EFFECTS OF LASER POWER AND EXPOSURE TIME ON THE AVIAN EYE: IMPLICATIONS FOR THE USE OF BIRD DETERRENTS

by

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Dedicated to my friends and family for constantly supporting me.

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ABSTRACT

Laser deterrents have been used as a method of deterring birds from problem areas such as fisheries, agricultural fields and airports. This method is considered a nonlethal means of control although lasers are known to cause visual lesions and loss of visual acuity in humans and other animals. Birds have a complex visual system which is necessary for behaviors critical to their survival, such as hunting and foraging, and predator vigilance. The purpose of this study is to determine the safety of laser deterrents for avian eyes using two species of birds: house sparrows (*Passer domesticus*) and European Starlings (*Sturnus vulgaris*). We found evidence that laser exposure can cause corneal edema, cataracts, retinal atrophy, displacement of the photoreceptor nuclei, and degeneration of the scleral cartilage. The laser exposure time was an important factor in the likelihood of developing corneal edema and retinal atrophy in starlings. Our findings suggest that lasers may not be completely safe for use as bird deterrents, but further research should be done to find possible solutions to improve laser safety from the avian viewpoint.

CHAPTER 1. INTRODUCTION

Birds are involved in multiple types of human-wildlife conflicts. In the blueberry industry alone birds cost thousands of dollars per hectare and millions statewide in crop damage (Anderson et al. 2013). Almost 97% of wildlife-airplane strikes reported to the Federal Aviation Administration from 1990 to 2014 involved birds (Dolbeer et al. 2015). In addition, bycatch is one of the top 10 threats to seabird species globally (Croxall et al. 2012). Species of conservation concern such as the Black-footed Albatross and other seabirds can become entangled in fishing nets or caught on hooks and consequently drown (Bergin 1997). In fact, pelagic longlining was the largest cause of Black-footed Albatross mortality in the early 2000s, affecting 1.9-5% of the population each year, and populations are still recovering (Lewison and Crowder 2003, Croxall et al. 2012). Different methods have been used to mitigate these issues, some lethal (i.e. poison), but also nonlethal methods to repel birds, such as chemical repellants that target smell or taste, auditory repellants such as propane cannons, tactile repellents that are oily or sticky, or visual repellents in the form of high intensity lights (Clark 1998, Werner and Clark 2003). Lasers are one of the nonlethal forms of control for birds (Blackwell et al 2002, Lustick 1973).

Lasers can trigger birds to initiate an avoidance (Lustick 1973). However, while lasers have been found to be effective in scaring birds away from areas for a short time, the results appear to be species-specific, and work better in conjunction with other methods (e.g. auditory) and under certain environmental conditions (Blackwell et al. 2002, Soldatini et al. 2008). In a study testing the efficacy of laser deterrents at a refuse dump, yellow-legged gulls appeared to leave the area, while black-headed gulls moved but stayed nearby (Soldatini et al. 2008). While the lasers did significantly reduce the probability of birds landing, they eventually became habituated and returned (Soldatini et al. 2008). Another study found that when paired with distress calls, ring-

billed gulls became sensitized to the laser treatment over time (Lecker et al. 2015). Another study on American crows in urban night roosts found that while the birds dispersed initially, they usually returned to roost a few minutes later and never left for a full night (Gorenzel et al. 2002). This suggests that the laser light may startle the birds, but does not indicate a significant threat (Gorenzel et al. 2002).

Humans have used lasers in various scenarios, from laser pointers in classrooms to medical applications, since the 1960's (Lustick 1973, Zweng et al. 1964). When not used appropriately, lasers can harm the human eye, sometimes irreversibly (Wolbarsht 1996). Laser injuries can negatively affect different parts of the eye: cornea, lens, retina, etc. Corneal edema (i.e., swelling of the cornea with fluid) can occur during laser trabeculoplasty – a treatment for glaucoma (Knickelbein et al. 2014). Cataracts (i.e., opacification of the lens) have been reported after treatments like retinal photocoagulation or accidental exposure during laser hair removal (McCanna et al. 1982, Shapiro et al. 1984, Brilakis and Holland 2004). Several military case studies have reported hemorrhaging in the vitreous, retinal pigment epithelium, and retina, resulting in a permanent loss in visual acuity that worsens over time (Harris et al. 2003). Commercial airplane pilots have been maliciously targeted with lasers, and in such cases, have experienced immediate irritation such as blurry vision, flash blindness, or retinal burns which could affect their ability to operate the aircraft (Palakkamanil and Fielden 2015, Gosling et al. 2016). In many cases children have been affected by laser pointers and experience blurry vision, blindspots and temporary retinal damage, one even resulting in a hole in the retina (Turaka et al. 2012, Xu et al. 2016, Dhoot et al. 2016, Zhang et al. 2016).

The negative effects of lasers have also been reported in other animals. Cynomologous monkeys suffered immediate vitreal hemorrhage, which can lead to retinal fibrosis, when exposed

to two pulses of 3 to 6 mJ on either side of the fovea (Zwick et al. 1994). Multiple studies reported a change in photoreceptor function – measured by ERG spectral sensitivity – after monkeys experienced foveal and parafoveal laser retinal lesions (Zwick et al. 1994, Zwick et al. 1974, Zwick et al. 1989, and Zwick et al. 1992). Research using rabbits have shown severe corneal burns, clouding of the cornea, formation of corneal craters, corneal scarring, and development of blood vessels in the corneal stroma which could be caused by oxygen deprivation, some of which were still present after 60 days, when exposed to energies above 100,000 mJ (Fine et al. 1968, Leibowitz and Peacock 1969). When exposed to energies of 29,500 mJ/cu cm, Dutch-belted rabbits suffered corneal edema, severe and permanent damage to the iris – including depigmentation, atrophy, and holes – cataracts, vitreous hemorrhage, and severe retinal burn (Leibowitz and Luzzio 1970). When Chinchilla rabbits were exposed to laser energies varying from 0.01 mJ to 0.05 mJ in the form of 1-4 pulses of 20 nano secs, they suffered retinal swelling and hemorrhages, which worsened as laser energy increased and over time (Brandeis et al. 2005). Severe damage to the inner retina and retinal detachment was observed 24 hrs after 225 lesions were applied to the peripheral retina of pigmented rabbits by a laser at 500 mW for 0.1 s, followed by the loss of all layers of the retina in damaged areas after 30 days (Leibu et al. 1999). A laser exposure of 100 mW for 0.05 s in rats caused significant disruption of the photoreceptors and outer nuclear layer when at least 129 lesions were produced on half or a quarter of each retina, and produced lesions that grew 50-70% after 3 days when 6 lesions were applied per retina (Ben-Schlomo et al. 2006, Belokopytov et al. 2005, Belokopytov et al. 2010). Colubrid snakes, such as checkered garter snakes, Great plains rat snakes and western coachwhip snakes, have experienced changes in photoreceptor size and shape including elongation and enlargement due to laser exposure (Zwick et al. 1999, Zwick et al. 2005). Checkered garter snakes had lesions characterized by severe central

photoreceptor vaporization surrounded by swollen photoreceptors, damage to the retinal pigmented epithelium cells, ultimately distorting retinal ganglion cell response when exposed to a lasers (Zwick et al. 2005, 2008, Glickman et al. 2007).

Despite the amount of literature available for other species, very little research has been done on the effects of lasers on bird eye injury. There has only been one publication testing the effects of lasers on a single species of birds: Double-crested Cormorant. Lasers varying in output between 3 and 5 mW were targeted to birds placed at 3, 13, and 33 m (Glahn et al. 2000). After ophthalmic exams performed by a veterinarian 24 hrs after exposure, ERGs done 96 hours after exams, and histopathological exams, no damage was detected (Glahn et al. 2000). However, the study used 3 treatment birds and 2 control birds (Glahn et al. 2000); such reduced sample size could have challenged the detection of injuries. Additionally, there are substantial difference in the anatomy of the different components of the eye between different bird species (Bowmaker and Knowles 1977, Blackwell 2002). Therefore, it is important to re-examine the question of whether avian eyes can be injury after laser exposure, as it can have important implications for safely using lasers to deter birds.

The purpose of this study was to determine if laser exposure would cause injury to bird eyes by testing the effects of a laser bird deterrent on the eyes of European starlings (*Sturnus vulgaris*) and house sparrows (*Passer domesticus*). We performed two different experiments in which the birds were exposed to a wide range of laser energies. During the first experiment, we exposed one eye of each starling to a laser and used the other eye as a control to take into consideration potential variation within individuals. We used 2 different laser powers and 4 different laser exposure times to assess the effects of lasers on the probability of eye injury. The second experiment involving house sparrows, included a laser exposed group of individuals (i.e.,

where both eyes were exposed to the laser) and a control group of individuals (no eye exposed to the laser). We exposed the birds to 7 different laser powers and 7 different laser exposure times, and evaluated the effects of lasers on the probability of eye injury. We designed both experiments to have different power and exposure times in each, so no conclusions can be drawn to compare results between species. The probability of eye injury was assessed using histopathological criteria based on a visual analysis of the cornea, conjunctiva, lens, iris, retinal pigment epithelium, retina and sclera for different types of injuries (corneal edema, ulceration, stromal fibrosis, keratitis, conjunctivitis, cataracts, uveitis, RPE atrophy, RPE hypertrophy, retinal atrophy, retinoschisis, retinal photoreceptor displaced nuclei, and scleral cartilage degeneration).

CHAPTER 2. METHODS

2.1 Animal use

All birds (24 European starlings, 84 house sparrows) were trapped in Lafayette and West Lafayette, Indiana, and transported to the lab in cloth bags where they were banded, sexed, aged, and randomly assigned to different treatment conditions. European starlings were trapped using potter traps and house sparrows using traps designed for this species in particular (https://sparrowtraps.net/). We kept the birds in 61 x 61 x 76 cm wired cages on a 14-h light/10-h dark light cycle, where they had access to water *ad libitum* and food. We kept 4 starlings per cage, and fed them a mix of Purina game bird chow and dry cat food *ad libitum*. We kept 6 house sparrows per cage, and fed them 80 g per day of a mix of Purina game bird chow, black oiled sunflower seeds, millet seeds and dried meal worms per cage. The care and protocols involving live animals were approved by Purdue Animal Care and Use Committee (protocol number 1707001594).

2.2 Experimental setup

On the day of a treatment, we transported birds to a separate room for anesthesia. We dilated both pupils with rocuronium bromide as recommended by Dr. Wendy Townsend, a veterinarian in the Department of Veterinary Clinical Sciences at Purdue University's College of Veterinary Medicine. We anesthetized each bird via injection in the breast muscle to prevent ocular movements during laser exposure that could affect the amount of laser light entering the eye (Lund et al. 2007). The anesthesia dosages were adjusted based on Velez et al. (2015). Once the birds were anesthetized and the eyes fully dilated, we placed the bird inside a bag and onto a heating pad, and transported them to the laser exposure arena (Fig. 1). We used Velcro straps to hold birds

in a foam cradle and held the eye open with a speculum. The cradle was positioned so that the eye was centered in front of a laser receiver 1 m away from the laser. Each bird was exposed to the laser for the assigned power and exposure time 3 times. The rationale of multiple exposures was to mimic natural situations. For instance, in longline fisheries, one fishing set can last several hours. When a laser deterrent is active during this time, an individual bird may be exposed to the laser multiple times per set (Ed Melvin Senior Scientist at Washington Sea Grant, personal communication). We allowed the eye tissue to cool for 5 s between each exposure (recommended by Bruce Stuck, Director of the Ocular Trauma Research Division at the U.S. Army Institute of Surgical Research in San Antonio Texas until 2013) in order to prevent additive damage caused by temperature increase (Thomsen 1991, Lund and Sliney 2014). Once exposed, we gently moved the bird to a heated pad to help with thermoregulation, where they remained until the anesthesia completely wore off and they could be returned to their cages.

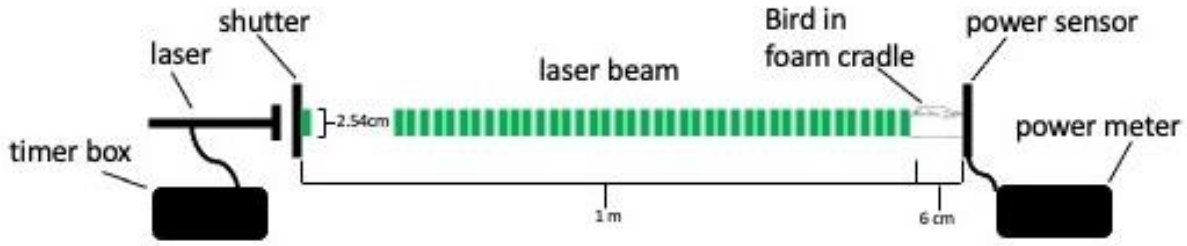


Figure 1. The experimental arena.

The laser we used was a prototype of the Seabird Saver (https://savewave.eu/seabirdsaver/), a Class IV continuous wave laser which has an adjustable power (0-1,000 mW), a beam diameter of 4cm at aperture, beam divergence of 0.5 mrad, and 532 nm wavelength. Class IV lasers are those that operate at over 500 mW making them hazardous under all viewing conditions including diffuse viewing and viewing of reflections (OSHA Technical Manual 2014). The laser was fitted with a "Thor labs 1-inch optical beam shutter" which converted the beam diameter to 2.54 cm, and a timer to control the time for which the shutter opened to expose the laser light. The laser unit was secured to a table exactly 1 m away from a power sensor (Ophir 30A-BB-18 power sensor). The laser was visually aligned with the sensor such that the desired laser power for exposure was displayed on the power meter (Ophir Vega laser power meter). We marked the location of the center of the sensor and moved it approximately 6 cm backward to make room for a bird in a foam cradle. This allowed the bird's eye to be positioned exactly 1 m from the laser aperture. This setup was approved by Purdue Radiological and Environmental Management (REM) and proper safety regulations were followed including signage alerting to the presence and safety hazards of the laser, a light outside the laser room that was turned on while the laser was in use, a barrier between the door and laser, and people in the room required to use laser safety glasses during the experimental procedures.

2.3 Laser energy calculations

To calculate the range of laser energies to which we would expose the birds, we used the American National Standard for Safe Use of Lasers and the International Commission on Non-Ionizing Radiation Protection, which are based on experiments mostly in non-human primates. Based on these guidelines, the threshold of injury is the dose at which an individual has a 50% probability of having damage, also known as the ED50 (ANSI 2014). We wanted to estimate the

range of predicted ED50 for birds and use it as a guideline to empirically assess the actual injury threshold (observed ED50). Once the range of predicted ED50 was estimated, we used these values as median doses to expose birds to values 3 times below the lowest predicted ED50 and 3 times above the highest predicted ED50 . This would give us a wide enough range of values to explore the actual injury threshold (observed ED50). We first calculated the maximum permissible exposure (MPE), which is one tenth of the ED50 (ANSI 2014). Both the ED50 and the MPE can be expressed as a corneal irradiance, or the radiant energy per unit area in $\frac{mJ}{cm^2}$ (ANSI 2014). The MPE of continuous wave lasers (like our case) that are 400-700 nm for laser exposure times between 5 μs and 10 s can be calculated using the following equation:

$$MPE = 1.8 * t^{0.75}$$

Where t is laser exposure time in s (ANSI 2014). We chose laser exposure times (0.1, 0.25, 0.4, 0.55, 0.7, 0.85, 1.0 s) based on times found in the literature that were within the capabilities of our equipment (Leibu et al. 1999, Ham et al. 1970). The above equation is for the human pupil of 7 mm, so we corrected the MPE values for a 4 mm house sparrow pupil by multiplying the MPE by the ratio (approximately 3.06) of human pupil area (38.48 mm^2) to house sparrow pupil area (12.57 mm^2, measured on a dilated eye). We kept the same ratio (approximately 3.06) for European starling calculations because the size of the pupil was similar. Once corrected, we had a range of MPE values from 0.98 mJ/cm^2 to 5.51 mJ/cm^2 that we multiplied by 10 to get the predicted ED50's. As mentioned previously, we exposed each bird to the laser 3 times, so we divided our ED50 by 3. The final predicted ED50 values ranged from 3.27 to 18.37 mJ/cm^2, and represented the predicted threshold of laser eye injury for these species when exposed to a laser 3 times for 0.1 to 1s. We then estimated the corneal irradiances that approximately 3 times below the lowest

predicted ED50 and 3 times above the highest predicted ED50 to obtain our final irradiances to be used in the experiments. Our range of corneal irradiances were 1.09 to 55.11 mJ/cm^2. To apply the corneal irradiances using the laser we had to manipulate two factors: laser power and laser exposure time following this mathematical relationship which we modified from the ANSI guidelines:

$$\left(\frac{\text{Power } mW}{\text{Beam Area at Cornea } cm^2}\right) * \text{Time } s = \text{Corneal Irradiance} \frac{mJ}{cm^2}$$

where the beam area at cornea was 5.07 cm^2, based on the 2.54cm diameter of the shutter we fitted to the laser. We chose 7 laser powers (60, 90, 130, 165, 200, 235, 270 mW), on the lower end of our possible range of powers (0-1000 mW), to substitute into this equation that would give us final irradiances that were within the range we wanted. We assumed beam size did not change from aperture to the cornea due to a low beam divergence of 0.5mrad. This process gave us 49 different irradiances ranging from 1.18-53.3 mJ/ cm^2. We then converted them to total intraocular energies (TIEs) by multiplying the values by the area of a 4mm house sparrow pupil, which gave values in mJ that were comparable to those found in the literature.

2.4 Experiment 1: European starlings

Before exposure, starlings were given 30 μL of rocuronium bromide per eye and injected with a dose of 80 mg/kg ketamine and 9 mg/kg xylazine to anesthetize them. We randomly chose one eye to be exposed to the laser and one to be used as a control for each individual. We exposed starlings to eight energies (1.24, 1.86, 3.11, 4.66, 6.21, 9.32, 12.43, 18.64 mJ). We chose to use higher laser powers (500 and 750 mW) because they were on the higher end of our possible range (0-1000 mW) and we exposed house sparrows to lower power levels. The exposure times were

kept the similar, but we had a smaller number of starlings and chose to use only 4 different times (0.1, 0.25, 0.5, and 1s). We euthanized them seven days after laser exposure.

2.5 Experiment 2: House Sparrows

The house sparrows we used were part of a behavioral experiment to examine the effects of laser exposure on their foraging behavior (Blumenthal 2020). For the purposes of this study, house sparrows were administered 20 μL per eye of rocuronium bromide and an anesthesia dose of 8 mg/kg ketamine, 2mg/kg xylazine and 4mg/kg midazolam. House sparrows had both eyes exposed to the laser. We used 7 laser power levels (60, 90, 130, 165, 200, 235, 270 mW) and 7 laser exposure times (0.1, 0.25, 0.4, 0.55, 0.7, 0.85, 1.0 s) to expose them to 49 different energies from 0.15 to 6.71 mJ (0.15, 0.24, 0.32, 0.37, 0.41, 0.50, 0.58, 0.59, 0.60, 0.67, 0.81, 0.82, 0.94, 1.02, 1.04, 1.24, 1.27, 1.29, 1.30, 1.46, 1.49, 1.64, 1.65, 1.67, 1.77, 1.98, 2.00, 2.25, 2.26, 2.33, 2.36, 2.68, 2.73, 2.74, 2.86, 3.21, 3.22, 3.47, 3.48, 3.68, 4.08, 4.09, 4.22, 4.69, 4.95, 4.96, 5.69, 5.83, 6.70 mJ). House sparrows were euthanized eight days after laser exposure.

2.6 Histopathological analysis

We euthanized each bird with CO_2 following (Fernandez-Juricic et al. 2013, Tyrell et al. 2019). We removed each eye and fixed it in a solution of 10% neutral buffered formalin for at least 24 hrs. Afterwards, we sent them to the Comparative Ocular Pathology Lab, University of Wisconsin, Madison (https://www.vetmed.wisc.edu/pbs/dubielzig/pages/coplow/main.html). After reaching their destination, the eyes were sectioned in a parasagittal plane and underwent histologic processing for paraffin imbedding. Processing consisted of dehydration, clearing and filling with paraffin wax in a VIP 5 Sakura tissue processor and embedding in paraffin with a Leica Embedding Center. This was followed by 5-μm sectioning of paraffin blocks with a Leica microtome and

staining with a Leica X/Y Auto Stainer in which slides were heated, deparaffinized, hydrated to water, stained with hematoxylin and eosin (H&E), and dehydrated to xylene. The remainder of the paraffin blocks were conserved in the event that more sections were needed for sampling.

The H&E-stained slides were analyzed visually by a masked, board-certified veterinary pathologist (LT) using a bright field microscope (Olympus BX43, Melville, NY) and images were captured by a mounted digital camera (Olympus DP72, Melville, NY) and image analysis software (CellSence Dimension 1.4, Olympus, Melville, NY).

The sections were inspected for lesions in the ocular tissues, which were registered and graded in an excel spreadsheet. There is no single scoring method or grading scale that applies to all cases, so pathologists usually create a grading scale on a case-by-case basis (Treuting and Boyd 2018) However, there are several methods of creating a grading scale. We created a scoring system that corresponds to the ordinal method of histological scoring described in Gibson-Corley et al. (2013), in which the lesions are assigned to ranked categories based on the severity of the lesion. All lesions, with the exception of cataracts, were graded through a semi-quantitative histopathological scoring system that takes in account their degree of severity and distribution (Table 1). The lesions in the lens (cataracts) were graded by a different scoring system (also created using the ordinal method) which includes more specific criteria for each score, described at Table 2. We checked for 13 different injuries (corneal edema, corneal ulceration, stromal fibrosis, keratitis, conjunctivitis, cataract, uveitis, RPE atrophy, RPE hypertrophy, retinal atrophy, retinoschisis, retinal photoreceptor displaced nuclei, and scleral cartilage degeneration), and we recorded the presence of five different types of lesions (corneal edema, cataracts, retinal atrophy, retinal photoreceptor displaced nuclei, and scleral cartilage degeneration) (Appendix A). Corneal edema is characterized by the cornea swelling with fluid. The signs of corneal edema included a

broken endothelium layer which would allow fluid into the cornea and a noticeable swelling in any area of the cornea. We assigned the cornea's severity scores (1-mild to 4-severe) based on the size of the swelling and a distribution score (1-focal to 4-diffuse) based on how much of the cornea was affected (Table 1). Also, retinal atrophy, which is the degeneration of photoreceptor nuclei. Our samples were considered to have retinal atrophy if the outer nuclear layer (ONL) of the retina appeared to be nonuniform or if there was a loss of density of photoreceptor nuclei when compared to a healthy retina. Retinal atrophy was graded on severity (1-mild to 4-severe) based on the level of retinal thinning or loss of density of photoreceptor nuclei and distribution (1-focal to 4-diffuse) based on the amount of area of the ONL experienced atrophy (Table 1). We also observed a more specific category of retinal atrophy, retinal photoreceptor displaced nuclei, where photoreceptor nuclei are displaced from the outer nuclear layer into the photoreceptor layer. The photoreceptor nuclei appeared as purple spots which should be tightly packed in the ONL of the retina. However, a sample was considered to have retinal photoreceptor displaced nuclei if one of these purple spots appeared outside of the ONL, usually just below in the photoreceptor layer. This was also graded on severity (1-mild to 4-severe) and distribution (1-focal to 4-diffuse) based on how many photoreceptor nuclei were displaced and in how many areas this occurred (Table 1). We observed the presence of scleral cartilage degeneration. In our samples, the sclera appeared as a pink line surrounding the back of the eye, and the scleral cartilage appeared as a thick, purple line inside of the sclera. Scleral cartilage degeneration was identified by breaking, clumping, or thinning of this purple line. We gave severity (1-mild to 4-severe) and distribution (1-focal to 4-diffuse) scores based on the size of clumps or breaks in the cartilage and how many areas in which this occurred (Table 1). Finally, we recorded the presence of cataracts, which showed a number of signs including the degeneration of lens fibers, nucleated lens epithelial cells migrating inward and the

formation of small deposits of protein called morgagnian globules. Degenerated lens fibers appeared in our samples as white half circles around the edge of the lens which represented the curled edge of a lens fiber after an accumulation of fluid, whereas the normal edge of a lens fiber would appear as a straight white line. The nuclei of the lens epithelial cells appear as purple spots on two sides of the lens, but a lens with cataracts will have these spots towards the center. Morgagnian globules have a similar appearance to degenerated lens fibers and appear in the same areas. We were able to distinguish the two because morgagnian globules would usually form a complete circle. The cataract grading scale implemented all of these signs in addition to the distribution – whether the signs appeared all around the lens or only in single spots – to assign scores from 1 to 4 (Table 2).

Table 1. The grading scale (score 0-8) for corneal edema, retinal atrophy, retinal photoreceptor displaced nuclei, and scleral cartilage degeneration. We substituted the descriptive terms for the corresponding numerical score and added both numerical scores for a final score**.** Ex: Mild/diffuse = ¼ = final score 5.

Degree of severity	Numerical score	Degree of distribution	Numerical score
None	0	None	0
Mild	1	Focal- Damage occurs in 1 small area	1
Moderate	2	Multifocal- Damage occurs in multiple small areas	2
Marked	3	Focally extensive- Damage occurs across a wide area	3
Severe	4	Diffuse- Damage is found throughout the entire media	4

Table 2**.** The cataract grading scale (score 0-4).

Grade 0	No lesions
Grade 1	Focal distribution, internal migration of nucleated lens epithelial cells (IMNLEC)
Grade 2	Multifocal distribution (equatorial and anterior lens capsule), IMNLEC, formation of bladder cells
Grade 3	Focally extensive distribution (anterior, equatorial and posterior lens capsule), IMNLEC, formation of bladder cells and morgagnian globules
Grade 4	Focally extensive to circumferential distribution, IMNLEC, formation of bladder cells and morgagnian globules, lens fibers vacuolization, liquefaction and mineralization.

Each slide contained 2-4 cross-sections of one eye of one individual. We chose which cross-section to grade for the cornea and sclera based on which was the most intact and had the least amount of artifact, damage or deformity from processing. An intact cornea would appear as a slightly rounded strip on the front end of the eye and the sclera, a thick pink layer around the back of the eye containing a thick purple layer which was the scleral cartilage. We sampled both the cornea and the sclera by scanning from one end to the other searching for signs of damage.. To sample the retina, we first chose a section from the slide that contained the optic nerve, which appeared as a large pink bubble in the center of the retina, and the least amount of artifact. We first looked on either side of the optic nerve and scanned to the outer ends of the retina searching for signs of retinal atrophy and retinal photoreceptor displaced nuclei. The cataract appeared as a large, pink, ovular body in the front of the eye, behind the cornea, and we chose which section to grade based on which had the least amount of artifact. Most of the signs of cataract could be found in the cortex, the outer edge of the lens which contains the lens fibers (National Toxicology Program 2014). We scanned around the cortex for degenerated lens fibers and morgagnian globules, but also scanned inward toward the center of the lens for nucleated epithelial cells. If other sections on the same slide contained little to no artifact, we also checked these to confirm the injuries.

We assessed if the qualitative lesion grading would be supported by a quantitative image analysis using Image J software. Using slides containing sections of house sparrow eyes, we took photos using a Carl Zeiss AxioCam attached to a Carl Zeiss Axio Scope.A1 and Zen Blue 2.3 pro software. We randomly chose 10 retinas of different individuals with injury and took 6 samples of the outer nuclear layer (ONL) of each, 3 damaged and 3 non-damaged sections (Appendix B). The size of the samples were different for each retina depending on the width of the ONL and the length of the retina that was not misshapen or damaged due to histologic processing. Signs of retinal

atrophy include disorganization and decrease in density of photoreceptor nuclei, both of which leave empty "white" space in the ONL (Al Mouiee et al. 2021). We used the adjust threshold function in Image J to measure the area (pixels) of the sample that corresponded to retinal atrophy. The adjust threshold function assigns values to colors ranging from 0 (black) to 255 (white), therefore it was necessary to convert each photo to 8-bit black and white to use this function. We were able to choose a range of values that represented the damaged areas and measure them. We chose the values based on a histogram shown by the adjust threshold window, which shows the amount of pixels that correspond to each value. For our samples, there was always a peak or several peaks in the center and a decline in the histogram towards the 255 (white) value. We chose the range of values to measure by highlighting all values after the last peak in the histogram which represented the whiter areas. We divided the area of highlighted pixels by the total area of the sample which gave us a percentage of damage. Similar steps were taken with photos of the lens. We randomly chose 10 lenses of different individuals with injury and took 8 rectangular samples of the cortex of each, 4 damaged and 4 non-damaged sections (Appendix B). Cataracts appear in our sections as whitish circles or half circles indicating the curled edge of a degenerated lens fiber, alongside normal fibers which will be straight whitish lines. We used the multipoint tool in Image J to count each shape. We recorded the number of circles and half circles per section, the number of normal fibers, and calculated the percentage of damage per sample.

We analyzed this quantitative analysis by running a general linear mixed model in R using package afex and measured calculated mean probabilities using the emmeans package. We wanted to find out if the samples of the retina that were damaged had a higher proportion of damage in pixels and if the samples of the lens that were damaged had a higher percentage of curled lens fibers than non-damaged samples. We found that images of damaged sections of the retina's

photoreceptor layer had a significantly higher proportion of white pixels than non-damaged sections (F 1, 49 = 23.7, P < 0.001) (Fig. 2). The effect size (h = 0.105) was less than 0.2 which is considered small. Images of damaged sections of the lens had a significantly higher proportion of curled lens fibers than images of non-damaged sections (F 1, 69 = 250, P < 0.001) (Fig. 2). Damaged images had an effect size (h = 0.933) larger than 0.8 which is large.

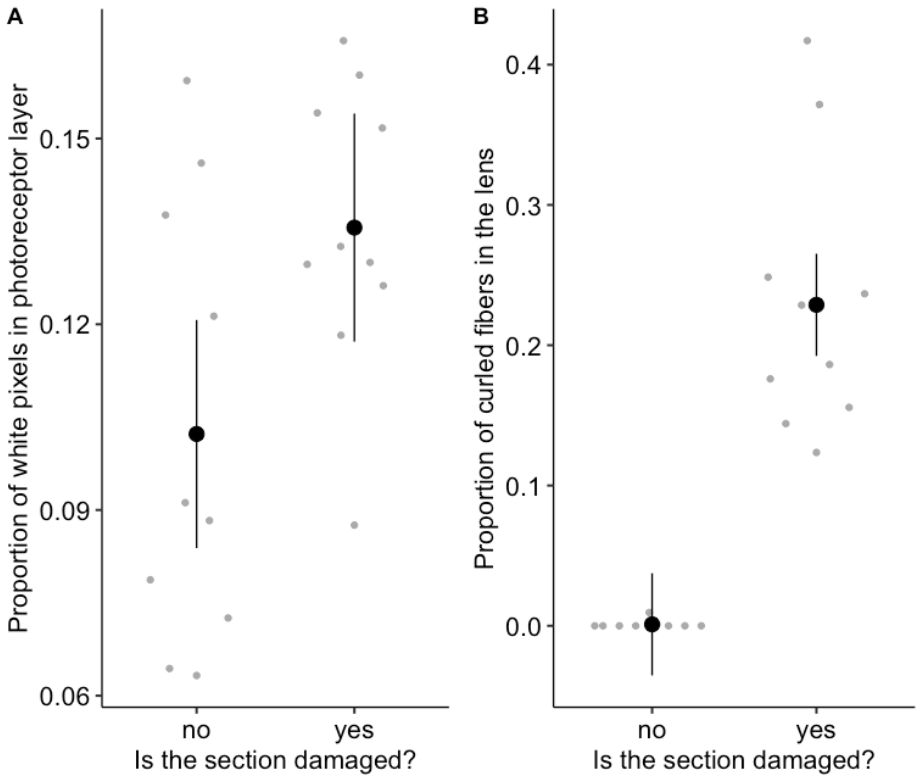


Figure 2. The relationship between a qualitative damage score on (a) the proportion of white pixels in the photoreceptor layer of the retina and (b) the proportion of curled fibers in the lens. Results from a general linear mixed model.

2.7 Statistical analyses

For the European starlings, we did 3 separate analyses: laser exposure effects, laser energy effects and a nested dichotomy analysis. The laser exposure effects analysis answered the question of whether birds that were exposed to the laser had a higher probability of damage than control birds.

In the laser energy effects analysis, we tested the effects of laser power and laser exposure time on the probability of damage. We used a binary, presence-absence dataset for both of these analyses and 5 dependent variables: corneal edema, cataracts, retinal atrophy, retinal photoreceptor displaced nuclei, and scleral cartilage degeneration. We also analyzed the effects of energy (laser power and laser exposure time) on the level of eye injury. We could have done this with a multinomial analysis, but the results were difficult to interpret due to some dependent variables having up to 7 different possible values. These values were based on the scoring system described in Table 1 and Table 2. We opted to use a nested dichotomy analysis which separates multilevel data into several binary analyses (Appendix C). The nested dichotomy analysis consists of a tree that splits a multilevel analysis into smaller and smaller sets of binary analyses (Frank and Kramer 2004). Figure 3 shows an example of a nested dichotomy for one of our damage categories. The first dichotomy is a binary analysis comparing scores of 0 to all other scores present for that category. We already completed this analysis previously in our first laser energy effects analysis using our binary dataset, therefore the resulting statistics for these two analyses will be exactly the same. The second binary analysis compares scores of 4 and 5 to scores of 6. Recall from Table 1 that each final score is a combination of a severity score and a distribution score. In this case, all scores of 5 and 6 shared the same distribution score and differed only in severity. There was only one final score of 4 observed in this damage category which would cause problems for the software during data analysis. We decided to group it with scores of 5 because both final scores 4 and 5 had a severity score of 1, unlike final scores of 6 which all had severity scores of 2. We labeled the first binary analysis of a tree dichotomy 1, the second dichotomy 2, and so on.

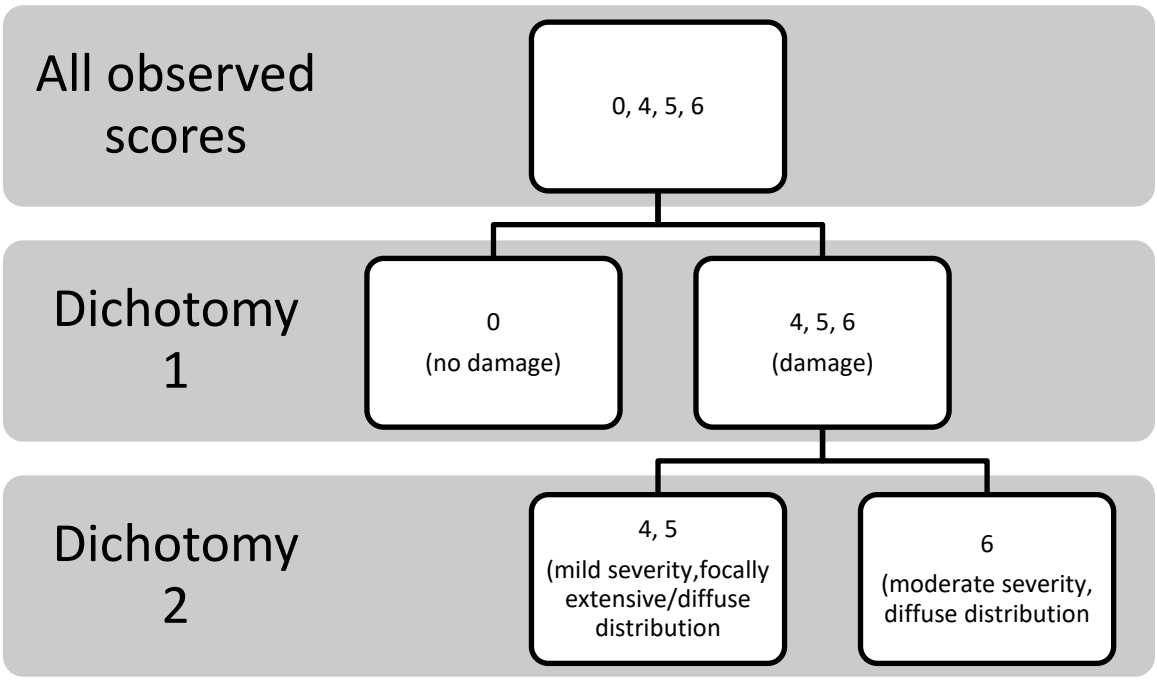


Figure 3**.** The nested dichotomies for corneal edema in house sparrows.

For house sparrows we also did laser exposure effects, laser energy effects, and nested dichotomy analyses. However there were some differences in these analyses from the way they were completed with European starlings. There were only 4 dependent variables tested for house sparrows: corneal edema, cataracts, retinal atrophy, and scleral cartilage degeneration. During the laser energy effects analysis, we tested the effects of laser energy, but not the individual effects of laser power and laser exposure time. We made this decision based on the difference in the experimental designs. The house sparrows were exposed to many different laser powers and laser exposure times, while the European starlings were only exposed to 4 different laser exposure times and 2 different laser powers. Both of these analyses used binary, presence-absence data. We were able to create an extra nested dichotomy structure with different binary analyses based on the distribution score for retinal atrophy in the house sparrows. We were unable to do this with other damage categories because the majority of final scores had the same distribution scores. As

previously mentioned, the house sparrows took part in a separate behavioral study also. From this we were able to get data on the body mass of the house sparrows over the course of 10 or more days and test the effects of laser exposure, sex, and age on house sparrow body mass. We compared the body mass from before laser exposure to after laser exposure and tested the interaction effects between exposure, sex and age.

For the laser exposure effects analysis in both bird species, we used R to run generalized linear mixed models with package afex. We used the same package to run generalized linear models for the laser energy effects and nested dichotomy analyses in both birds. Afex was also used to run general linear mixed models for the quantitative analysis of qualitative criteria. We estimated all mean probabilities using the emmeans package. From these means we were able to estimate Cohen's h, a measure of effect size for proportions, for most analyses using the package pwr. We used a general linear mixed model in afex to analyze the body mass in house sparrows. We calculated Cohen's d, a measure of effect size for means, with package effectsize. We also ran a pairwise comparison to analyze the interaction between age and time of recording of body mass in relation to laser exposure.

CHAPTER 3. RESULTS

3.1 Experiment 1: European Starlings

We found that laser exposed eyes had a significantly higher probability of experiencing the following five injuries than control eyes (Fig. 4): corneal edema (χ^2 1 = 15.2, P < 0.001), cataracts ($\chi^2$1 = 17.6, P < 0.001), retinal atrophy ($\chi^2$1 = 27.9, P < 0.001), retinal photoreceptor displaced nuclei ($\chi^2$1 = 12.7, P < 0.001) and scleral cartilage degeneration ($\chi^2$1 = 35.8, P < 0.001). All effect sizes (Cohen's h) were considered large (>0.8) with the following decreasing order of relevance: scleral cartilage degeneration (h = 1.889), retinal atrophy (h = 1.823), cataracts (h = 1.252), retinal photoreceptor displaced nuclei (h = 1.230), and corneal edema (h = 1.210).

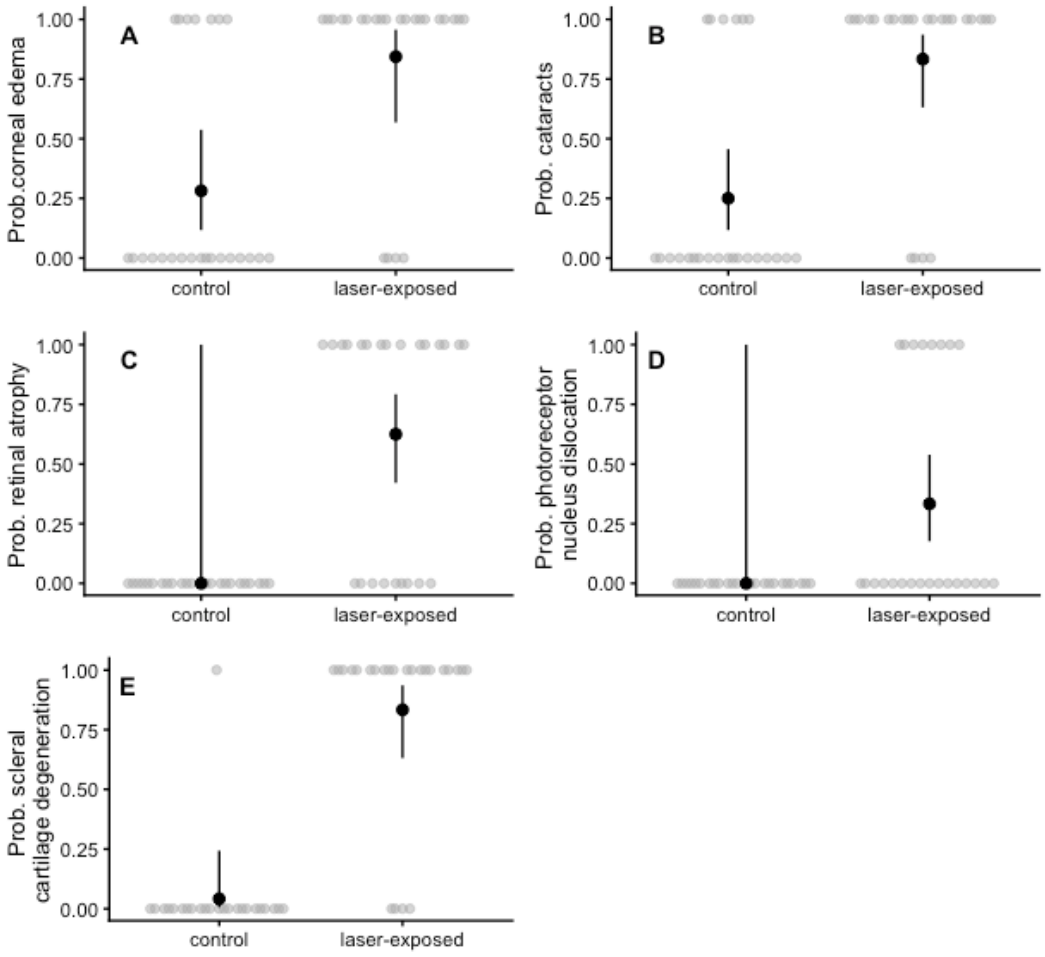


Figure 4**.** The effects of laser treatment and control treatment on the probability of (a) corneal edema, (b) cataracts, (c) retinal atrophy, (d) retinal photoreceptor displaces nuclei, and (e) scleral cartilage degeneration in European starlings. Results from a general linear mixed model.

We analyzed the effects of laser energy, a combination of laser power and laser exposure time, and found that laser exposure time had a significant effect on corneal edema and retinal atrophy (Table 3). Figure 5 shows the mean probabilities of corneal edema and retinal atrophy for each laser exposure time. Laser exposure time was not significant for cataracts, retinal photoreceptor displaced nuclei, or scleral cartilage degeneration (Table 3). Laser power was not significant for any of the five injuries: cornea edema, cataracts, retinal atrophy, retinal photoreceptor displaced nuclei, and scleral cartilage degeneration (Table 3). Table 4 lists Cohen's

h effect size for the energy effects analyses. Effect size was generally the highest between higher laser exposure times except for corneal edema in which the highest effect size was for the lowest times. In the nested dichotomy analysis, laser exposure time was significant for edema dichotomy 1, indicating that laser exposure time had a significant effect on the presence of corneal edema (Fig. 6). Cataract dichotomy 2 compared damage scores of 1 (focal distribution and one sign of cataract) to higher scores of 2 and 3 (wider distributions and 2 or more signs of cataract) (Table 2) and was also significant (Fig. 6), which could mean that increasing energy is associated with more widely distributed and severe cases of cataracts. Laser exposure time was not significant for any other dichotomies (Table 3). Laser power was not significant for any dichotomies: edema dichotomy 1, edema dichotomy 2, cataract dichotomy 1, cataract dichotomy 2, cataract dichotomy 3 sclera dichotomy 1 or sclera dichotomy 2 (Table 3). Both dichotomies for corneal edema showed a negative trend in effect size as laser exposure time increased, and cataract dichotomy 2 showed a positive trend in effect size as laser exposure time increased (Table 4). Cataract dichotomy 1, cataract dichotomy 2, and sclera dichotomy 2 had large effect sizes for 0.5 vs. 1 s (Table 4).

Table 3**.** Deviances, p-values and pseudo R^2 for the European starling energy effects analysis using a generalized linear model. An asterisk in the p-value column represents a significant effect.

	Pseudo R2	***Independent Factor***	***Deviance***	***Df***	***Residual Deviance***	***Residual Df***	***P-value***
Corneal Edema	0.4487147	Power	1.2468	1	20.38	22	0.26416
		Time	8.4575	3	11.923	19	0.03744*
Edema Dichotomy 1	0.4487147	Power	1.2468	1	20.38	22	0.26416
		Time	8.4575	3	11.923	19	0.03744*
Edema Dichotomy 2	0.0567783	Power	0.20238	1	27.523	18	0.6528
		Time	1.37185	3	26.152	15	0.7121
Cataract	0.1468215	Power	0	1	21.627	22	1
		Time	3.1753	3	18.452	19	0.3654
Cataract Dichotomy 1	0.1468215	Power	0	1	21.627	22	1
		Time	3.1753	3	18.452	19	0.3654
Cataract Dichotomy 2	0.3906867	Power	0.8402	1	26.08	18	0.35933
		Time	9.6772	3	16.403	15	0.02152*
Cataract Dichotomy 3	0.531419	Power	1.1947	1	15.1059	10	0.27437
		Time	7.4677	3	7.6382	7	0.05839
Retinal Atrophy	0.2645764	Power	0.1781	1	31.577	22	0.67305
		Time	8.2236	3	23.353	19	0.04161*
Retinal photoreceptor displaced nuclei	0.1767654	Power	0.756	1	29.797	22	0.3846
		Time	4.6447	3	25.152	19	0.1997
Sclera	0.2108713	Power	1.2468	1	20.38	22	0.2642
		Time	3.3137	3	17.066	19	0.3457
Sclera Dichotomy 1	0.2108713	Power	1.2468	1	20.38	22	0.2642
		Time	3.3137	3	17.066	19	0.3457
Sclera Dichotomy 2	0.2269304	Power	2.2276	1	24.693	18	0.1356
		Time	3.8815	3	20.811	15	0.2745

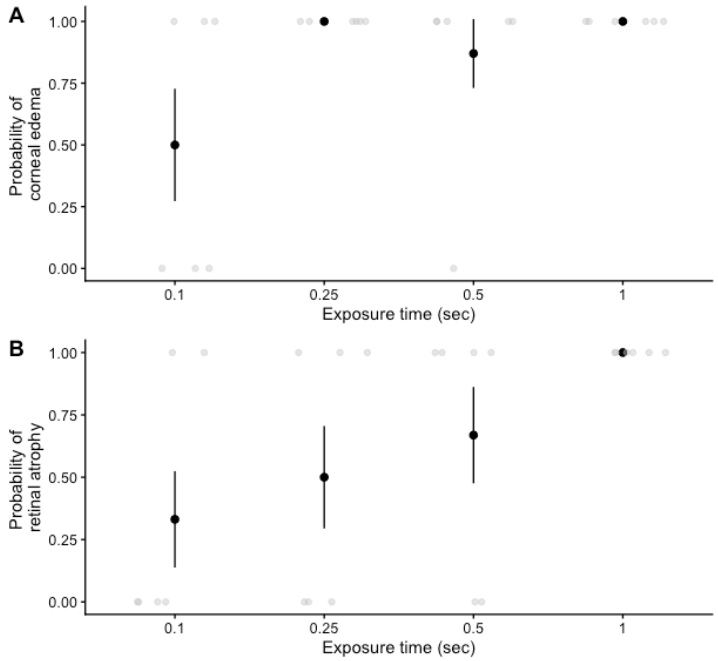


Figure 5**.** The effects of laser exposure time on (a) the probability of corneal edema and (b) the probability of retinal atrophy. Results from a general linear mixed model.

Table 4**.** Cohen's h effect size for the European starling energy effects analysis. Cohen's h is small when h = 0.2, medium when h = 0.5, and large when h = 0.8.

	Power (mW)		***Time (s)***	
	500 vs 750	*0.1 vs 0.25*	*0.25 vs 0.5*	*0.5 vs 1*
Corneal edema	0	1.570796	0.7376085	0.7376085
Edema Dichotomy 1	0	1.570796	0.7376085	0.7376085
Edema Dichotomy 2	0.2795871	0.7367132	0.5789946	0.2320777
Cataract	0	0.3898891	0	0.8410696
Cataract Dichotomy 1	0	0.3898891	0	0.8410696
Cataract Dichotomy 2	0.1121223	0.6344865	0.7937934	1.411489
Cataract Dichotomy 3	1.594799	0	3.141593	0
Retinal atrophy	0.05200174	0.3444277	0.3444277	1.226369
Retinal photoreceptor displaced nuclei	0.4352892	0.4011584	0	1.116301
Sclera	0.07828337	0.4099648	0	0.7792853
Sclera Dichotomy 1	0.07828337	0.4099648	0	0.7792853
Sclera Dichotomy 2	0.8977093	0.4319271	0.3188421	1.096676

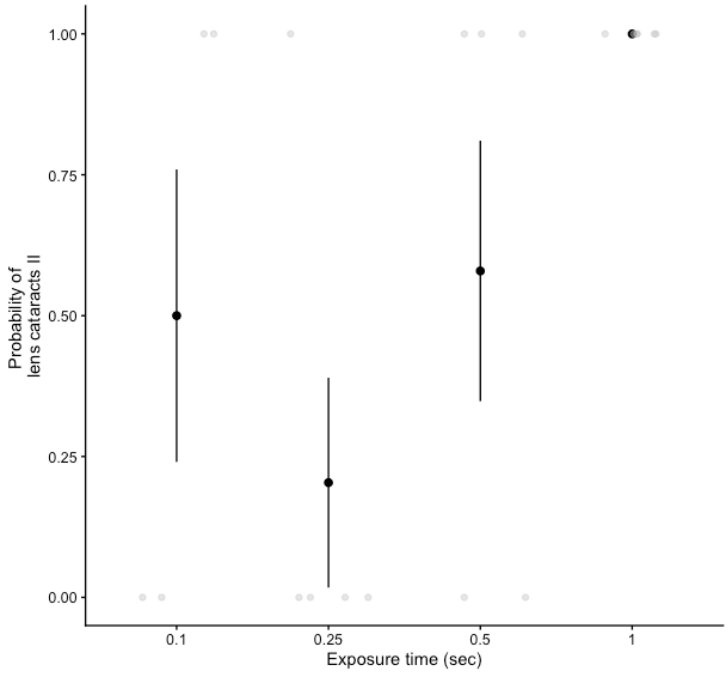


Figure 6**.** The effect of laser exposure time on the probability of cataracts in cataract dichotomy 2 in starlings. Results from a generalized linear mixed model.

3.2 Experiment 2: House Sparrows

Laser-exposed birds had a significantly higher probability of experiencing four injuries (Fig. 7): corneal edema (χ^2 1 = 18.5, P < 0.001), cataracts (χ^2 1 = 39.5, P < 0.001), retinal atrophy (χ^2 1 = 38.1, P < 0.001), and scleral cartilage degeneration (χ^2 1 = 20.0, P < 0.001). Effect sizes for all injuries were considered large (>0.8) and are listed in decreasing order: retinal atrophy (h = 3.119), cataracts (h = 3.117), scleral cartilage degeneration (h = 3.06), and corneal edema (h = 3.03). There was not a significant difference between adults and juveniles for any of the four injuries: corneal edema (χ^2 1 = 0.155, P = 0.694), cataracts (χ^2 1 = 0.035, P = 0.852), retinal atrophy (χ^2 1 = 0.387, P = 0.534), and scleral cartilage degeneration (χ^2 1 = 0.030, P = 0.862). Sex did not have a significant effect on the probabilities of any injuries: corneal edema (χ^2 1 = 0.004, P = 0.949),

cataracts (χ^2 1 = 0.013, P = 0.910), retinal atrophy (χ^2 1 = 0.022, P = 0.883), and scleral cartilage degeneration (χ^2 1 = 0.020, P = 0.888). We also did not find a significant difference between left and right eyes of the birds for any injuries: corneal edema (χ^2 1 = 0.168, P = 0.682), cataracts (χ^2 1 = 0.278, P = 0.598), retinal atrophy (χ^2 1 = 2.18, P = 0.140), and scleral cartilage degeneration (χ^2 1 = 0.00, P = 1.00). The effect sizes for age, sex, and eye were all less than 0.2 which is considered small (Table 6).

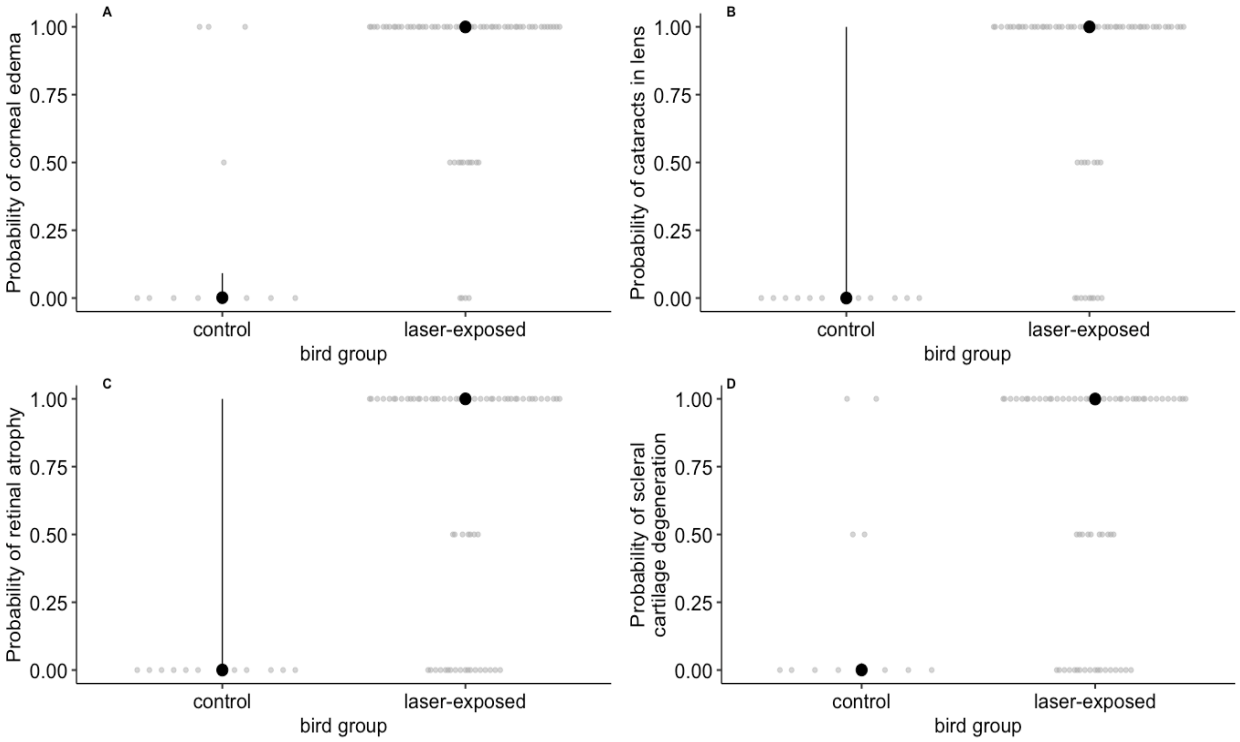


Figure 7. The effect of laser treatment and control treatment on the probability of (a) corneal edema, (b) cataracts, (c) retinal atrophy, and (d) scleral cartilage degeneration in house sparrows. Results from a general linear mixed model.

We found that energy did not have a significant effect on the probability of injury: corneal edema, cataracts, retinal atrophy, and scleral cartilage degeneration (Table 5). Despite these non-significant results, we observed some trends: positive trends between energy and the probabilities

of both corneal edema and scleral cartilage degeneration, no trend between energy and the probability of cataracts, and a negative trend between energy and the probability of retinal atrophy (Fig. 8). Age was not significant for any injuries: corneal edema, cataracts, retinal atrophy, and scleral cartilage degeneration (Table 5). Sex also did not significantly affect the probability of injury: corneal edema, cataracts, retinal atrophy, and scleral cartilage degeneration (Table 5). The effect sizes for age and sex were small (Table 6).

Table 5**.** Chi-square (X^2) and p-values for the house sparrow energy effects analysis using a generalized linear mixed model. An asterisk in the p-value column represents a significant effect.

	X2	***Independent Factor***	***Df***	***P-value***
Corneal Edema	0.0998	Energy	1	0.7520
	0.4023	Age	1	0.5259
	0.3665	Sex	1	0.5449
Edema Severity Dichotomy 1	0.0998	Energy	1	0.7520
	0.4023	Age	1	0.5259
	0.3665	Sex	1	0.5449
Edema Severity Dichotomy 2	0.0517	Energy	1	0.8200
	0.5373	Age	1	0.4636
Edema Distribution Dichotomy 1	0.0998	Energy	1	0.7520
	0.4023	Age	1	0.5259
	0.3665	Sex	1	0.5449
Cataract	0.0018	Energy	1	0.9662
	0.0366	Age	1	0.8483
	0.0129	Sex	1	0.9097
Cataract Dichotomy 1	0.0018	Energy	1	0.9662
	0.0366	Age	1	0.8483
	0.0129	Sex	1	0.9097
Cataract Dichotomy 2	5.3941	Energy	1	0.02021*
	0.1688	Age	1	0.68120
	4.0687	Sex	1	0.04369*
Cataract Dichotomy 3	2.2777	Energy	1	0.13124
	0.0480	Age	1	0.82653
	3.1098	Sex	1	0.07782
Retinal Atrophy	0.3506	Energy	1	0.5538
	0.5007	Age	1	0.4792
	0.0200	Sex	1	0.8874
Atrophy Severity Dichotomy 1	0.3506	Energy	1	0.5538
	0.5007	Age	1	0.4792
	0.0200	Sex	1	0.8874
Atrophy Severity Dichotomy 2	1.1670	Energy	1	0.280028
	10.3704	Age	1	0.001281*
	1.1406	Sex	1	0.285529
Atrophy Severity Dichotomy 3	8.7613	Energy	1	0.003077*
	0.0019	Age	1	0.965216

	0.3880	Sex	1	0.533355

Table 5 continued

Atrophy Distribution Dichotomy 1	*0.3506*	*Energy*	*1*	*0.5538*
	0.5007	Age	1	0.4792
	0.0200	Sex	1	0.8874
Atrophy Distribution Dichotomy 2	4.5571	Energy	1	0.03278*
	2.1945	Age	1	0.13851
	1.1217	Sex	1	0.28955
Atrophy Distribution Dichotomy 3	3.3081	Energy	1	0.068939
	8.4871	Age	1	0.003577*
	1.1626	Sex	1	0.280937
Sclera	0.2511	Energy	1	0.6163
	0.1307	Age	1	0.7177
	0.0324	Sex	1	0.8571
Sclera Severity Dichotomy 1	0.2511	Energy	1	0.6163
	0.1307	Age	1	0.7177
	0.0324	Sex	1	0.8571
Sclera Severity Dichotomy 2	3.9836	Energy	1	0.04595*
	0.0246	Age	1	0.87542
	0.5873	Sex	1	0.44345
Sclera Severity Dichotomy 3	0.0170	Energy	1	0.8962
	0.1172	Age	1	0.7321
	0.0892	Sex	1	0.7652
Sclera Distribution Dichotomy 1	0.2511	Energy	1	0.6163
	0.1307	Age	1	0.7177
	0.0324	Sex	1	0.8571

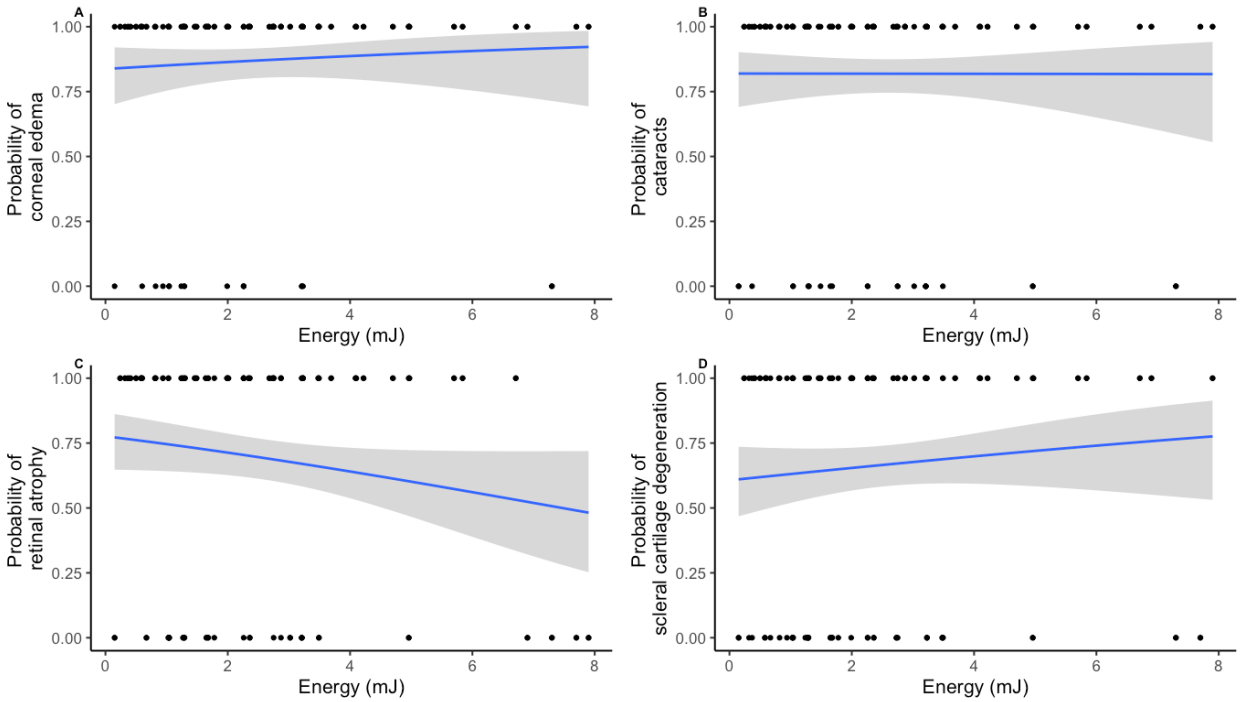


Figure 8**.** The effects of laser energy on the probability of developing (a) corneal edema, (b) cataracts, (c) retinal atrophy, and (d) scleral cartilage degeneration in house sparrows. Results from a generalized linear mixed model.

Table 6. Cohen's h effect size for the house sparrow analysis. Cohen's h is small when h = 0.2, medium when h = 0.5, and large when h = 0.8.

	Eye	***Age (Adult vs. Juvenile)***		***Sex (Male vs. Female***	
	L vs R	*Treatment Effects*	*Energy Effects*	*Treatment Effects*	*Energy Effects*
Corneal edema	0.1644417	0.2934907	0.03012553	0.04634945	0.02740157
Edema Sev. Dichotomy 1			0.03012553		0.02740157
Edema Sev. Dichotomy 2			0.1410035		0.03826096
Edema Distr. Dichotomy 1			0.03012553		0.02740157
Cataract	0.01798755	0.01135345	0.004764968	0.006878372	0.008284881
Cataract Dichotomy 1			0.004764968		0.008284881
Cataract Dichotomy 2			0.09720008		0.4654105
Cataract Dichotomy 3			0.05390462		0.4817442
Retinal atrophy	0.03872739	0.02900225	0.02049929	0.006728759	0.006357534
Atrophy Sev. Dichotomy 1			0.02049929		0.006357534
Atrophy Sev. Dichotomy 2			0.8259852		0.2493993
Atrophy Sev. Dichotomy 3			0.01445544		0.1984761
Atrophy Distr. Dichotomy 1			0.02049929		0.006357534
Atrophy Distr. Dichotomy 2			0.4283594		0.2995074
Atrophy Distr. Dichotomy 3			0.9684351		0.3329913
Sclera	0	0.1276344	0.011851	0.101286	0.008196114
Sclera Sev. Dichotomy 1			0.011851		0.008196114
Sclera Sev. Dichotomy 2			0.0384315		0.1761538
Sclera Sev. Dichotomy 3			0.09762347		0.08386665
Sclera Distr. Dichotomy 1			0.011851		0.008196114

Similarly to the European starling analysis, we did a nested dichotomy analysis. We created two separate dichotomous structures for the retinal atrophy, one with categories formed based on the severity score and one based on the distribution score for the injury. We also tried to develop two separate dichotomies for corneal edema and scleral cartilage degeneration, however, we were unable to make dichotomies based on the distribution score beyond dichotomy 1 for corneal edema and scleral cartilage degeneration because the majority of cases of these two injuries had the same distribution score and only differed in severity. Cataract had a different grading scale that was not based on severity and distribution (Table 2), and the dichotomies were created by separating the lowest score. However the first dichotomy will always have the same raw data values as the binary injury data tested previously, and thus will have the same p-values, effect sizes etc. This is true for all injuries and the dichotomies based on severity and distribution.

Energy was significant for cataract dichotomy 2, atrophy severity dichotomy 3, atrophy distribution dichotomy 2, and sclera severity dichotomy 2 (Table 5) and the linear relationships are shown in Figure 9. Cataract dichotomy 2 compared those injuries with a focal distribution and one sign of cataracts to those injuries that were more widely distributed and shows two or more signs of cataract, which means that increasing energy could be associated with a widely distributed and more severe cataract. Atrophy severity dichotomy 3 compared retinal atrophy scores with mild severity and focal distribution to those with mild severity and focally extensive distribution, and atrophy distribution dichotomy 2 compared injuries with a focal distribution to injuries with a focally extensive distribution. Both of those results could imply that increasing energy is associated with more widely distributed retinal atrophy. Sclera severity dichotomy 2 compares injuries with mild severity to more severe cases, indicating that higher energy is associated with more severe scleral cartilage degeneration. Energy was not significant in any other dichotomies:

edema severity dichotomy 1, edema severity dichotomy 2, edema distribution dichotomy 1, cataract dichotomy 1, cataract dichotomy 3, atrophy severity dichotomy 1, atrophy severity dichotomy 2, atrophy distribution dichotomy 1, atrophy distribution dichotomy 3, sclera severity dichotomy 1, sclera severity dichotomy 3, and sclera distribution dichotomy 1 (Table 5).

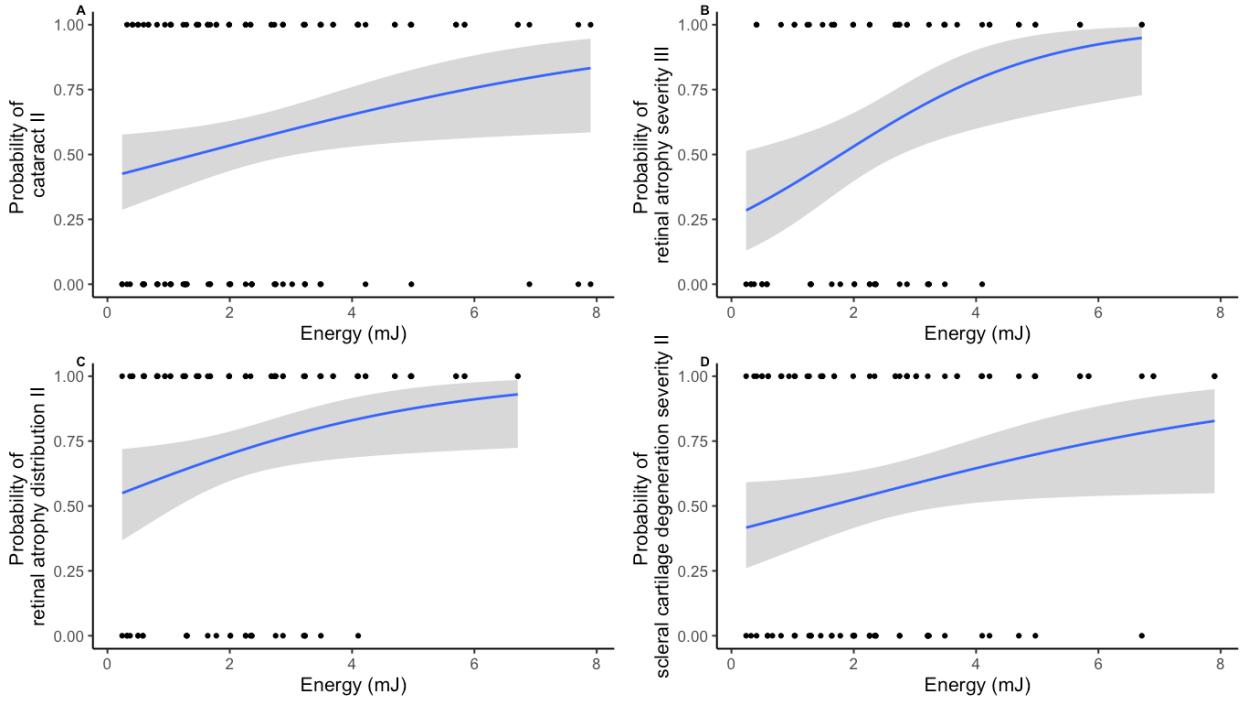


Figure 9**.** The effects of laser energy on the probability of damage in (a) cataract dichotomy 2, (b) retinal atrophy severity dichotomy 3, (c) retinal atrophy distribution dichotomy 2, and (d) scleral cartilage dichotomy 2 in house sparrows. Results from a generalized linear model.

Age was significant for atrophy severity dichotomy 2 and atrophy distribution dichotomy 3 (Table 5), and both had large effect sizes greater than 0.8 (Table 6). This indicates that juvenile house sparrows suffered more severe cases of retinal atrophy. Sex was only significant for cataract dichotomy 2 (Table 5) and had a medium effect size close to 0.5 (Table 6), indicating that females suffered more widely distributed and severe cataracts. Age was not significant for other dichotomies: edema severity dichotomy 1, edema severity dichotomy 2, edema distribution

dichotomy 1, cataract dichotomy 1, cataract dichotomy 2, cataract dichotomy 3, atrophy severity dichotomy 1, atrophy severity dichotomy 3, atrophy distribution dichotomy 1, atrophy distribution dichotomy 2, sclera severity dichotomy 1, sclera severity dichotomy 2, sclera severity dichotomy 3, and sclera distribution dichotomy 1(Table 5). Sex was not significant for edema severity dichotomy 1, edema severity dichotomy 2, edema distribution dichotomy 1, cataract dichotomy 1, cataract dichotomy 3, atrophy severity dichotomy 1, atrophy severity dichotomy 2, atrophy severity dichotomy 3, atrophy distribution dichotomy 1, atrophy distribution dichotomy 2, atrophy distribution dichotomy 3, sclera severity dichotomy 1, sclera severity dichotomy 2, sclera severity dichotomy 3, and sclera distribution dichotomy 1 (Table 5).

We tested the effects of laser exposure on house sparrow body mass. We found that laser-exposed birds were more likely to have a higher body mass before laser exposure than after ($F_{1, 80} = 16.3$, $P < 0.001$) (Figure 10). There was not a significant difference in the body mass of control birds before and after treatment ($F_{1, 11} = 1.25$, $P = 0.289$). Laser-exposed males had a significantly higher body mass than laser-exposed female house sparrows ($F_{1, 71} = 5.48$, $P = 0.022$) (Figure 10), but the difference between male and female control birds was not significant ($F_{1, 12} = 3.84$, $P = 0.074$). Age did not have a significant effect on the body mass of laser-exposed birds ($F_{1, 87} = 2.97$, $P = 0.088$) or control birds ($F_{1, 12} = 0.515$, $P = 0.487$). The effect sizes (Cohen's d) were small for age (0.32), sex (0.44) and time of recording of body mass in relation to laser exposure (0.22). There was a significant interaction effect between age and time of recording of body mass of laser-exposed birds ($F_{1, 80} = 7.95$, $P = 0.006$), in which juveniles had a higher body mass before and after laser exposure than adult house sparrows (Figure 11). The interaction was not significant for control birds ($F_{1, 12} = 3.62$, $P = 0.081$). The interaction between

sex and the time of the recording of body mass was not significant for laser-exposed birds (F 1, 65 = 0.055, P = 0.815) or control birds (F 1, 12 = 1.02, P 0.331).

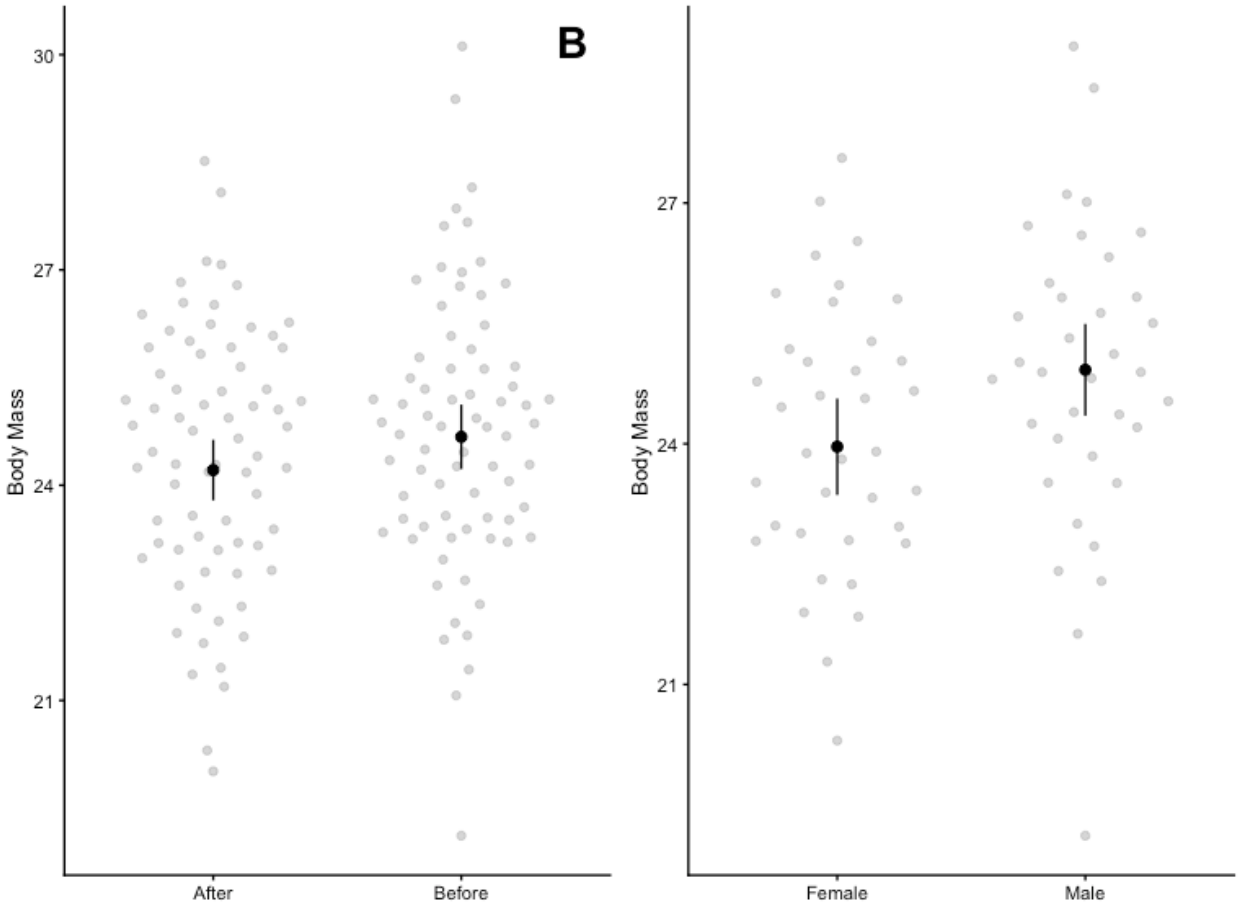


Figure 10**.** The effects of (a) age and (b) sex on house sparrows body mass. Results from a general linear mixed model.

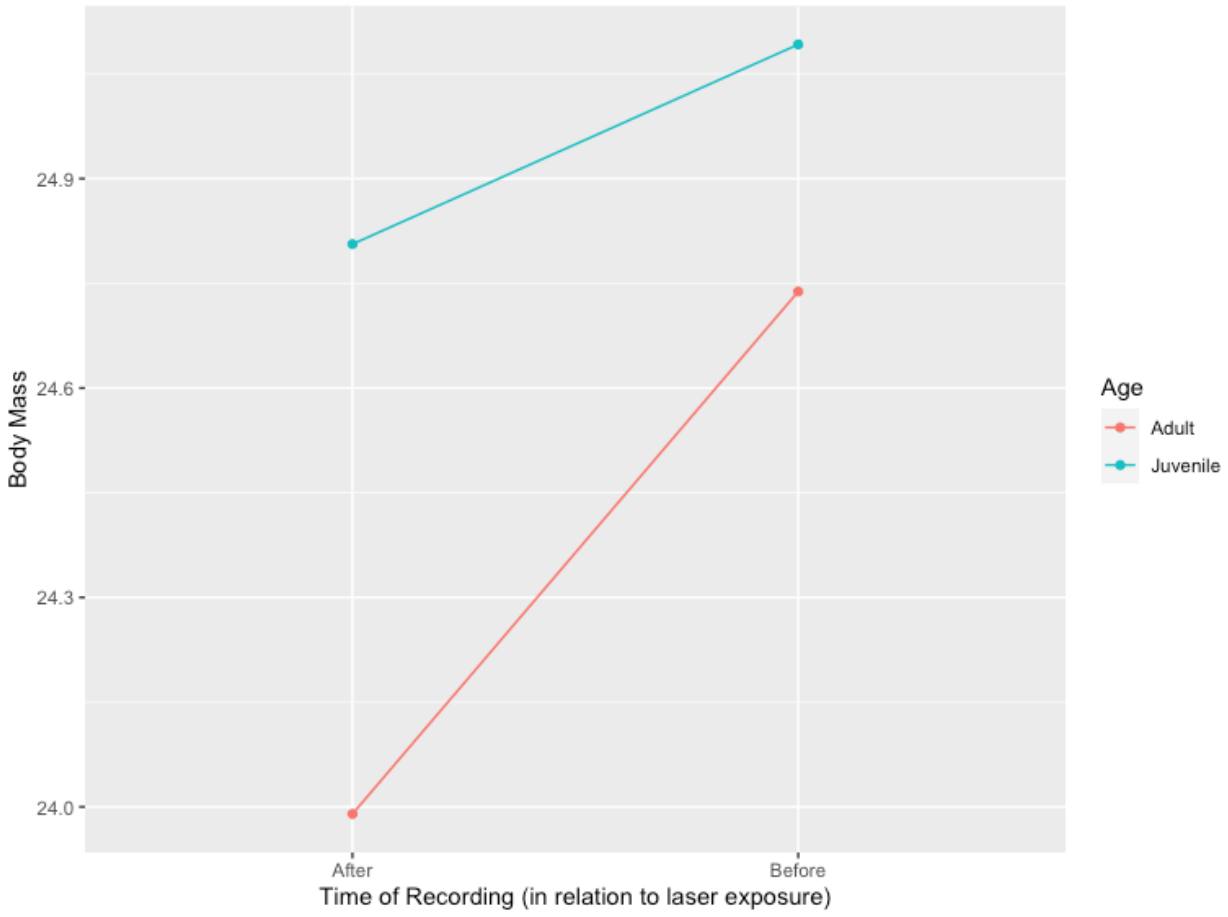


Figure 11**.** The interaction effect between age and time of recording of body mass on house sparrow body mass. Results from a general linear mixed model.

CHAPTER 4. DISCUSSION

In European starlings, we found a total of 5 different types of injury including corneal edema, cataracts, retinal atrophy, retinal photoreceptor displaced nuclei, and degeneration of the scleral cartilage. House sparrows experienced four injuries: corneal edema, cataracts, retinal atrophy, and scleral cartilage degeneration. For all injuries in both species, there was a significant difference between the probability of damage observed in control birds versus laser-exposed birds with the laser exposed treatment having a higher probability of damage. From this we can conclude that there is evidence of injury to the bird eyes as a result of laser exposure. Corneal edema, cataracts, retinal atrophy, and retinal photoreceptor displaces nuclei have all been noted in other species as a result of laser exposure such as rabbits, rats, humans and non-human primates (Leibowitz and Luzzio 1970, Belokopytov 2010, McCanna et al. 1982, Paulus et al. 2011). However, these species would not have experienced scleral cartilage degeneration because scleral cartilage is a characteristic that is not present in placental mammals (Walls 1942). Only one other study has tested the effects of lasers on bird eyes and found no injury as a result of the laser, however, it was very different from this study (Glahn et al. 2000). We used European starlings and house sparrows and the past study used double-crested cormorants as research subjects. According to Lustick (1973), different species can react very differently to laser light, with some being less sensitive. The past study also exposed 2 of their 3 birds at distances of 13 and 33 m, while we exposed 73 birds at a distance of 1 m from the laser. Their sample size was very small which could be a limitation in finding injuries. While the distances may have been considered "safe" for their laser, other types of lasers can still be dangerous at longer distances (Barkana and Belkin 2000). Glahn et al. (2000) also determined that some injuries were pre-existing. We could not determine this because all eye exams in our study were done post-euthanasia, which could be a limitation of our

experiment. However, the cormorant study did not include the amount of time each bird was exposed or a pulse duration, which are very important factors when determining whether or not damage will occur (Marshall 1998, Harris et al 2003).

There are 3 mechanisms of damage from laser exposure: thermal, photochemical, and mechanical. Laser energy and pulse duration, or what we call laser exposure time, are important factors in determining the mechanism of damage and thus the extent of injury (Harris et al. 2003, Marshall 1998). Thermal damage occurs when the laser energy absorbed by the eye turns into heat and the temperature of the tissues increases, which then causes denaturation of proteins resulting in a lesion (Mellerio 1966). This rise in temperature can cause denaturation of proteins which then causes a lesion and happens at exposure times from a few nanoseconds up to 10 seconds (Priebe et al. 1975, Marshall 1998). Photochemical damage is caused by longer exposure times (greater than 10 seconds) at wavelengths from 400 to 550 nm and lower power levels that will not increase the temperature by more than a few degrees Celsius (Ham et al. 1980, Marshall 1998). At these shorter wavelengths, energetic photons induce chemical reactions and directly break bonds in nucleic acids and structural proteins (Zuclich 1989, Barkana and Belkin 2000). Mechanical damage results from ionization or plasma formation when pulse duration is extremely short, less than a few nanoseconds (Ham et al. 1980, Marshall 1998). We found that laser exposure time was significant in the probability of corneal edema and retinal atrophy being present in starlings. Laser energy did not have a significant effect on the probability of damage being present in house sparrows, although the relationship was positive. Laser exposure time was also significant in the nested dichotomy analysis for starlings, in cataract dichotomy 2. These results provide evidence that laser exposure time could also be important in determining the presence and extent of some injuries in birds which is supported by the literature, however we cannot conclude this for laser

energy. We did, however, find that energy was significant in the nested dichotomy analysis for house sparrows. Energy was significant in cataract dichotomy 2, retinal atrophy severity dichotomy 3, retinal atrophy distribution dichotomy 2 and sclera severity dichotomy 2. The results from these analysis suggest that higher energy can cause retinal atrophy to be more severe in and widely distributed across the retina in house sparrows. Higher energy levels may also cause more severe cases of cataracts and scleral cartilage degeneration. This could be evidence that while laser energy does not affect the presence of these injuries in house sparrows, it could be affecting the extent of the injuries.

We tested the effects of laser exposure on the body mass of the house sparrows and found that body mass was higher before laser exposure than after. There was also an interaction effect in which juveniles had a higher body mass before and after laser exposure than adult house sparrows. If laser induced eye injuries create a challenge for birds during foraging, they could lose weight. However this is somewhat inconsistent with a previous study that found that although house sparrows changed their foraging behaviors after laser exposure, they still consumed the same amount of food as before (Blumenthal 2020). However the weight loss could have been stress-induced. Chronic or acute stress can cause decreased growth rates and weight loss in birds (Siegel 1980). Captivity can be one cause of stress, however Dickens et al. (2009) found that although birds lost weight and entered a state of chronic stress after initial capture, they gained weight within the first 10 days of captivity. But handling could also lead to stres according to Le Maho et al. (1992) which determined that geese had high stress indices even during a routine handling to which they had been accustomed for weeks. Stress from handling would be a reasonable explanation for the loss of body mass observed in our birds, because they were handled almost daily for weighing, laser treatments, and some were handled for a separate behavioral study as well.

One limitation of this study is that we were not able to check damage at the fovea using our methods. A lesion can cause a loss in visual acuity if it is foveal or if it is parafoveal and spreads to the fovea (Harris et al. 2003). However, there is already some evidence of losses in visual acuity in birds as a previous study of the effects of lasers on avian foraging behavior concluded that there was some loss in visual function as a result of laser exposure (Blumenthal 2020). There was a significant difference between several behaviors, including a faster approach to the food patch, reduction of binocular vision use, and increased pecking rate during foraging before and after exposure (Blumenthal 2020). We also did not account for distance in our study. We exposed all of our birds at 1m away from the laser, but lasers can be used, when deterring birds, at far distances over 1km (Baxter 2007). However, we know from cases in airplane pilots and military staff that lasers can still be injurious to the eye in humans at far distances up to several hundred meters and even at 3 km through a scope (Harris et al. 2003, Gosling et al. 2016). As mentioned previously, we also did not assess the bird's eyes before exposing them to the laser. This means that we cannot control for pre-existing injuries or conclude that every injury was a result of laser treatment. Future studies should consider these factors in their experimental design to get the most accurate results.

APPENDIX A. LASER INJURIES

Table 7**.** The injuries that we did not find as a possible result of laser exposure.

Injury	***Description***
Corneal ulceration	A break in the corneal epithelium which causes an open sore on the eye and may involve the underlying stroma (Bordin 2020)
Stromal fibrosis	Scarring of the corneal stroma as a result of injury that may appear as a haze or mild opacity in the cornea (Medeiros et al. 2018)
Keratitis	Inflammation of the cornea, often a precursor of corneal ulceration (Cope et al. 2016)
Conjunctivitis	Inflammation of the conjunctiva (Hutnik et al. 2010)
Uveitis	Inflammation of the uvea which can occur alongside lesions in the lens or cornea (Cummings et al. 2020)
RPE atrophy	The RPE degenerates resulting in areas missing. (Blair 1975)
RPE hypertrophy	Abnormal thickness of the RPE (Gass 1989)
Retinoschisis	Splitting of the retina into layers with the site of splitting dependent on the cause (Yanoff et al. 1968)

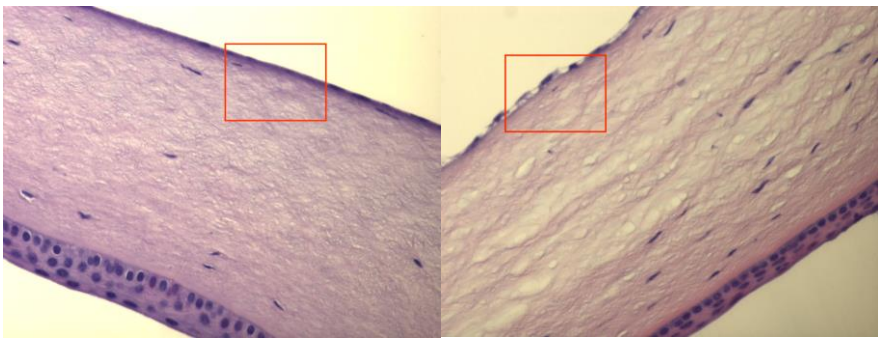


Figure 12**.** A healthy cornea (left) appears smooth with a solid endothelium (boxed). With corneal edema (right) the endothelium deteriorates, which appears as a broken line along the cornea. This allows the cornea to fill with fluid causing the stroma to appear swollen.

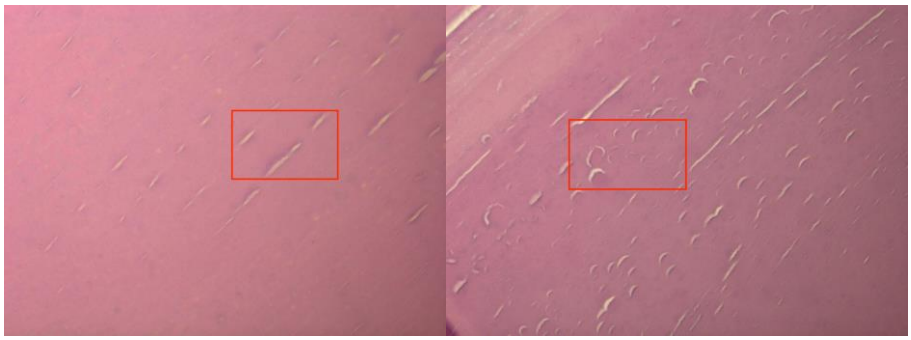


Figure 13**.** Healthy lens fibers (left) appear as straight lines. One sign of cataracts (right) is when the fibers become filled with fluid and the ends curl, causing them to appear as "c" shapes.

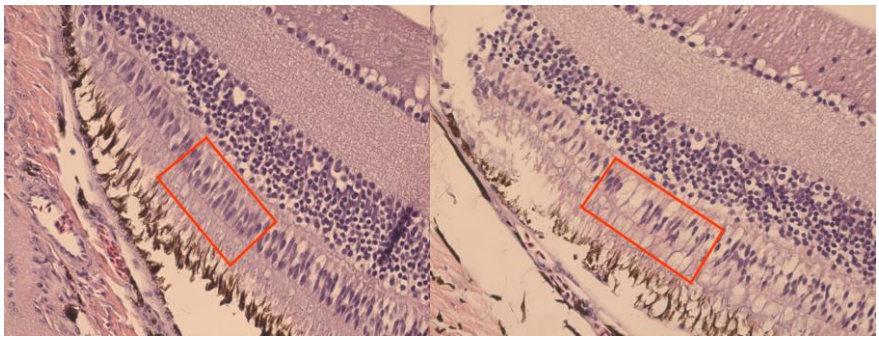


Figure 14**.** In a healthy retina (left) the photoreceptor layer (boxed) will appear organized with photoreceptors packed tightly together. Retinal atrophy (right) will show a decrease in density of photoreceptor nuclei.

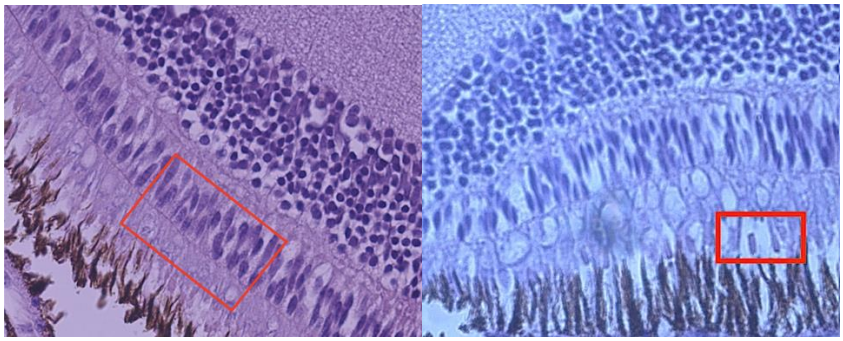


Figure 15**.** In a healthy retina (left) the photoreceptor nuclei (boxed) will be in the outer nuclear layer (ONL) and appear separately from the photoreceptors. When retinal photoreceptor displaced nuclei (pdn) (right) occurs, the photoreceptor nuclei appear in the photoreceptor layer as opposed to the ONL.

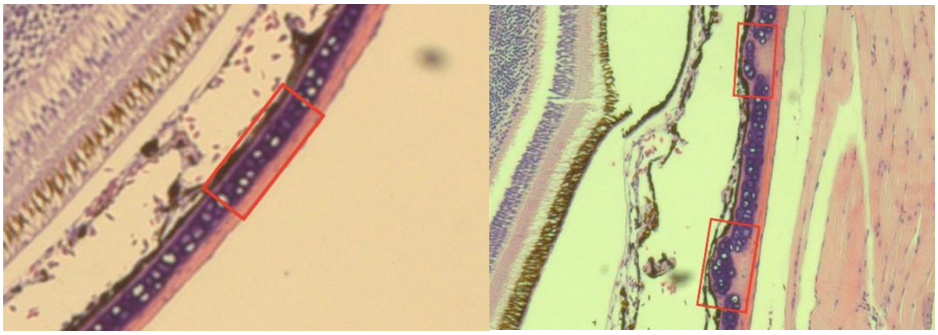
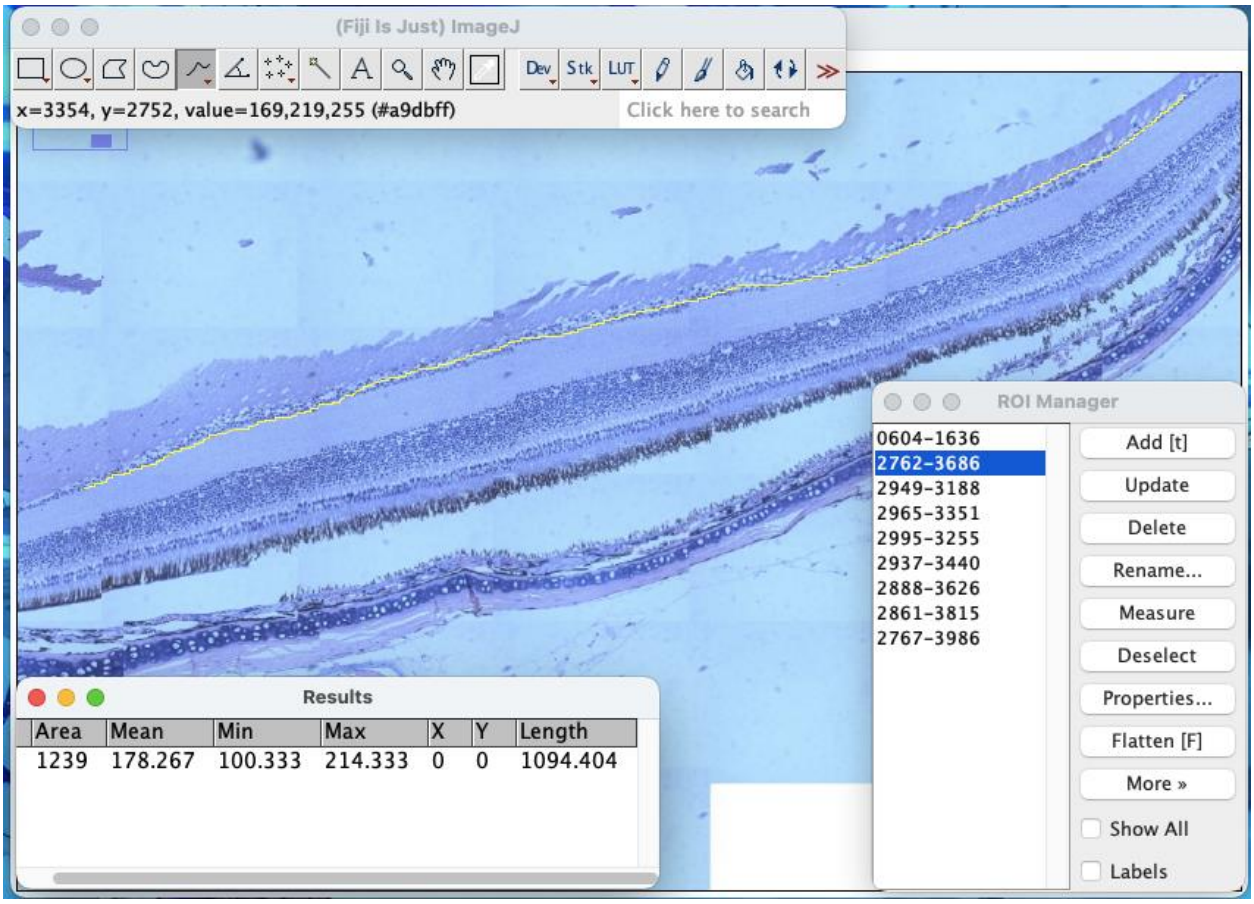


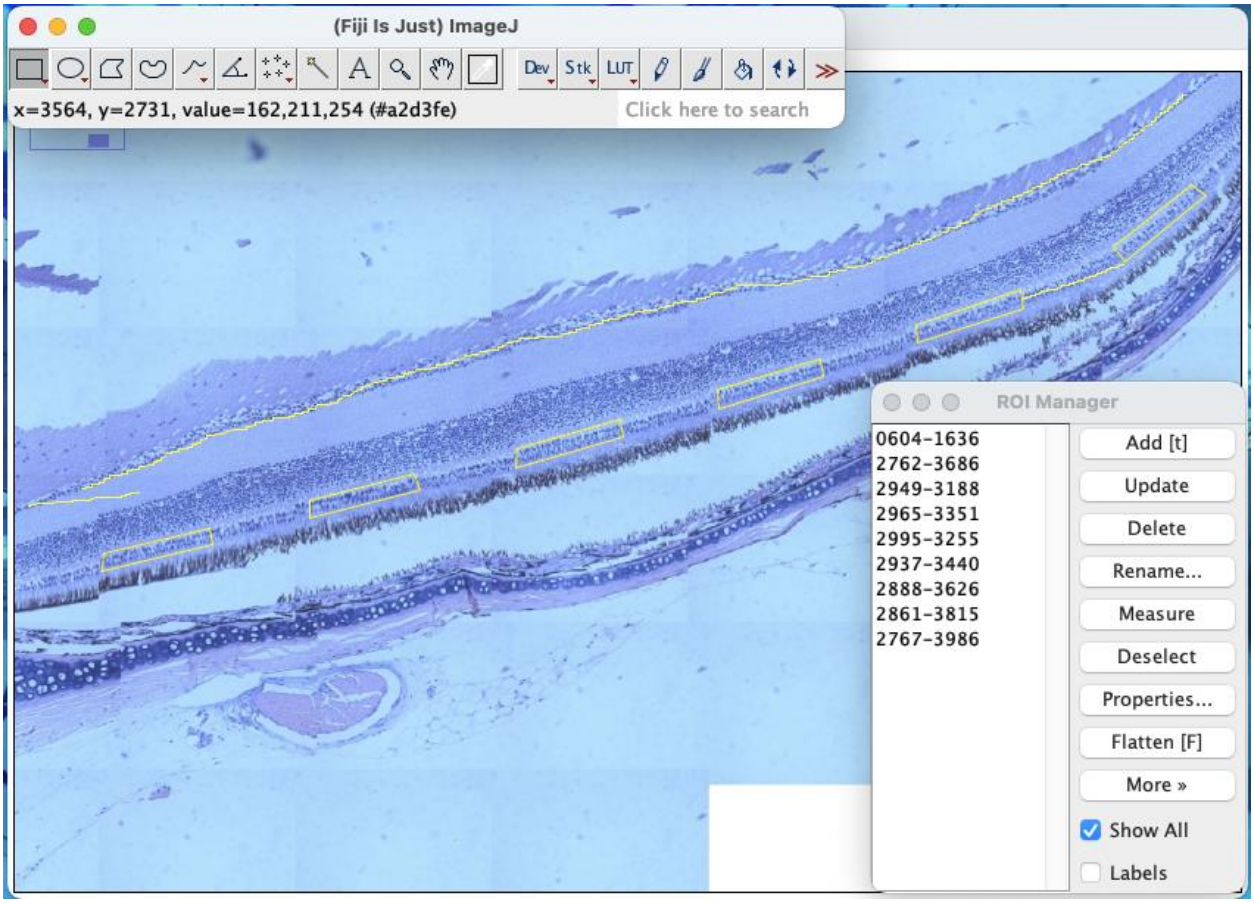
Figure 16**.** A healthy sclera (left) will have a thick layer of cartilage (boxed). When scleral cartilage degeneration (right) occurs, the cartilage will begin to clump and break (boxed).

APPENDIX B. QUANTITATIVE IMAGE ANALYSIS IN IMAGE J

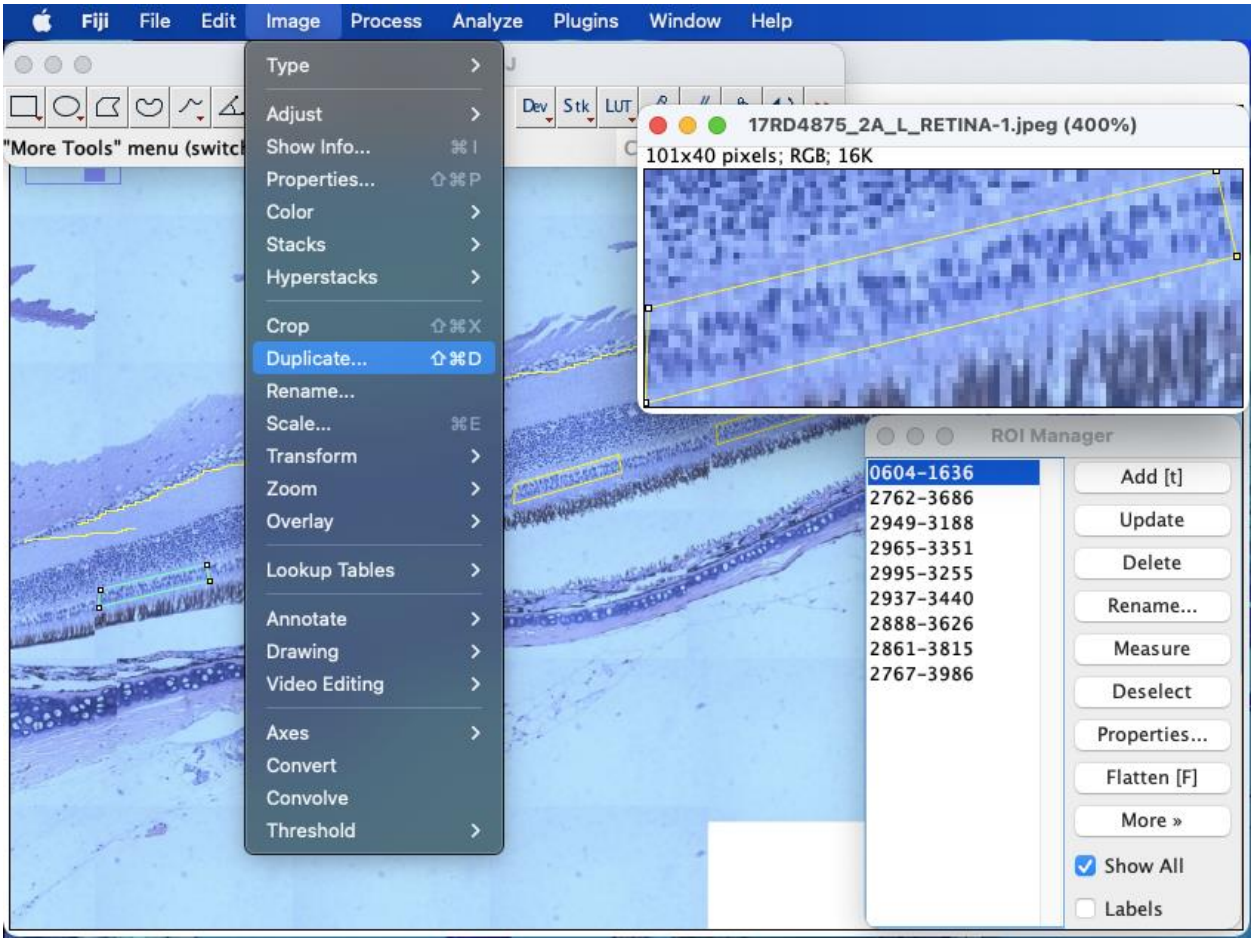
Retina



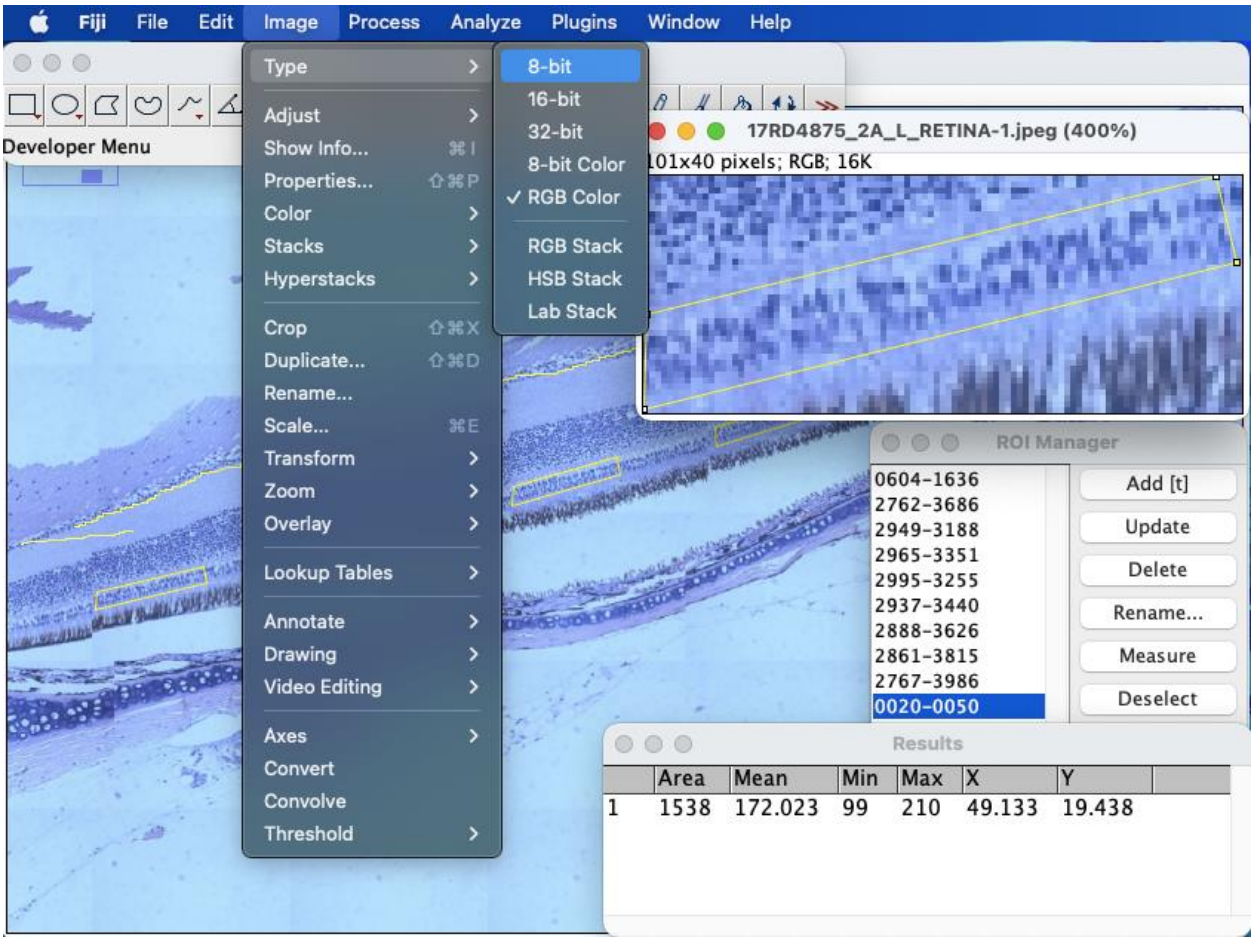
Step 1: Draw a line along the intact areas of the retina using the freehand line tool on the toolbar, and measure the length of the line. Save the line using the add button in the ROI manager.



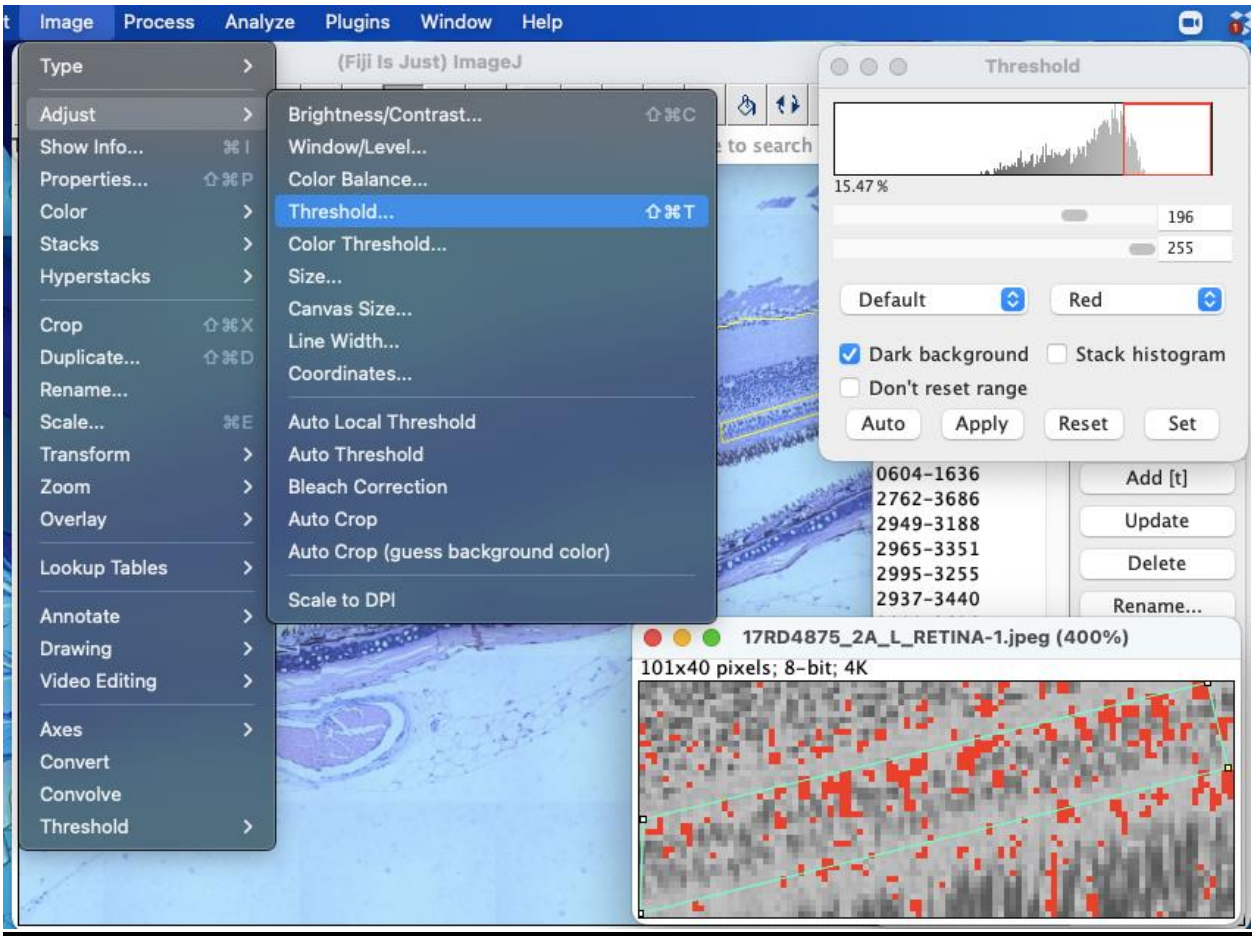
Step 2: Place 6 samples onto the ONL of the retina using the rectangle tool on the toolbar and add them to the ROI manager. The size of the boxes should be such that 6 boxes can fit within the length of the line in step 1 and be the same distance apart from each other, and the width should only be as wide as the ONL of the retina. Once the size of the samples is decided, they can be placed so that there are 3 damaged samples and 3 undamaged samples.



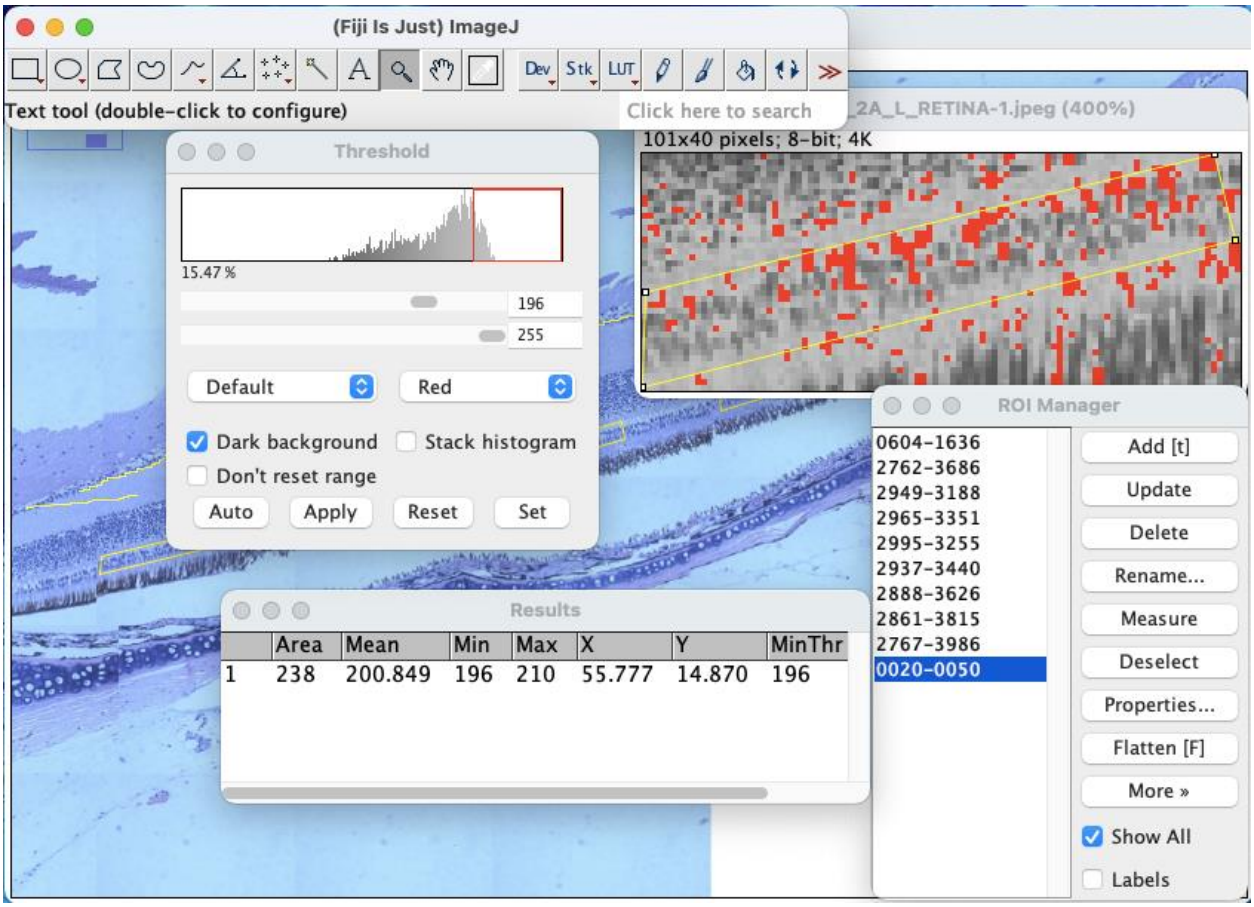
Step 3: Choose the first sample in the ROI manager and use the Duplicate function from the image menu to enlarge the sample. Add this enlarged sample to the ROI Manager.



Step 4: Change the enlarged sample to black and white using the 8-bit function under Type in the Image menu. Choose the sample in the ROI manager and measure it. The area is the total number of pixels in the sample.

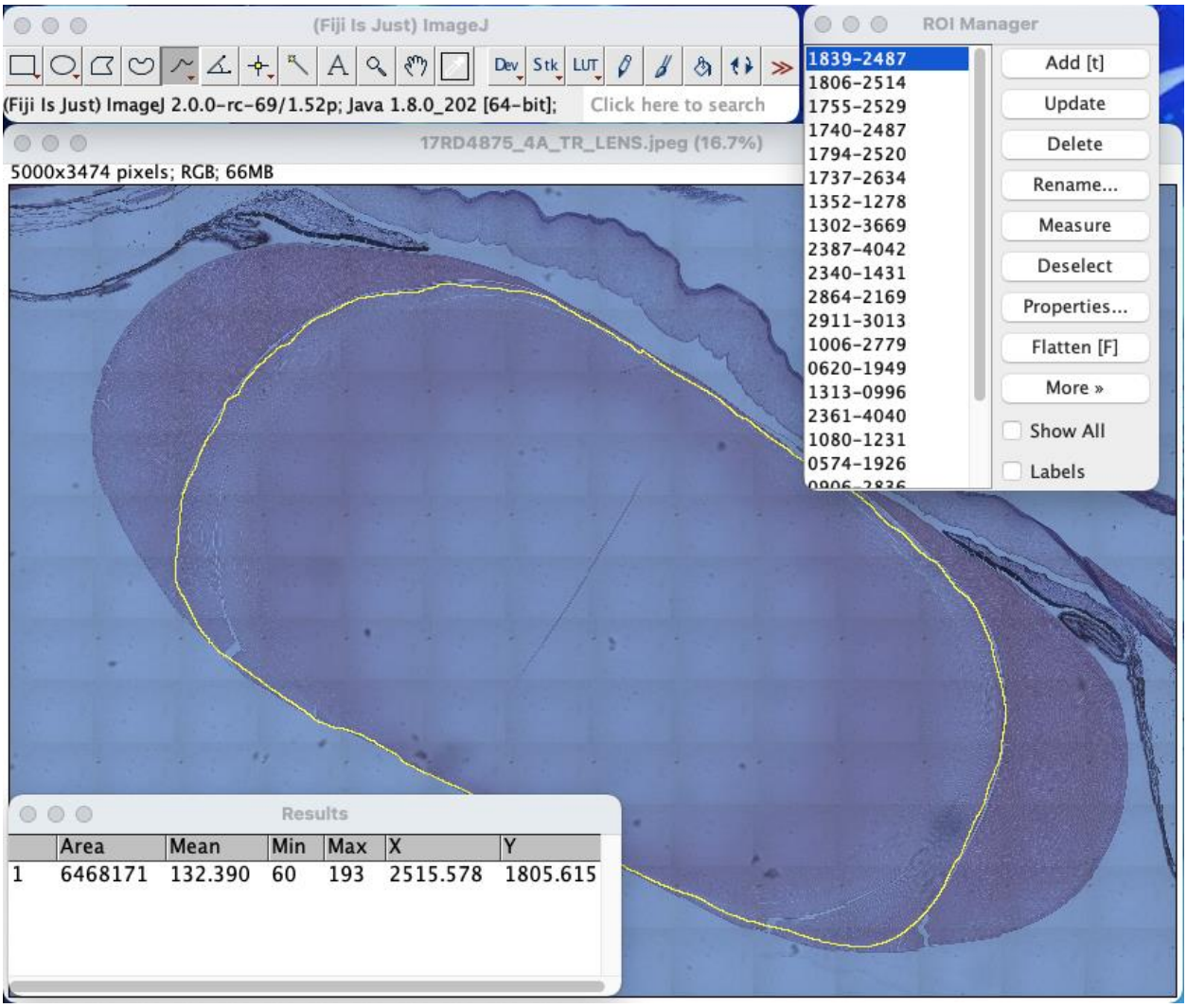


Step 5: Use the Threshold function under Adjust in the Image menu to highlight the empty space in the ONL. Use the slider bars in the Threshold box to highlight everything to the left of the farthest right high peak of the graph, which represent the whitest areas of the sample.

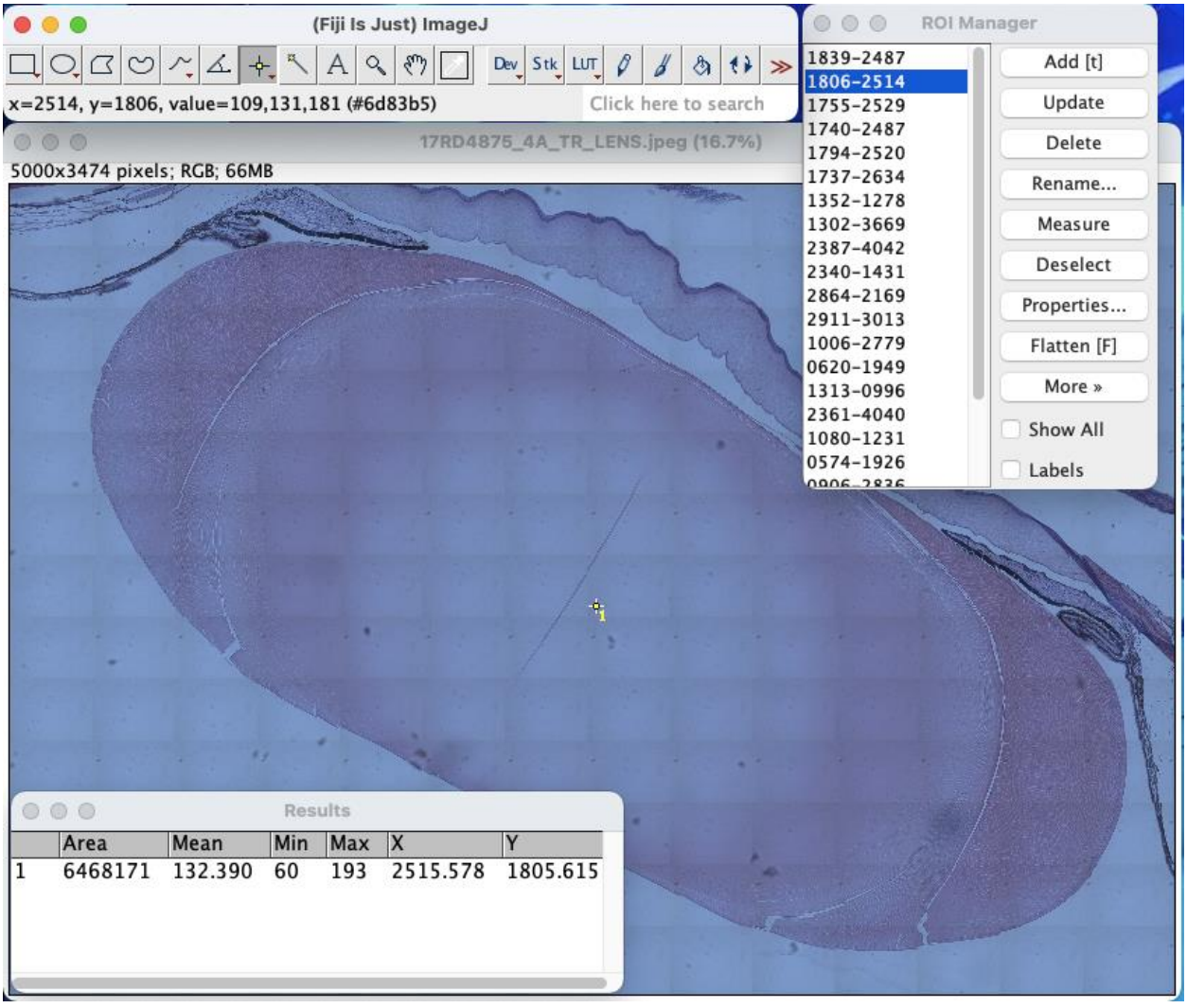


Step 6: Highlight the enlarged sample in the ROI manager and measure it. The area represents the white spaces which are highlighted in red, and can be divided by the total area in step 4 to get the proportion of damaged pixels in the sample. Repeat steps 3-6 for each sample.

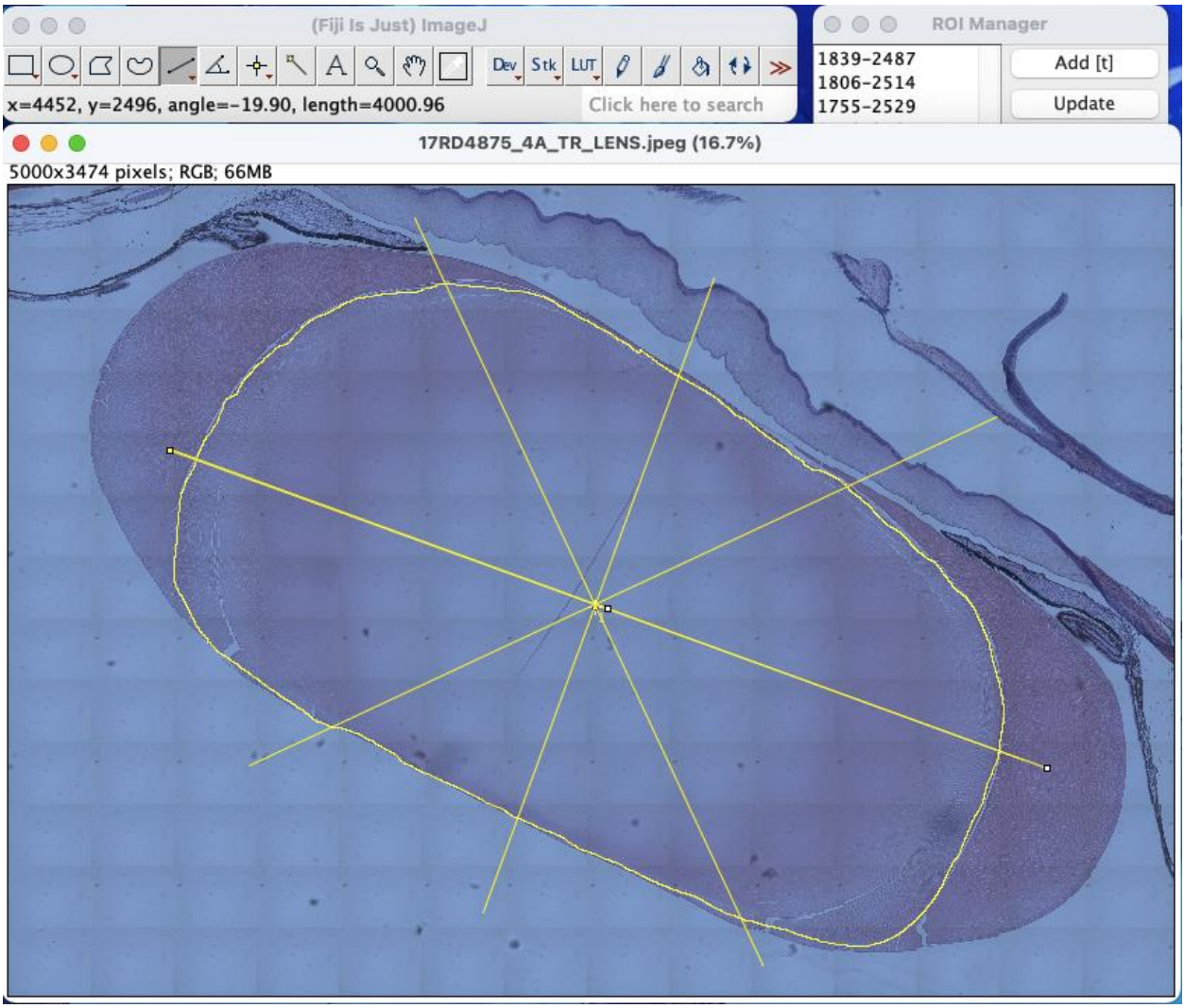
Lens



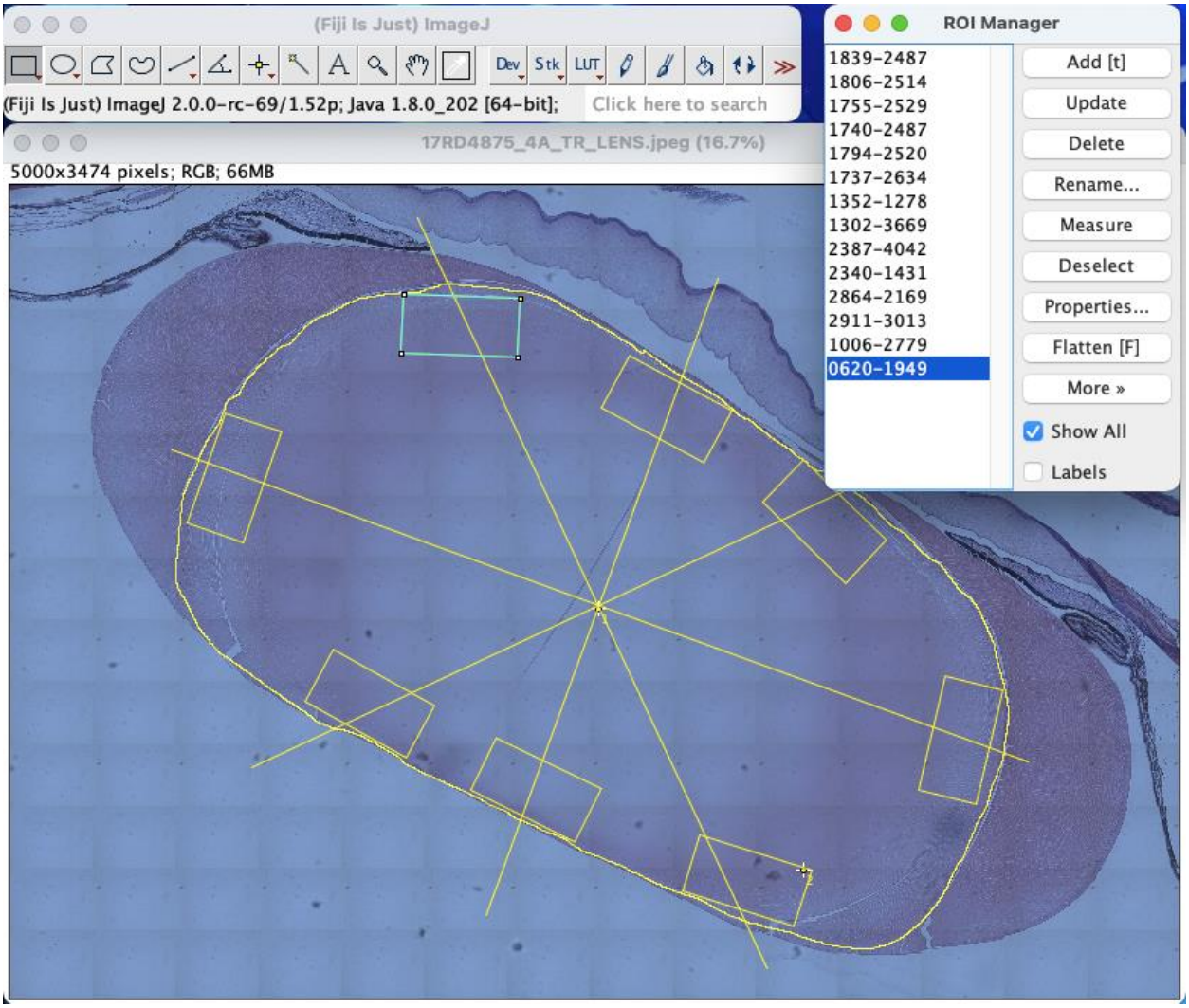
Step 1: Use the freehand line tool to draw a line around the perimeter of the lens cortex and use the add button in the ROI manager to save it. Select the ROI in the manager and measure. The center of the shape is given in X and Y coordinates.



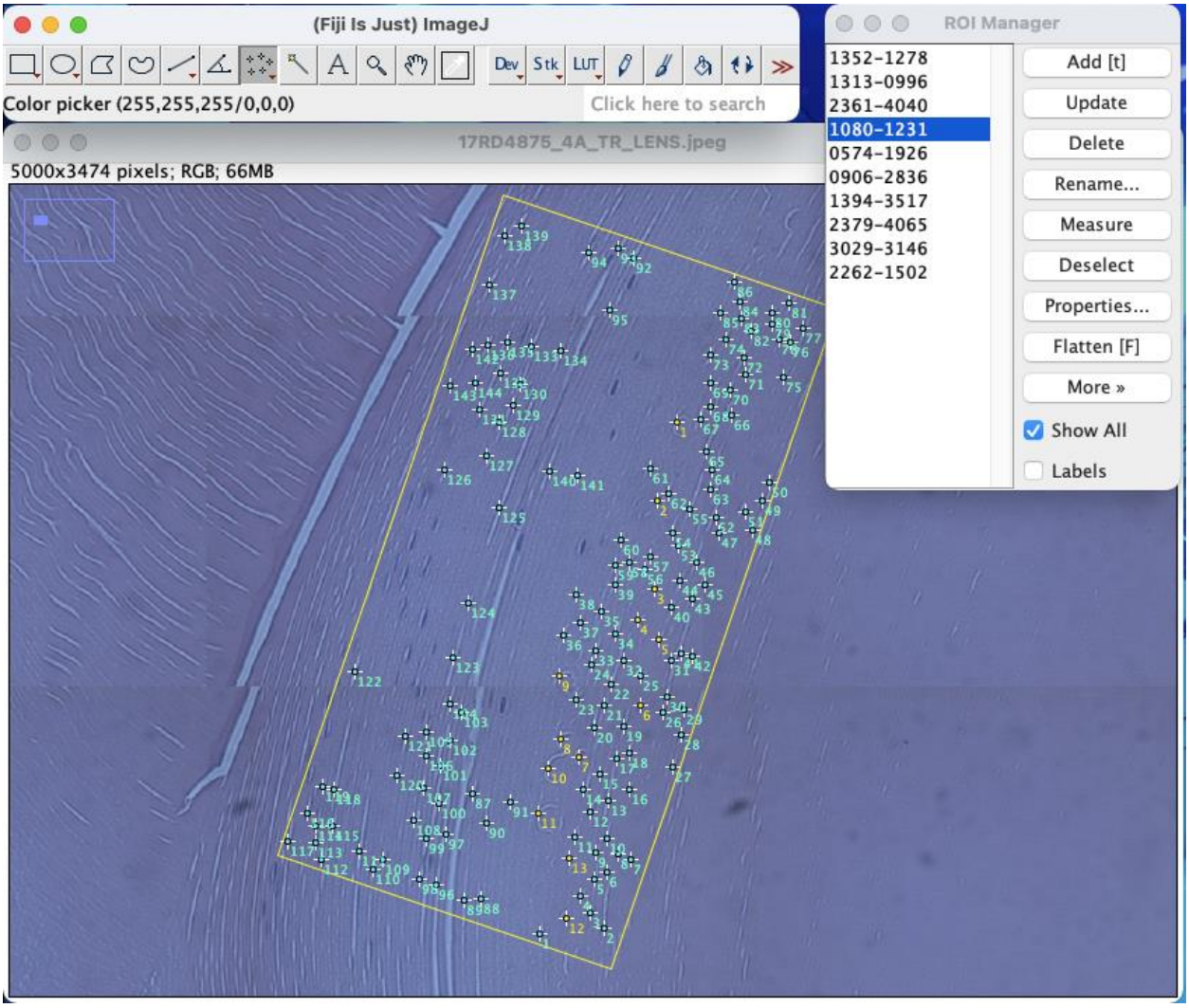
Step 2: Use the point tool to place a point at the X and Y coordinates or as close as possible to them. Add the point to the ROI manager.



Step 3: Use the straight line tool to separate the lens into sections. Start by drawing one line through the center point and take note of the angle of the line given by Image J. Add this line to the ROI manager. Add 45 to the angle measurement of the first line and draw another line through the center point at that angle. It may be easier to draw the line starting at the center point, then adjust the length of the line to extend across the entire lens. Repeat 2 more times, adding all the lines to the ROI manager. This will create 8 45° sections.



Step 4: Draw boxes in each of the 8 sections using the rectangle tool. The box should be big enough that the width of it expands across the entire area where lens fibers are visible. You can make multiple boxes of the same size by drawing one box and adding it to the ROI manager, then move the box to a new position and adding that to the ROI manager. Adjust the position of the boxes so that there are 4 samples containing damaged fibers and 4 that are not containing damaged fibers.



Step 5: Zoom into the desired section. Use the multipoint tool to count all of the curled lens fibers (yellow) and add them to the ROI manager. Then use the same tool to count the healthy fibers (blue) and add them to the ROI manager. Add these two numbers together to get the total number of fibers and find the percentage of damaged fibers. Repeat this step for the remaining 7 samples.

APPENDIX C. NESTED DICHOTOMIES

European Starling Dichotomies

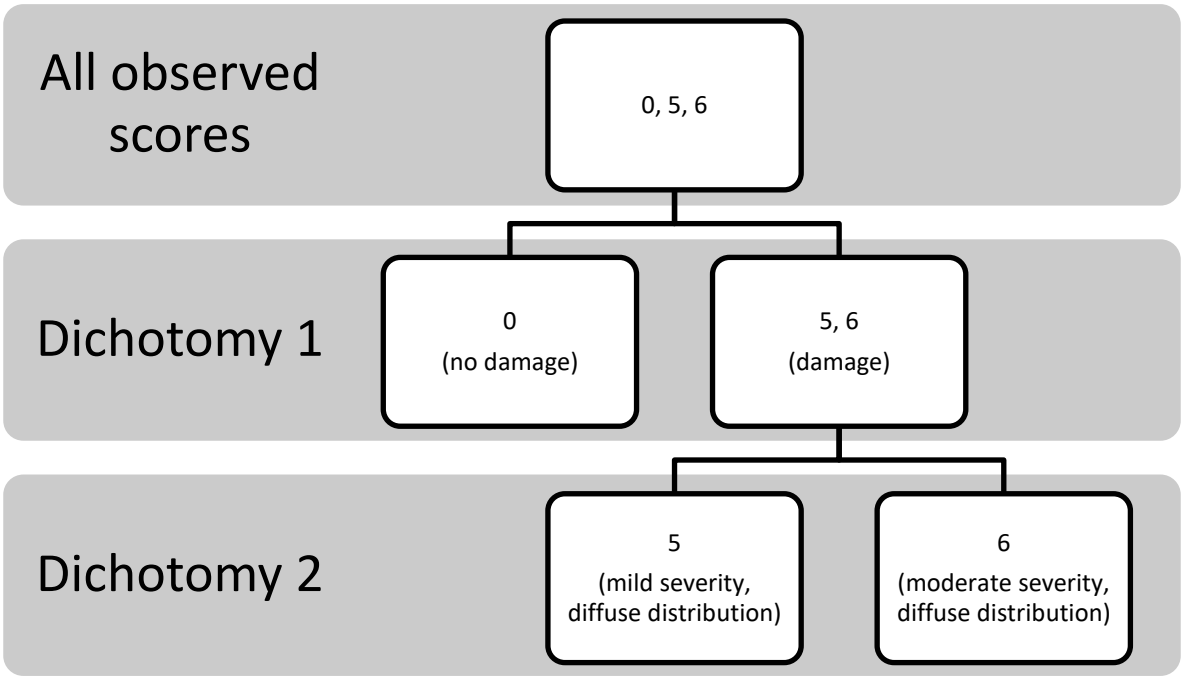


Figure 17**.** The nested dichotomies for corneal edema in European Starlings.

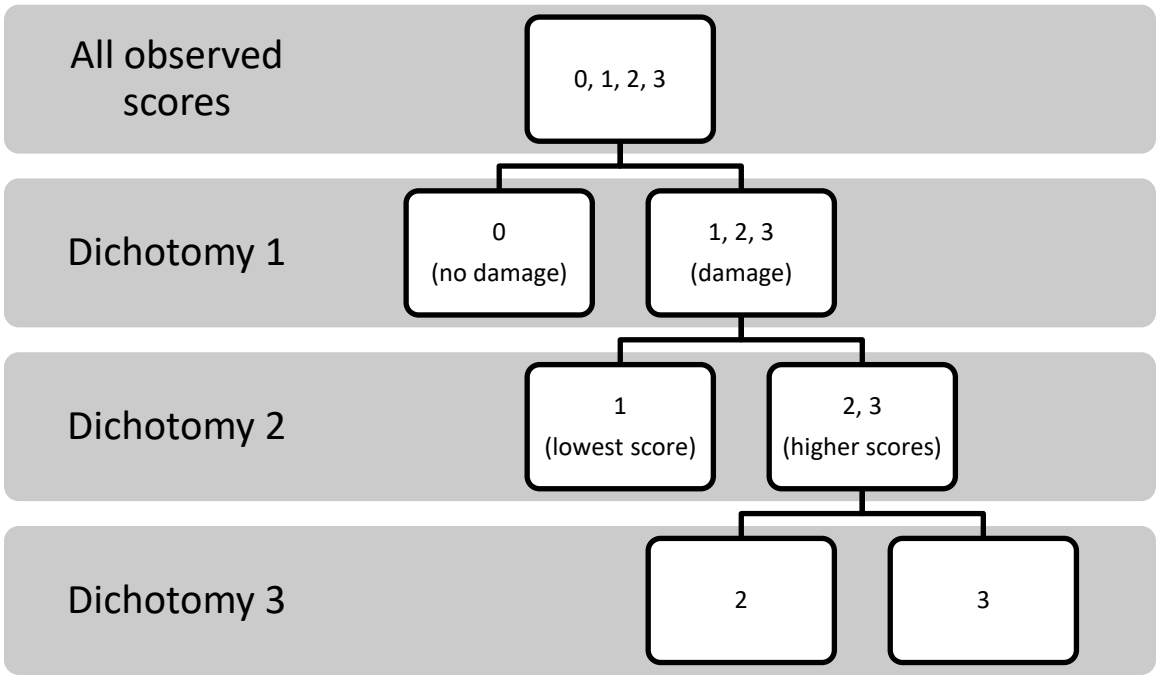


Figure 18**.** The nested dichotomies for cataracts in European starlings.

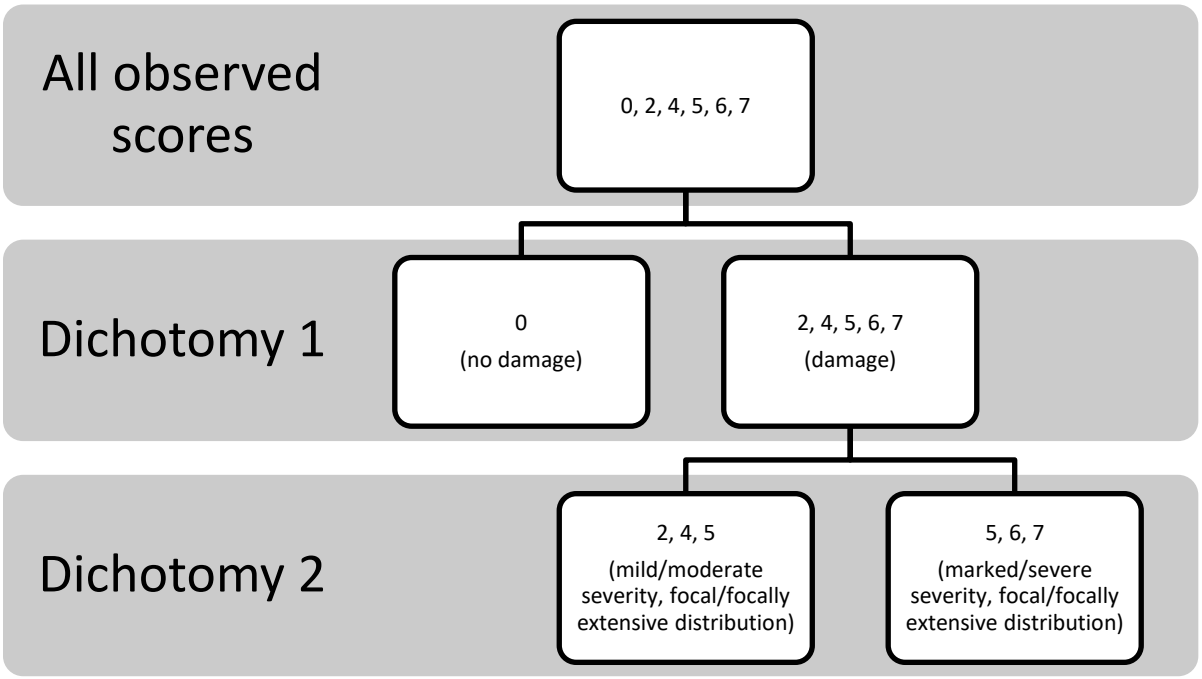


Figure 19**.** The nested dichotomies for scleral cartilage degeneration in European starlings.

House Sparrow Dichotomies

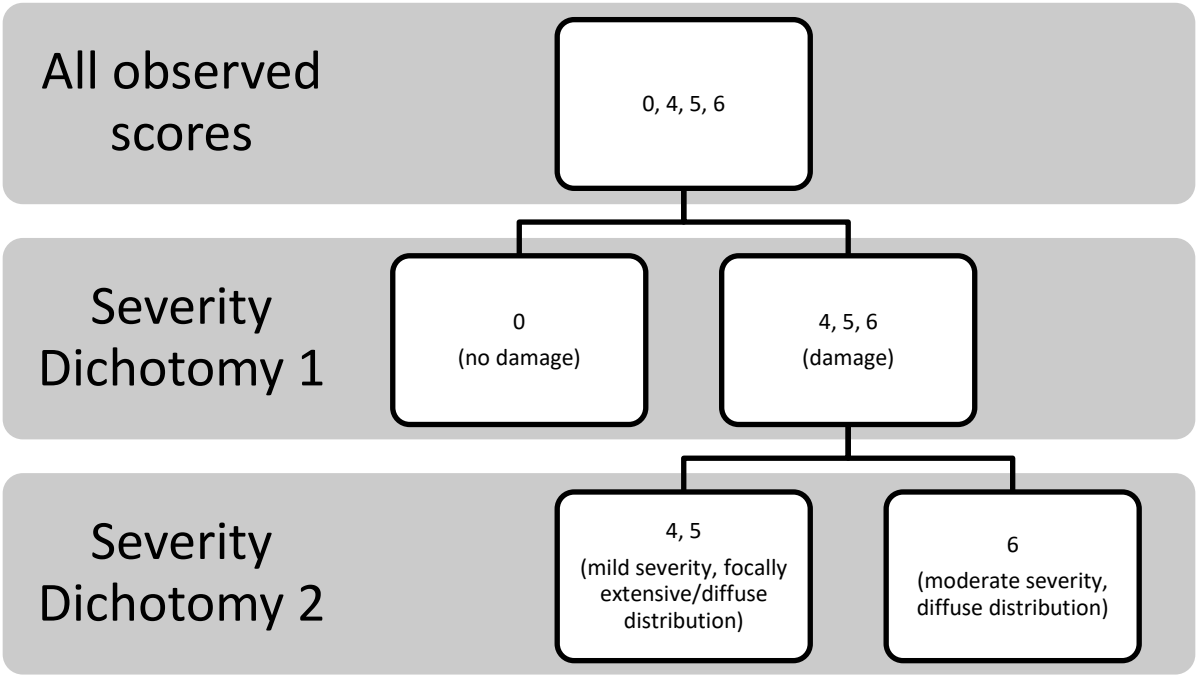


Figure 20**.** The nested dichotomies for corneal edema in house sparrows.

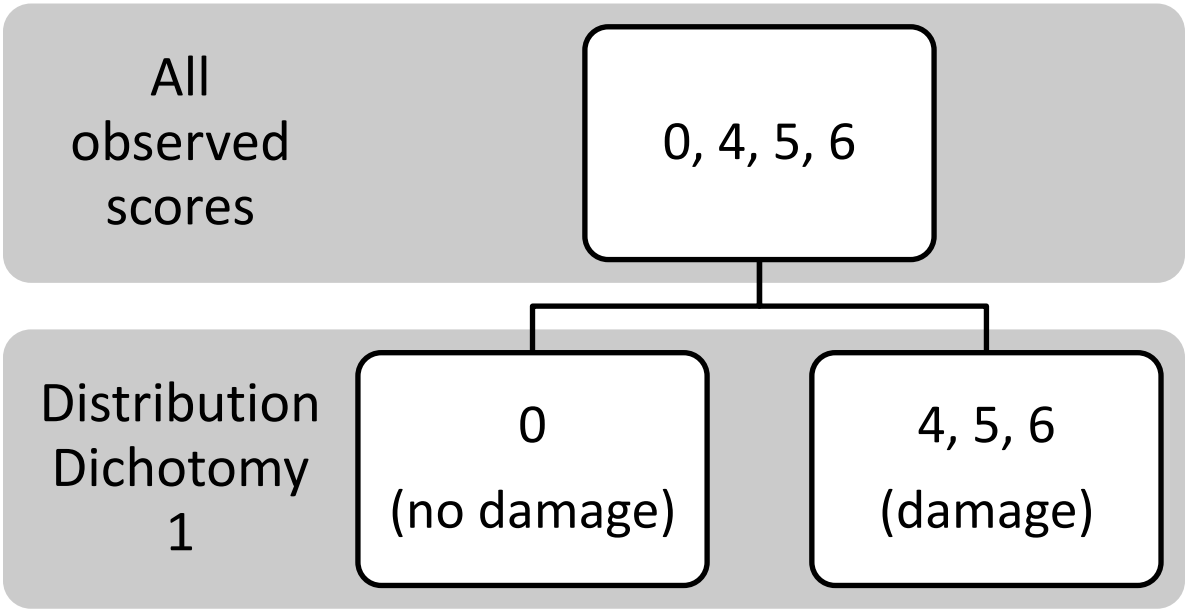


Figure 21**.** The scores had the same distribution score and differed only in level of severity in corneal edema in house sparrows, so we could not create a nested dichotomy based on distribution.

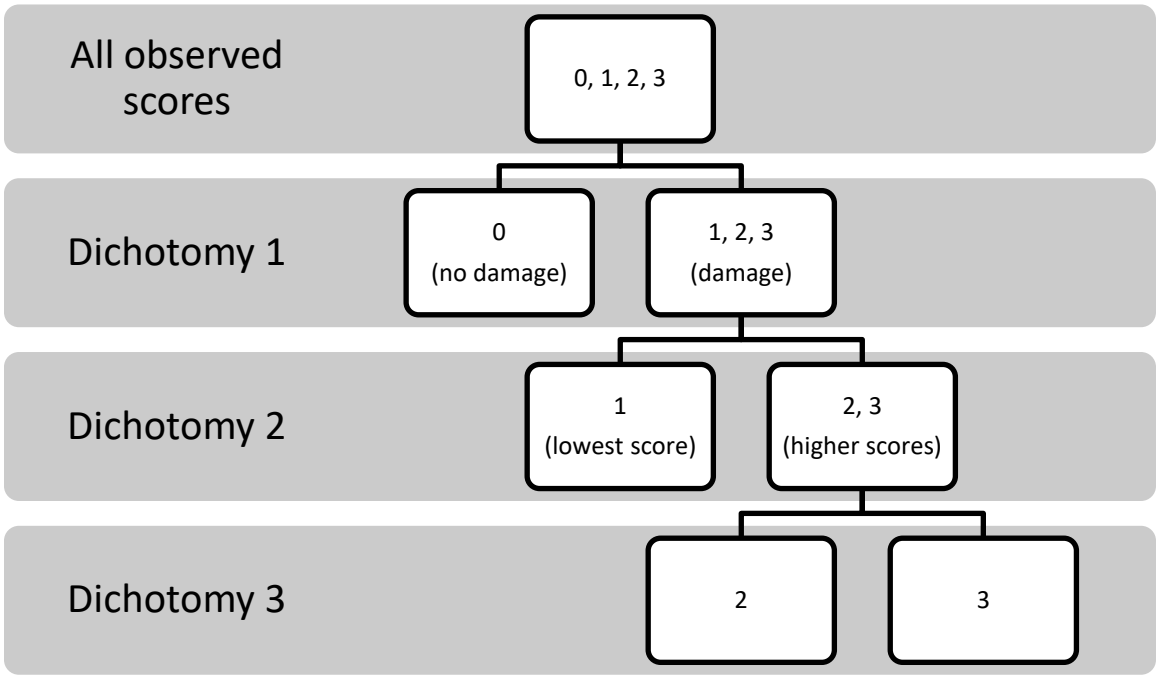


Figure 22**.** The cataracts nested dichotomies in house sparrows.

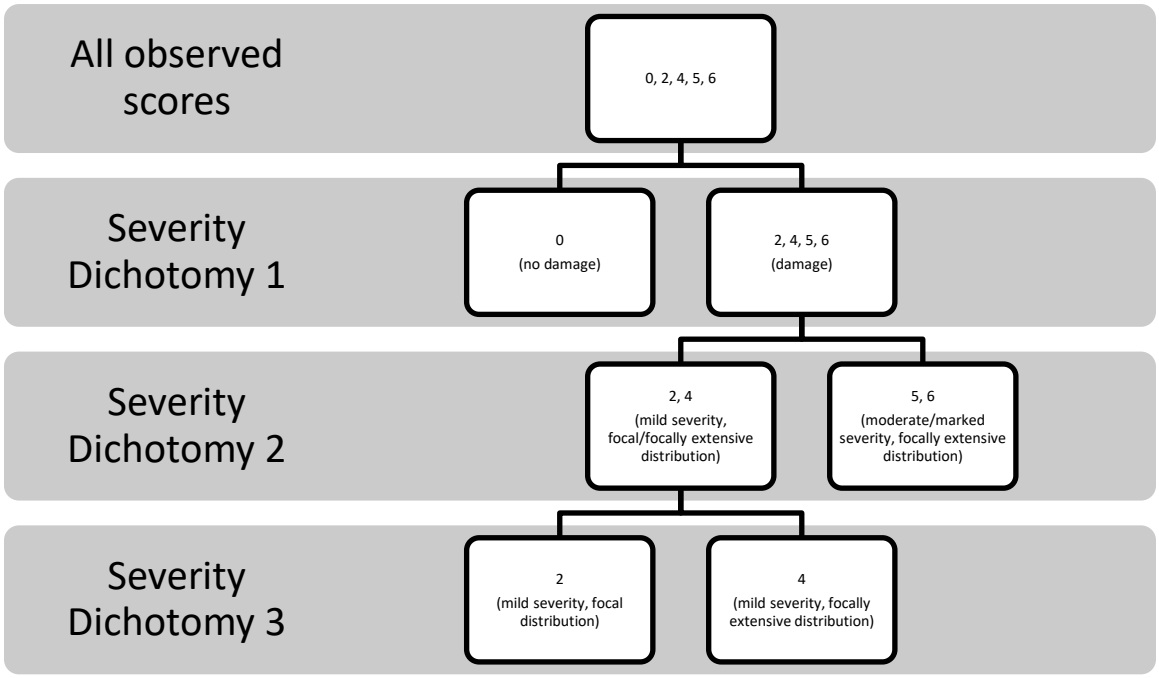


Figure 23**.** The nested dichotomies for retinal atrophy in house sparrows based on severity scores.

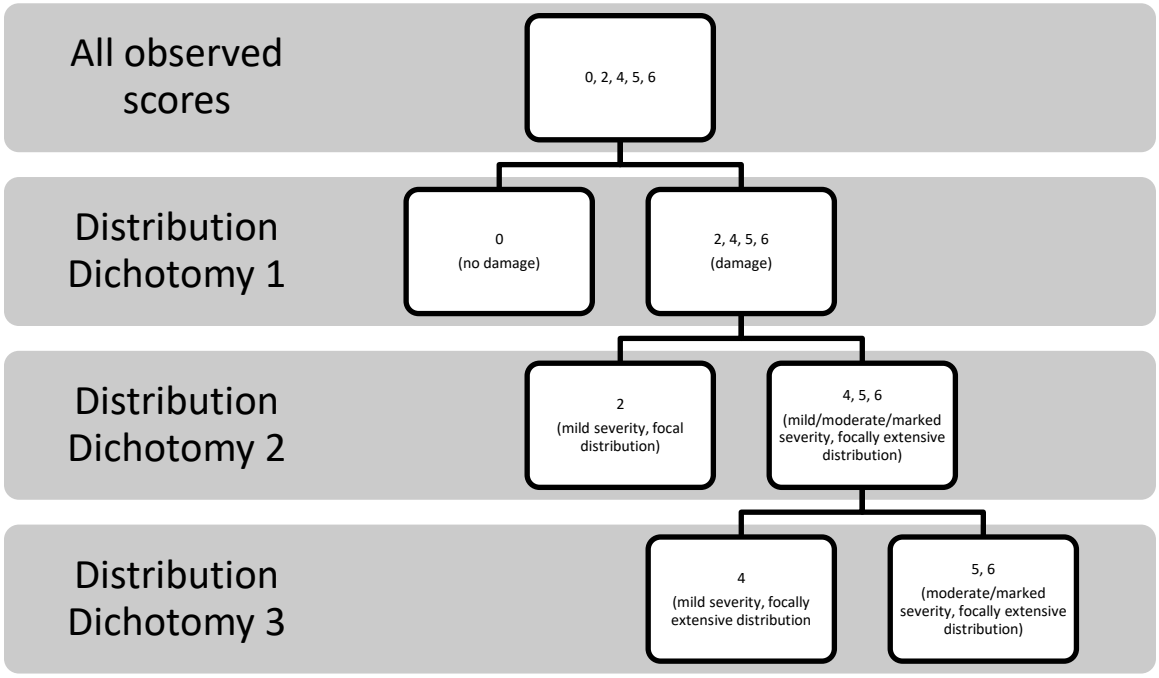


Figure 24 The nested dichotomy for retinal atrophy in house sparrows based on distribution scores.

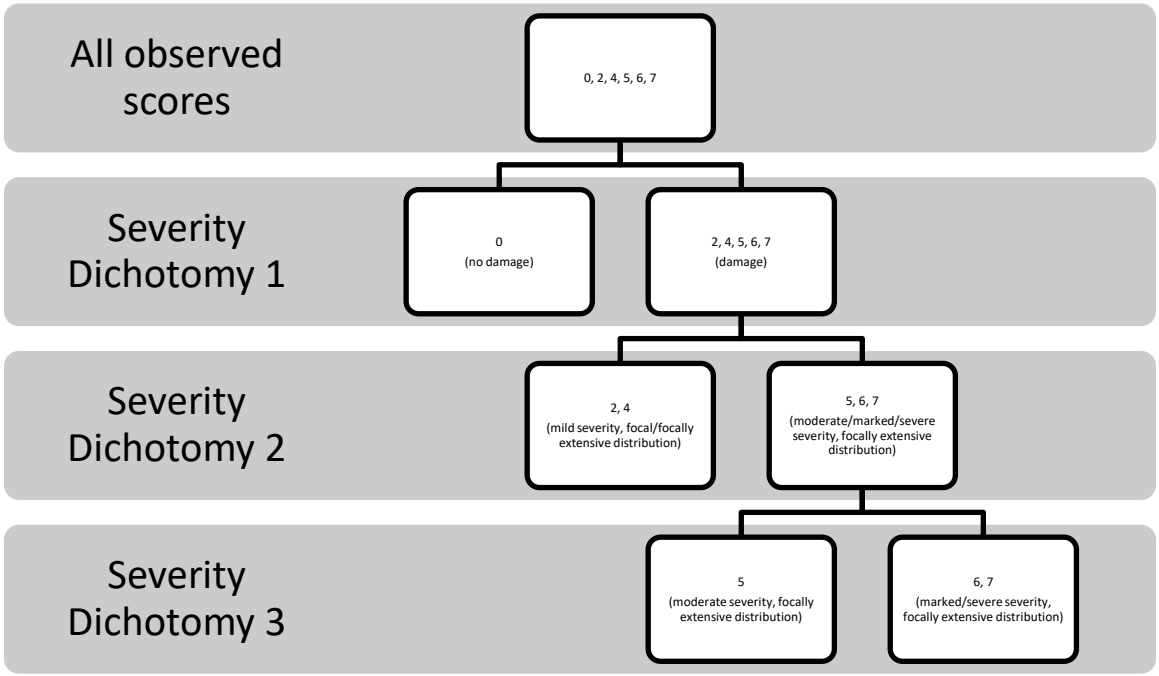


Figure 25. These scleral cartilage degeneration dichotomies for house sparrows based on severity scores.

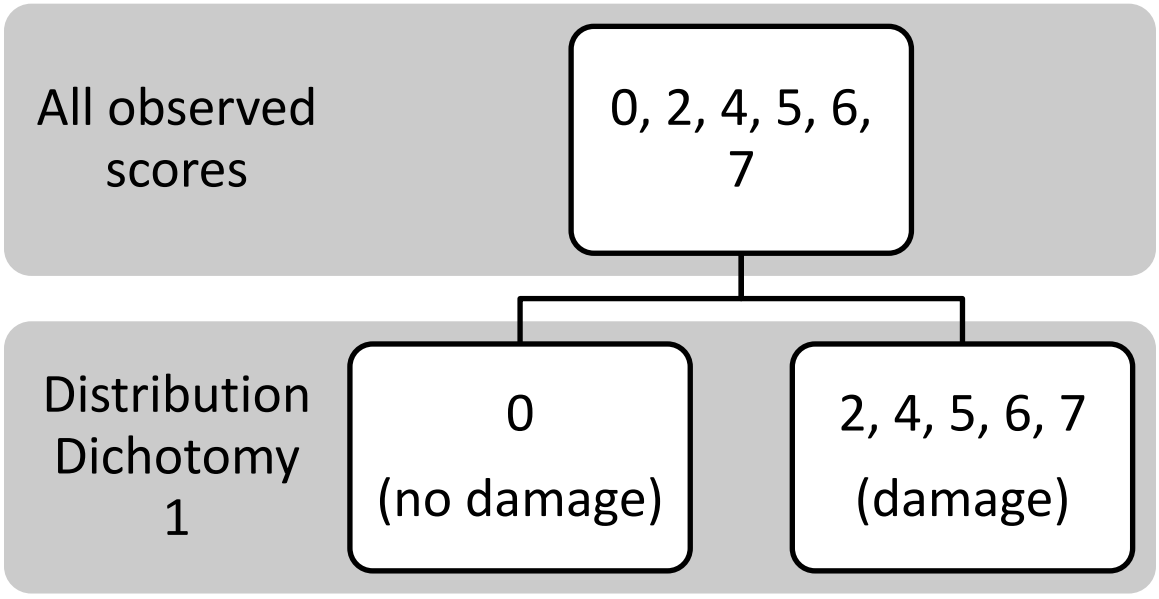


Figure 26**.** We could not make dichotomies based on distribution scores for scleral cartilage degeneration in house sparrows because the majority of overall scores had the same distribution score.

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