alpha < 0.05$. We excluded data from 390 - 410 nm to limit influence of neighboring points across bins. Analyses were conducted with the R packages *lme4*, *lsmeans* [35], and *multcompView* [36].

3. Experiment one: UV-reflective cork treatments, MgCO_3

3.1 Methods

To test whether UV-reflective substrates are a useful foraging cue for PIWO, we employed a two-choice behavioral assay using six adult, trained, cue-naïve PIWO. If so, then behavioral responses for UV-reflective corks ($n = 360$) would be greater than those for control corks ($n = 360$). Each subject participated in 10 trials (two per day) following the previously described methods, schedule of food-deprivation, and trial times (General Methods 2.2).

To reduce the likelihood that MgCO_3 transferred from corks to poles would confuse birds during subsequent trials, we rinsed each hole and the immediate surrounding surface area with demineralized water after each trial. Subject 108 sustained extensive abrasions on the tongue between Trials 9 and 10, and was subsequently placed under veterinary care. The data from Trial 10 for this subject were excluded from analyses.

3.2 Results and discussion

Study subjects were more likely to contact treatment corks ($T = 86.0\%$, $C = 76.2\%$; $F_{1, 691.85} = 11.1$, $P = 0.001$; Fig. 2A) and more likely to remove treatment corks than control corks ($T = 83.0\%$, $C = 69.8\%$; $F_{1, 691.83} = 16.6$, $P < 0.001$; Fig 2F). With one outlier removed (one T substrate, Subject 103, Trial 1), CHT of treatment corks was greater than control corks ($T = 13.1$ s, $C = 9.5$ s; $F_{1, 542.79} = 27.1$, $P < 0.001$; Fig. 2K). There was no difference in the order that corks were removed ($P = 0.111$). These behavioral responses aligned with our predictions, except for the order removed variable.

Figure 2. Results of generalized linear mixed model analyses (point = mean, error bars = standard error) for contact, removed, and cork handling time (CHT) variables (Open = Control,

Black = Treatment) from Experiments 1-5 with Pileated Woodpeckers (*Dryocopus pileatus*). * denotes statistical differences ($P < 0.05$).

To test whether cue-naïve subjects exhibited a predisposition to forage at UV-reflective substrates, we analyzed data from Trial 1 separately. Probability estimates for cork contact ($P = 0.25$) and corks removed ($P = 0.75$), and the order corks were removed ($P = 0.92$) were not different. However, after removing one outlier (previously reported), the estimate for CHT was greater for control corks than treatment corks ($T = 37.8$, $C = 44.6$; $F_{1, 53.121}$, $P < 0.001$; Fig. 3G).

Fig. 3. Results of generalized linear mixed model analyses (point = mean, error bars = standard error) for contact, removed, cork handling time (CHT), and order removed variables (Open = Control, Black = Treatment) for trials with cue-naïve Pileated Woodpeckers (*Dryocopus pileatus*) associated with Experiments 1, 3, and 4. * denotes statistical differences ($P < 0.05$).

The reflectance of control and treatment corks from Experiment 1 were different in the UV bin (Z ratio = 17.58, $P < 0.001$) and were not different in the visible bin (Z ratio = 0.16, $P =$

309 0.999; Fig. 4, Exp 1). Based on preliminary work with these substrates, these were the expected
310 spectral differences.

Fig. 4. Mean reflectance spectra (left) of treatment (T) and control (C) substrates used in Experiments 1 – 3 with Pileated Woodpeckers (*Dryocopus pileatus*) over 300 – 700 nm, 10 nm average (error bars not shown for clarity). Relative reflectance values below 5% are considered to be not visible, represented by horizontal dashed lines. Mean departure values (right) were calculated as the percent difference from the mean reflectance of the opposite substrate (represented as decimals) over 300 – 700 nm. The mean reflectance value is centered on 1.0 (i.e. 100%).

We measured the reflectance of each cork presented to study subjects, and thereby ensured that differences between control and treatment corks occurred in the UV range (300 - 400 nm) rather than in the human visible range (400 - 700 nm). The mean reflectance spectra do not appear markedly different at first glance. However, the mean treatment spectrum is elevated above 5% relative reflectance (i.e. threshold of vision [37]) throughout the UV range, whereas

the mean control spectrum is at or below 5% for a portion of the UV range (Fig. 4, Exp 1). Additionally, these mean spectra may not truly represent the visual images perceived by study subjects. Images of our experimental substrates taken with a UV-only camera show that some grooves and pits of the treatment cork surfaces were even more UV-reflective (brighter) than the flat surfaces from which spectral measurements were collected (Fig. S1). Thus, the differences between treatment and control corks were likely greater than can be demonstrated by our spectral data. Since MgCO_3 is considered odorless and tasteless, and because we controlled for mealworm odor and differences in resonance between control and treatment holes, we conclude that the subjects were responding to the spectral differences between control and treatment corks as they perceived them.

Cue-naïve study subjects did not demonstrate a preference toward UV-reflective treatments, and in the case of handling time, the preference was toward control substrates. These results were contrary to our predictions. After analyzing the combined results of 10 trials, subjects' responses aligned with our *a priori* predictions. Together, these results suggest that the observed positive responses toward UV-reflective corks were conditioned, and that a predisposition toward UV-absorbing substrates may in fact be present in PIWO.

4. Experiment two: UV-absorptive cork treatments, MgCO_3 + UV Killer

4.1 Methods

To test whether the application of a UV-absorbing substance to UV-reflective substrates negatively impacted previously conditioned PIWO's ability to locate food, we employed another two-choice behavioral assay. If so, then behavioral responses should be similar (i.e. not different) between control and treatment substrates. We used the same subjects from Experiment 1, except for study subject 108 which had been removed from participation. Test poles

contained UV-absorbing (n=300) and control (n=300) corks. Treatment corks were prepared by submerging UV-reflective corks in a bath of UVK for 20 s. All other parameters were as previously described.

4.2 Results and discussion

Study subjects were more likely to contact treatment corks ($T = 94.1\%$, $C = 88.7\%$; $F_{1, 585} = 7.982$, $P = 0.004$; Fig. 2B) and were more likely to remove treatment corks than control corks ($T = 93.9\%$, $C = 88.0\%$; $F_{1, 585} = 9.005$, $P = 0.003$; Fig. 2G). Handling time was greater for treatment corks than control corks ($T = 8.8$ s, $C = 7.3$ s; $F_{1, 493.64} = 32.108$, $P < 0.001$; Fig. 2L). There was no difference in the order that corks were removed ($P = 0.45$). These behavioral responses were aligned with our predictions (see Introduction), except for the order removed variable.

The reflectance values of control and treatment corks from Experiment 2 were different in the UV bin (Z ratio = -23.55 , $P < 0.001$) and in the visible bin (Z ratio = -3.72 , $P = 0.001$; Fig. 4, Exp 2). We anticipated there would be no difference in the visible bin (410 - 700 nm), because corks treated with UVK did not appear to have a human visible residue. Additionally, a preliminary analysis of treatment and control reflectance spectra with a model of avian visual perception suggests that the statistical difference of the visible bin did not translate into a perceptual difference for the study subjects (Appendix 2). Therefore, we are confident that the substrates were only different in the UV spectrum when viewed by study subjects.

In this experiment, we tested whether decreasing the UV reflectance of substrates would impair the subjects' ability to locate food items. We selected the UVK product for the reduction of UV wavelengths because we believed it would produce corks with reflectance spectra similar to controls, and because there was no human visible residue on the corks when dried. The UVK

treatment did not result in spectrally similar control and treatment corks (Fig. 4, Exp 2). Rather, the treatment corks exhibited a depression in UV reflectance compared to the controls. The performance of the study subjects suggests that they were able to distinguish this spectral relationship equally as well as that experienced with UV-reflective treatments in Experiment 1. This performance also suggests they were able to re-learn the food-associated cue during the course of the experiment.

Aspects of PIWO sensory ecology such as olfaction and taste perception are presently undescribed. Though we considered it unlikely that either of these senses aided subjects in distinguishing control and treatment corks in our experiments, we could not definitively rule out the possibility of confounding influences because we included MgCO_3 substrates in both experiments. Therefore, we initiated an experiment that attempted to rule out properties of MgCO_3 other than UV reflectance (i.e. Experiment 3).

5. Experiment 3: UV-absorptive cork treatment, UV Killer

5.1 Methods

To test whether PIWO may have identified treatment corks in Experiments 1 or 2 based upon a property of MgCO_3 other than UV reflectance (e.g. taste, odor), we conducted another two-choice behavioral assay. If so, then behavioral responses should be similar (i.e. not different) between treatment and control substrates when MgCO_3 was not present. This experiment was conducted with five adult, trained, cue-naïve PIWO randomly selected from a population of 15 available birds. Experimental substrates presented on test poles were UV-absorptive corks ($n = 300$) and control corks ($n = 300$). All other conditions were as previously described.

5.2 Results and discussion

Study subjects were more likely to contact treatment corks ($T = 88.0\%$, $C = 75.3\%$, $F_{1, 585.09} = 15.9$, $P < 0.001$; Fig. 2C) and more likely to remove treatment corks than control corks ($T = 85.8\%$, $C = 73.4\%$, $F_{1, 585.08} = 14.2$, $P < 0.001$; Fig. 2H). With two outliers removed (two T substrates, one each Trials 1 and 2, subject 712), CHT of treatment corks was greater than control corks ($T = 9.2$ s, $C = 5.7$ s, $F_{1, 514.99} = 201.8$, $P < 0.001$; Fig. 2M). There was no difference in the order that corks were removed ($P = 0.2$). These behavioral responses aligned with our predictions, except for the order removed variable.

To test whether cue-naïve subjects exhibited a predisposition to forage at UV-absorptive substrates, we analyzed the data from Trial 1 separately. Probability estimates for cork contact were not different ($P = 0.32$), but the probability of removal was greater for treatments than controls ($T = 87.4\%$, $C = 63.9\%$; $F_{1, 54} = 4.2$, $P = 0.042$; Fig. 3E). After removing one outlier (one T substrate, subject 712), CHT was greater for treatment corks than control corks ($T = 9.2$, $C = 5.7$; $F_{1, 44.782} = 18.7$, $P < 0.001$; Fig. 3H). There was no difference in the order that corks were removed ($P = 0.33$). These results support the greater control substrate (decreased UV) handling time observed with cue-naïve subjects in Experiment 1.

The reflectance of treatment and control corks from Experiment 3 were different in the UV bin (Z ratio = -21.54 , $P < 0.001$), and there was no difference in the visible bin (Z ratio = 1.35 , $P = 0.53$; Fig. 4, Exp 3). These were the expected spectral differences.

Subjects in Experiment 3 responded similarly to those in Experiment 2; both sets of birds demonstrated greater behavioral responses towards UV-absorbing treatment corks than towards control corks. Since $MgCO_3$ was not included in this experiment, there could be no influence of its odor, taste, or other sensory-related properties. We therefore conclude that subjects in both

experiments with UV-absorbing substrates were responding to the reduction of UV reflectance relative to the control substrates, and not merely to the presence of MgCO_3 .

6. Experiment 4: UV-reflective wood wafers, wood decay fungi

6.1 Methods

To test whether decayed wood is a useful foraging cue for PIWO, we conducted a two-choice behavioral assay. If so, then behavioral responses towards decayed (treatment) substrates should be greater than towards control (sound; not decayed) substrates. We used 10 adult, trained, cue-naïve individuals randomly selected from a population of 15 available study subjects. We selected five study subjects from each of the east and west sides of the building to account for potential location differences in ambient light condition among individual aviaries. Experimental substrates presented in test poles included decayed ($n = 600$; 236 unique) and control ($n = 600$; 239 unique) red pine wafers adhered to corks (Size #10, Carolina Biological); all other conditions were as previously described.

6.2 Results and Discussion

Study subjects were more likely to contact treatment substrates ($T = 97.4\%$, $C = 94.7\%$, $F_{1, 1174} = 13.9$, $P < 0.001$; Fig. 2D) and more likely to remove treatment substrates than control substrates ($T = 86.6\%$, $C = 75.5\%$, $F_{1, 1172.1} = 23.8$, $P < 0.001$; Fig. 2I). Handling time of treatment substrates was greater than control substrates ($T = 6.5$ s, $C = 6.1$ s; $F_{1, 1071.5} = 4.3$, $P = 0.037$; Fig. 2N). There was no difference in the order that substrates were removed ($P = 0.18$). These behavioral responses aligned with our predictions, except for the order removed variable. There was considerable variation within Control substrates for the Contact variable (Fig. 2D). This variation was most likely influenced by four individual birds that consistently removed all

substrates presented in all trials (resulting in Contact probability of 100%), influencing the mean probability as well as the variance.

To test whether cue-naïve subjects exhibited a predisposition to forage at decayed substrates, we analyzed the data from Trial 1 separately. There was no difference in any of the response variables (Fig. 3), which suggests that the observed positive responses over 10 trials were conditioned.

The reflectance of treatment and control wafers from Experiment 4 were different in the UV bin (Z ratio = 5.12, $P < 0.001$) and in the visible bin (Z ratio = -7.83, $P < 0.001$; Fig. 5, Exp 4). Based on preliminary work with *P. pini*-decayed and control red pine substrates, these were the expected spectral differences.

Figure 5. Mean reflectance spectra (left) of treatment (T) and control (C), and decayed (D) and sound (S) red pine substrates used in Experiments 4 and 5, respectively, with Pileated

Woodpeckers (*Dryocopus pileatus*) over 300 - 700 nm, 10 nm average (error bars not shown for clarity). Treatment substrates (Exp 4) were exposed to the decay fungus *Porodaedalea pini*. All Exp 5 substrates were treated with an ultraviolet-absorbing liquid. Relative reflectance values below 5% are considered to be not visible, represented by horizontal dashed lines. Mean departure values (right) were calculated as the percent difference from the mean reflectance of the opposite substrate (represented as decimals) over 300 – 700 nm. The mean reflectance value is centered on 1.0 (i.e. 100%).

In this experiment, we tested whether study subjects could distinguish between decayed and sound pine wafers. The wafers used in these trials had been desiccated for at least eight days prior to use, in an effort to remove any influence of the decay fungus other than the spectral condition of the wafers. However, analysis of volatile organic compounds, often produced by wood decay fungi, was beyond the scope of this study. Therefore, we cannot conclude that subjects used only a visual cue (UV or otherwise) to distinguish between treatment and control wafers, just that they were able to make the distinction.

7. Experiment five: UV-absorptive decayed and sound wood wafers, UV Killer

7.1 Methods

To test whether PIWO could discriminate between decayed and sound wood wafers after both were treated with UVK, we conducted another two-choice behavioral assay. If so, then behavioral responses toward decayed substrates should be greater than toward sound substrates. Though both substrates were UV-absorbing after UVK treatment, they remained different in the human-visible spectrum, thus we predicted that study subjects would still be able to discriminate decayed from sound wafers. Subjects were those used in Experiment 4. Experimental substrates presented in test poles were decayed (D; n = 600; 197 unique) and sound (S; n = 600; 190 unique) red pine wafers adhered to corks, both of which were treated with UVK (20 s bath).

7.2 Results and Discussion

Study subjects were more likely to contact decayed substrates ($D = 96.3\%$, $S = 93.2\%$, $F_{1, 1180} = 12.2$, $P < 0.001$; Fig. 2E) and more likely to remove decayed substrates than sound substrates ($D = 83.1\%$, $S = 73.0\%$, $F_{1, 1180} = 23.8$, $P < 0.001$; Fig. 2J). There was no difference in handling time between decayed and sound substrates ($P = 0.12$; Fig. 2O), nor was there a difference in the order that wafers were removed ($P = 0.18$). These behavioral responses aligned with our initial predictions (see Introduction) except for the handling time and order removed variables. As with Experiment 4, the within Control substrate variation of the Contact variable was likely driven by the four individuals that continued to remove all substrates presented.

After treatment with UVK, the reflectance of decayed and sound wafers differed in both the UV (Z ratio = 5.52, $P < 0.001$) and visible bins (Z ratio = -11.4, $P < 0.001$; Fig. 5, Exp 5). For much of the UV range, the relative reflectance of both substrates was below 5% and where it was above 5%, there was little difference (Fig. 5). Additionally, a preliminary analysis of decayed and sound pine wafer reflectance spectra with a model of avian visual perception suggests that the difference in UV did not translate into a perceptible difference for the study subjects (Appendix 2).

In this experiment, we tested whether reducing the differences in UV reflectance between substrates would influence the subjects' ability to discriminate between decayed and sound wood wafers. While spectral differences were detected in the UV range, these did not translate into perceptible differences. The differences in contact and removal probability between Experiments 4 and 5 were less than 1%. Substrate handling time was not different in this experiment, but it was different in Experiment 4. These experiments were conducted sequentially with the same subjects, and as there was no difference in contact or removal

between Experiments 4 and 5. We attribute the change in handling time between experiments to the subjects becoming more adept at removing the corks rather than being influenced by the UV condition of the substrates. We therefore conclude that the subjects were not influenced by the application of UVK to the wood wafers.

8. General Discussion

Our results demonstrate that PIWO study subjects were able to distinguish between treatment and control substrates in each of the five experiments. In the first three experiments (i.e. chemically-treated corks), we artificially altered the UV reflectance of the substrates, and any variation to the human-visible reflectance was likely not perceptible (Appendix 2). We used experimental controls to account for both olfactory and resonance cues, and we also accounted for non-visual influences of MgCO_3 by removing it from the substrates (Experiment 3). We therefore conclude that PIWO are visually sensitive to UV wavelengths, and that this sensitivity translated into a useful foraging cue in our experiments. To our knowledge, this is the first documentation of UV sensitivity in the Piciformes, a widespread avian family of both ecological and economic importance.

Interestingly, subjects responded in the same manner whether the treatment substrates were UV-reflective or UV-absorptive. Thus, a change in UV reflectance relative to the untreated control substrates, regardless of the direction of the change, was perceptible by PIWOs. This result mirrors those found in behavioral trials with Red-winged Blackbirds (*Agelaius phoeniceus*) [28], and has implications for ecological interactions with wood decay fungi as well as for developing damage control strategies.

We tested the importance of UV reflectance in discriminating between decayed and sound red pine substrates in Experiments 4 and 5. The study subjects did not alter their behavior

when both decayed and sound substrates were UV-absorbing. Thus, for red pine decayed by *P. pini*, the difference in reflected light from 400 - 700 nm was sufficient for PIWOs to distinguish between decayed and sound wafers. There are at least 10,000 species of wood decay fungi [38], and some woodpecker species appear to exhibit a preference for placing nest and roost cavities within trees decayed by specific fungi [30], [39], [40], and [41]. It is possible that each decay species could create a specific substrate reflectance, which could vary among tree species as well [42]. A larger sampling of decayed substrates may reveal combinations of decay fungi and tree species that produce substrates for which UV reflectance or absorbance is greatly different from surrounding uninfected wood.

Cue-naïve subjects in Experiment 3 discriminated between UV-absorbing and control substrates, and the handling time of control corks by cue-naïve subjects in Experiment 1 was greater than that of UV-reflective treatment corks. These results suggest that UV-absorbing substrates may contain information for Pileated Woodpeckers, possibly as a foraging cue. Many flowering plants exhibit UV-absorbing “nectar guides” on flower petals which have co-evolved with UV-sensitive insect pollinators. Woodpeckers are known to transport spores of fungi present at cavity locations [20] and [31]. Thus woodpeckers and fungi have potentially evolved mutualisms analogous to those between plants and pollinators. Such mutualisms could enlist similar communication methods, an idea supported by our behavioral results.

Reducing the UV reflectance of decayed and sound wood did not alter the subjects’ response; therefore, it is unlikely that any product which only alters substrate UV reflectance would deter or repel woodpeckers from anthropogenic structures. However, products which pair a negative consequence with a UV cue could be useful in conditioning woodpecker avoidance of treated substrates. In behavioral tests with Red-winged Blackbirds, a UV-absorptive cue paired

with 9, 10-anthraquinone (a UV-absorptive compound with negative post-ingestive consequences) showed a synergistic effect in feeding repellency [43]. Since the woodpeckers' response to the UV condition of experimental substrates mirrored that of Red-winged Blackbirds, we speculate that Pileated Woodpeckers would demonstrate a similar avoidance response toward a post-ingestive repellent paired with a UV cue.

9. Conclusions

We demonstrated that Pileated Woodpeckers can be conditioned with UV cues. Additionally, Pileated Woodpeckers may be pre-disposed to target UV-absorptive substrates when foraging. Knowledge of woodpecker sensory systems is vital for understanding ecological interactions with fungi which form the basis for cavity nest webs, and our study provides an initial framework for understanding woodpecker visual ecology. Other sensory systems such as olfaction may be important to these interactions as well.

A behavior can be defined as an organism's response to some environmental stimulus [44]. Therefore, if a particular animal behavior is to be somehow manipulated, or "controlled", an understanding of that organism's sensory thresholds is imperative. Results from this study should be beneficial for future research seeking to control woodpecker excavation behaviors through visual cues.

Finally, our behavioral assay represents a novel approach to woodpecker research. With minimal training, subjects readily performed the task of removing corks in search of food items. This methodology offers the ability to research similar visual ecology questions with other species of woodpecker and primary cavity excavators (e.g. nuthatches (*Sitta* spp.)). Additionally, our design can be easily modified to investigate other sensory modalities (e.g. olfaction, vibration) which may be equally important to woodpeckers. We view this study as a

starting point, and hope that our work will lead to supplemental research regarding the sensory ecology of woodpeckers.

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Literature Cited

- [1] Goldsmith, T.H. 1990. Optimization, constraint, and history in the evolution of eyes. *The Quarterly Review of Biology*. 65: 281-322.
- [2] Ödeen, A., O. Håstad, and P. Alstrom. 2011. Evolution of ultraviolet vision in the largest avian radiation – the passerines. *BMC Evolutionary Biology*. 11: 313.
- [3] Burns, K. and A.J. Shultz. 2012. Widespread cryptic dichromatism and ultraviolet reflectance in the largest radiation of Neotropical songbirds: Implications of accounting for avian vision in the study of plumage evolution. *The Auk*. 129: 211-221.
- [4] Vorobyev, M. and D. Osorio. 1998. Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London, Series B*. 265: 351-358.
- [5] Hart, N.S. and D.M. Hunt. 2007. Avian visual pigments: Characteristics, spectral tuning, and evolution. *The American Naturalist*. 169: S7-S26.
- [6] Ödeen, A. and O. Håstad. 2003. Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Molecular Biology and Evolution* 20: 855-861.
- [7] Jane, S.D. and J.K. Bowmaker. 1988. Tetrachromatic colour vision in the duck (*Anas platyrhynchos* L.): microspectrophotometry of visual pigments and oil droplets. *Journal of Comparative Physiology A*. 162: 225-235.
- [8] Wilkie, S., P.R. Robinson, T.W. Cronin, S. Poopalasundaram, J.K. Bowmaker, and D.M. Hunt. 2000. Spectral tuning of avian violet- and ultraviolet-sensitive visual pigments. *Biochemistry*. 39: 7895-7901.
- [9] Endler, J.A. and P.W. Mielke. 2005. Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*. 86: 405-431.
- [10] Kemp, D.J., M.E. Herberstein, L.J. Fleishman, J.A. Endler, A.T.D. Bennett, A.G. Dyer, N.S. Hart, J. Marshall, and M.J. Whiting. 2015. An integrative framework for the appraisal of coloration in nature. *The American Naturalist*. 185: 705-724.
- [11] Bennett, A.T.D., I.C. Cuthill, J.C. Partridge, and K. Lunau. 1997. Ultraviolet plumage colors predict mate preferences in starlings. *Proceedings of the National Academy of Sciences of the USA*. 94: 8618-8621.
- [12] Hunt, S., I.C. Cuthill, A.T.D. Bennett, and R. Griffiths. 1999. Preferences for ultraviolet partners in the blue tit. *Animal Behaviour*. 58: 809-815.
- [13] Hill, G.E., S.M. Doucet, and R. Buchholz. 2005. The effect of coccidial infection on iridescent plumage coloration in wild turkeys. *Animal Behaviour*. 69: 387-394.
- [14] Siefferman, L. and G.E. Hill. 2005. UV-blue structural coloration and competition for

- nestboxes in male eastern bluebirds. *Animal Behaviour*. 69: 67-72.
- [15] Siitari, H., J. Honkavaara, and J. Viitala. 1999. Ultraviolet reflection of berries attracts foraging birds. A laboratory study with redwings (*Turdus iliacus*) and bilberries (*Vaccinium myrtillus*). *Proceedings of the Royal Society of London, Series B*. 266: 2125-2130.
- [16] Church, S.C., A.T.D. Bennett, I.C. Cuthill, and J.C. Partridge. 1998. Ultraviolet cues affect the foraging behaviour of blue tits. *Proceedings of the Royal Society of London, Series B*. 265: 1509-14. doi: 10.1098/rspb.1998.0465
- [17] Martin, K. and J. Eadie 1999. Nest webs: A community-wide approach to the management and conservation of cavity-nesting forest birds. *Forest Ecology and Management*. 115: 243-257.
- [18] Arnett, E.B., A.J. Kroll, and S.D. Duke. 2010. Avian foraging and nesting of created snags in intensively-managed forests of western Oregon, USA. *Forest Ecology and Management*. 1773-79.
- [19] Farris, K.L, M.J. Huss, and S. Zack. 2004. The role of foraging woodpeckers in the decomposition of ponderosa pine snags. *The Condor*. 106: 50-59.
- [20] Jusino, M.A., D.L. Lindner, M.T. Banik, K.R. Rose, and J.R. Walters. 2016. Experimental evidence of a symbiosis between red-cockaded woodpeckers and fungi. *Proceedings of the Royal Society of London, Series B*. 283: 20160106. doi: 10.1098/rspb.2016.0106
- [21] Bednarz, J.C., D. Ripper, P.M. Radley. 2004. Emerging Concepts and Research Directions in the Study of Cavity-Nesting Birds: Keystone Ecological Processes. *The Condor*. 106: 1-4.
- [22] Harness, R. and E. Walters. 2004. Woodpeckers and Utility Pole Damage. In: *Proceedings of the Institute of Electrical and Electronics Engineers/Industry Applications Society Rural Electric Power Conference, 2004*. Paper No. 04/B3: 1-7.
- [23] Conner, R.N., S.D. Jones, and G.D. Jones. 1994. Snag condition and woodpecker foraging ecology in a bottomland hardwood forest. *The Wilson Bulletin*. 106:2, 242-247.
- [24] Jackson, J. and B. Jackson. 2004. Ecological relationships between fungi and woodpecker cavity sites. *The Condor*. 106:37-39.
- [25] Zahner, V., L. Sikora, and G. Pasinelli. 2012. Heart rot as a key factor for cavity tree selection in the black woodpecker. *Forest Ecology and Management*. 271: 98-103.
- [26] Klapstein, K., F.L. Weichman, W.N. Bauer, and D.J. Kenway. 1989. Optical characteristics of wood stains and rot. *Applied Optics*. 28: 4450-4452.

- [27] Lytinen, A., R.V. Alatalo, L. Lindstrom, and J. Mappes. 2001. Can ultraviolet cues function as aposematic signals? *Behavioral Ecology*. 12: 65-70.
- [28] Werner, S.J., S.K. Tupper, J.C. Carlson, S.E. Pettit, J.W. Ellis, and G.M. Linz. 2012. The role of a generalized ultraviolet cue for blackbird food selection. *Physiology and Behavior*. 106: 597-601.
- [29] Ohsawa, K., T. Watanabe, R. Matsukawa, Y. Yoshimura, and K. Imaeda. 1984. The possible role of squalene and its peroxide of the sebum in the occurrence of sunburn and protection from the damage caused by UV irradiation. *The Journal of Toxicological Sciences*. 9: 151-59.
- [30] Steirly, C.C. 1957. Nesting ecology of the red-cockaded woodpecker in Virginia. *The Raven*. 28: 24-36.
- [31] Jusino, M.A., D.L. Lindner, M.T. Banik, and J.R. Walters. 2015. Heart rot hotel: fungal communities in red-cockaded woodpecker excavations. *Fungal Ecology*. 14: 33-43.
- [32] Koenig, W.D. 1991. The effects of tannins and lipids on digestion of acorns by acorn woodpeckers. *The Auk*. 108: 79-88.
- [33] Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-8. <http://CRAN.R-project.org/package=lme4>
- [34] R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. <http://www.R-project.org/>
- [35] Lenth, R.V. and M. Herve. 2015. lsmeans: Least-squares means. R package version 2.18. <http://CRAN.R-project.org/package=lsmeans>
- [36] Graves, S. H-P. Piepho, L. Selzer, and S. Dorai-Raj. 2015. multcompView: Visualizations of paired comparisons. R package version 0.1-7. <http://CRAN.Rproject.org/package=multcompView>
- [37] Eaton, M.D. and S.M. Lanyon. 2003. The ubiquity of avian ultraviolet plumage reflectance. *Proceedings of the Royal Society of London, Series B*. 270: 1721-1726.
- [38] Hibbett, D.S., and Donoghue, M.J. 2001. Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in Homobasidiomycetes. *Systematic Biology* 50:215-242.
- [39] Shigo, A. and L. Kilham. 1968. Sapsuckers and *Fomes ignarius* var. *populinus*. USDA Forest Service Research Note NE-84.
- [40] Conner, R.N., O.K. Miller, Jr. and C. S. Adkisson. 1976. Woodpecker dependence on trees

- infected by fungal heart rots. *The Wilson Bulletin*. 88: 575-581.
- [41] Winternitz, B.L. and H. Cahn. 1983. Nestholes in live and dead aspen. In: J. W. Davis, G. A. Goodwin, and R. A. Ockenfels [Tech. Coords.]. *Snag habitat management: proceedings of the symposium*. USDA Forest Service General Technical Report RM-99. 102-106.
- [42] O'Daniels, S.T. 2016. Light reflectance patterns of decayed wood with implications for the visual ecology woodpeckers. Master's Thesis. University of Missouri, Columbia, MO. pgs. 45-75.
- [43] Werner, S.J., S.T. DeLiberto, S.E. Pettit, and A.M. Mangan. 2014. Synergistic effect of an ultraviolet feeding cue for an avian repellent and protection of agricultural crops. *Applied Animal Behaviour Science*. 159: 107-113.
- [44] Levitis, D.A., W.Z. Lidicker, Jr., and G. Freund. 2009. Behavioural biologists don't agree on what constitutes behaviour. *Animal Behaviour*. 78: 103-110. doi:10.1016/j.anbehav.2009.03.018
- [45] York, D.L., J.E. Davis, Jr., J.L. Cummings, and E.A. Wilson. 1998. Pileated Woodpecker capture using a mist net and taped call. *North American Bird Bander*. 23: 81-82.
- [46] Tupper, S.K., W.F. Andelt, J.L. Cummings, C. Weisner, and R.E. Harness. 2010. Polyurea elastomer protects utility pole crossarms from damage by Pileated Woodpeckers. *Journal of Wildlife Management*. 74: 605-608.
- [47] Vorobyev, M., D. Osorio, A.T.D. Bennett, N.J. Marshall, and I.C. Cuthill. 1998. Tetrachromacy, oil droplets and bird plumage colours. *Journal of Comparative Physiology A*. 183: 621-633.
- [48] Iqic, B. P. Cassey, T. Grim, D.R. Greenwood, C. Moskat, J. Rutila, and M.E. Hauber. 2012. A shared chemical basis of avian host-parasite egg colour mimicry. *Proceedings of the Royal Society of London, Series B*. 279: 1068-76.
- [49] Olssen, P., O. Lind, and A. Kelber. 2015. Bird colour vision: behavioral thresholds reveal receptor noise. *Journal of Experimental Biology*. 218: 184-193.
- [50] Eaton, M. 2005. Human Vision Fails to Distinguish Widespread Sexual Dichromatism among Sexually "Monochromatic" Birds. *Proceedings of the National Academy of Sciences of the United States of America*. 102: 10942-10946.
- [51] Maia, R., C.M. Eliason, P-P. Bitton, S.M. Doucet, and M.D. Shawkey. 2013. Pavo: an R package for the analysis, visualization, and organization of spectral data. *Methods in Ecology and Evolution*. doi: 10.1111/2041-210X.12069
- [52] Endler, J.A. 1993. The color of light in forests and its implications. *Ecological*

Monographs. 63(1): 1-27.

- [53] Maier, E.J. & Bowmaker, J.K. 1993. Colour vision in the passeriform bird, *Leiothrix lutea*: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. *Journal of Comparative Physiology A*. 172: 295. doi:10.1007/BF00216611
- [54] Herrerra, G., J.C. Zagal, M. Diaz, M.J. Fernandez, A. Vielma, M. Cure, J. Martinez, F. Bozinovic, and A. G. Palacios. 2008. Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephanoides*). *Journal of Comparative Physiology A*. 194: 785-794.
- [55] Hart, N.S. 2001. The visual ecology of avian photoreceptors. *Progress in Retinal and Eye Research*. 20: 675-703.
- [56] Amano, H.E. and K. Eguchi. 2002. Foraging niches of introduced Red-billed *Leiothrix* and native species in Japan. *Ornithological Science*. 1: 123-131.

10. Supplementary Material

10.1 Supplementary Figures

Figure S1. Human-visible (left) and UV-only (right) images of control and UV-reflective treatment (MgCO_3) corks used in behavioral trials with Pileated Woodpeckers (*Dryocopus pileatus*). Untreated control corks ($n = 3$) are on the left and treatment corks ($n = 3$) are on the right of each image. The UV image shows that more MgCO_3 may have been deposited in the various depressions on the corks than on the flat surfaces, thus creating visually different substrates unable to be measured by the spectrometer used in this study. Digital images were created using a modified Nikon D200 (Nikon, Inc., Melville, NY, U.S.A.), CoastalOpt® 60 mm UV–VIS–IR APO macro (Jenoptik Optical Systems, Inc., Jupiter, FL, U.S.A.), Baader 300–400 nm bandpass filter (Alpine Astronomical, Eagle, ID, U.S.A.), and a modified Vivitar 285 HV flash (Vivitar, Edison, NJ, U.S.A.).

10.2 Appendix 1

Methods for the live-capture and care of Pileated Woodpecker (PIWO) study subjects, and a description of the testing facilities used in this study.

We captured individual PIWO from wild populations in Arkansas (n=20) and Missouri (n=1), USA, from 17 March – 8 May, 2014, and from 31 March – 10 April, 2015, from suitable habitats using mist nets combined with audio recordings of PIWO calls following York et al [45]. Captured woodpeckers were placed in individual temporary outdoor holding pens for 1 - 14 days until transport was possible. We conducted behavioral experiments at the Outdoor Animal Research Facility of the National Wildlife Research Center (NWRC), Ft. Collins, Colorado, USA. Woodpeckers were transported to NWRC in individual holding cages (50 cm x 61 cm x 50 cm) on 21 March, 2014, 18 April, 2014, 8 May, 2014, and 12 April, 2015. Each transport cage was fitted with a cage liner to prevent slipping, and each bird was supplied with maintenance diet *ad libitum* during transport.

At NWRC, woodpeckers were housed separately in outdoor aviaries (2.6 m x 2.6 m x 5.3 m). All temporary and permanent aviaries were lined with nylon mesh nets to reduce injury potential. After arriving at NWRC, all subjects underwent a 14 day acclimation/quarantine period during which the daily maintenance diet and fresh water were provided *ad libitum*.

Daily maintenance diet consisted of 20 g canned dog food (beef), 35 g live mealworms (40 g in 2015), and 50 g mixed fruit (apples, bananas, grapes, oranges) [46]. Each PIWO had access to food, water, and one untreated, 20 cm diameter x 90 cm section of southern yellow pine (*Pinus* spp.) enrichment pole. During testing periods, food restrictions were implemented (see General Methods 2.2). The capture, care, and use of birds were approved by NWRC's Institutional Animal Care and Use Committee (NWRC study protocols QA2242, QA2398).

10.3 Appendix 2

The receptor noise-limited (RNL) color discrimination model [4] has been widely used to estimate avian color discrimination capabilities [37], [47], [48], and [49]. The model calculates the difference between points (ΔS) within a theoretical color space as defined by the quantum catch of each receptor type within the retina of the species of interest. Values of $\Delta S \geq 1.0$ are considered to be theoretically distinguishable. The RNL model requires inputs for absorbance values of each cone cell type, each oil droplet type and ocular media. When possible, MSP data from the species of interest are used to populate the various model parameters. Since MSP is available for very few avian species, data from one species is frequently used to model visual perception in other species presumed to have similar visual systems (e.g. [50]). Due to the conservative nature of visual systems, such assumptions are not necessarily unwarranted.

Additionally, a value for the Weber fraction, an estimate of the noise inherent in the neural mechanisms of vision, is needed as this is a limiting model parameter. The RNL model requires input for only the LWS Weber fraction, with values for the remaining cone types calculated from their densities. It is important to emphasize that in the absence of species-specific MSP data, results obtained with the RNL model should be interpreted cautiously. While they may be used as a guide, changes to model parameters may produce very different results.

10.2.1 Methods

When unpredicted spectral differences were detected by the statistical models for Experiments 2 and 5, we employed the RNL model to assess whether those differences might have translated into perceptual differences for the study subjects, and thereby confounded the behavioral results of Experiments 2 and 5. The RNL model was implemented through the *pavo* package (version 0.5-2, [51]) in R as it is described in Vorobyev et al. [47]. We first calculated

ΔS for the mean reflectance spectra of treatment and control substrates (Table S1). We then created control and treatment reflectance spectra with equalized UV values by averaging the relative reflectance between control and treatments from 300 - 400 nm, and calculated ΔS values for these UV-equalized spectra. There are no published MSP data for any woodpecker species, but we have an estimate of SWS1 $\lambda_{\max}$ for GSWO at 405 nm [6]. Therefore, we compared ΔS values generated by two different models of woodpecker visual perception.

The first model was based on the receptor quantum catches for the average VS bird [9]. The second model (GSWO) was created with the `sensmodel()` function in *pavo*, using $\lambda_{\max}$ values of 405 nm, 452 nm, 505 nm, and 565 nm for each receptor. The latter three values are the average $\lambda_{\max}$ values for SWS2, MWS and LWS cones reported for VS species by Endler and Mielke [9]. Both models included average absorbance values for transparent/clear (459 nm), yellow (525 nm) and red (588 nm) oil droplets and for avian ocular media (362 nm; also from [9]). The only difference between the two models was the SWS1 $\lambda_{\max}$ value (Avg VS = 412 nm, GSWO = 405 nm). Additional parameters for both models included forest shade irradiance [52] and the mean reflectance of four test poles as the background spectra.

We ran the models with LWS Weber fraction values ranging from 0.05 - 0.1 (Table S1), which covers the range of published values for avian species [49]. We included a cone density ratio of 1:2:2:4 (SWS1:SWS2:MWS:LWS), from the Red-billed Leiothrix (*Leiothrix lutea*) [53] which has been used in at least two previous studies utilizing the RNL model [47] and [54]. While cone densities likely vary between species, there is evidence that such differences may be related to visual ecology [55 and references therein]. Since *L. lutea* is native to forest and woodland habitats from southern China to the western Himalayas [56], it is not unreasonable to assume this cone density ratio for a species of woodpecker.

10.2.2 Results and Discussion

10.2.2.1 Experiment 2

Comparing the mean treatment and control reflectance spectra of substrates from Experiment 2 produced a ΔS value of 1.3 (above threshold) under the GSWO model (LWS Weber fraction = 0.05). The only other model that produced a threshold or greater ΔS value was the GSWO model with an LWS Weber fraction of 0.06 (Table S1). When we set the UV portion of these mean reflectance spectra to equal in the model, so that the only differences were in the 400 - 700 nm range, ΔS dropped to below threshold (Table S1) in all models tested. This result suggests that any differences over that range (i.e. those detected by the statistical model, Section 4.2) were not perceptible.

Substrate 1	Substrate 2	Visual Model	ΔS	ΔS (UV equal)	LWS Weber Fraction
MgCO ₃ + UV Killer®	Control	Avg VS	0.7	0.2	0.05
Cork	Cork	GSWO	1.3	0.3	0.05
		GSWO	1.0	0.1	0.06

Table S1. Results from receptor noise-limited model analyses of mean control and treatment substrate spectra used in Experiment 2 with Pileated Woodpeckers (PIWO; *Dryocopus pileatus*). Values of $\Delta S < 1.0$ are considered below the threshold of discrimination (only one such result reported for comparison). The GSWO model includes the estimated SWS1 $\lambda_{\max}$ of the Great Spotted Woodpecker (*Dendrocopus major*), which we used to approximate the visual system of

PIWO. UV-equalized spectra produced below threshold values for both models suggesting that any differences outside of the UV range were not perceptible for the study subjects.

Assuming that our GSWO model is appropriate for Pileated Woodpeckers, these RNL model results might be used to infer two aspects of the PIWO visual system, SWS1 $\lambda_{\max}$ and the LWS Weber fraction. Threshold values of ΔS were produced only when the GSWO model LWS Weber fraction was ≤ 0.06 , and no threshold values were produced under the Average VS model (Table S1). Since the study subjects did behaviorally discriminate between control and treatment substrates, these perceptual model results suggest that the SWS1 $\lambda_{\max}$ of the Pileated Woodpecker is similar to that of the Great Spotted Woodpecker. This finding is consistent with the conservative nature of avian visual systems [10]. Similarly, if an LWS Weber fraction estimate for PIWO of 0.06 is accurate, it would fall within the published range of values for avian species [49].

10.2.2.2 Experiment 5

After treatment with UVK, the reflectance of decayed and sound wafers differed in both the UV (Z ratio = 5.52, $P < 0.001$) and visible bins (Z ratio = -11.4, $P < 0.001$; Fig. 4, Exp 5). Since the difference in the UV bin was not predicted, we again employed the RNL model to determine if the statistical difference translated into a perceptible difference for the subjects. The ΔS values comparing the mean decayed and sound substrate reflectance spectra were 2.4 and 1.4 (both above threshold) under the Average VS and GSWO models (LWS Weber fraction = 0.05), respectively. When we set the UV portion of the mean reflectance spectra to equal in the model, so that the only differences were in the visible bin (410 - 700 nm), there was no change in ΔS .

910 These results suggest that the perceptible differences were entirely within the visible bin, and
911 that the statistical difference in the UV bin was not perceptible to the study subjects.