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# A novel system for bi-ocular eye-tracking in vertebrates with laterally placed eyes

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## Summary

**1.** Animals use vision to gather information about their environment and then use that information to make behavioural decisions that affect fitness. They will often move their heads or eyes to inspect areas of interest with their centres of acute vision, such as foveae, to gather high resolution information about potential mates, predation risks, or other aspects of the environment. Few studies to date have been able to accurately determine where laterally eyed animals direct their visual attention and how they use their eyes to gather information.

**2.** We present a non-invasive eye-tracking system that can simultaneously track the gaze of two eyes. This is particularly useful for studying animals with laterally placed eyes (most vertebrates) where the two eyes are viewing different images. This system can also accommodate comparative studies using animals of varying size, including small animals that are not frequently used in eye-tracking studies due to constraints of existing eye-tracking systems. We conducted an eye-tracking experiment with European starlings (*Sturnus vulgaris*) to test the eye-tracking system, calibration methods and highlight relevant aspects of experimental design.

**3.** We were able to accurately track the gaze of European starlings with $< 5$ degrees of error. We also found that starlings are more likely to fixate on biologically relevant visual stimuli (e.g. predators and active prey) than simple stimuli (e.g. a dot) in video playbacks.

**4.** The method presented here can be used to address ecological and evolutionary questions about where animals direct their attention and how they visually inspect mates, food and predators, as well as address management questions about how animals inspect man-made objects. This method can also be used to answer fundamental questions about vision, such as how laterally eyed vertebrates coordinate the use of their eyes laterally and binocularly.

**Key-words:** vision, binocular, gaze, fovea, retina, sensory, animal behaviour, visual ecology

## Introduction

Fundamental questions in evolutionary ecology can require an understanding of the sensory and behavioural mechanisms animals use to gather information (Stevens 2013). Considerable research has been devoted to trying to identify the specific cues individuals attend to before making decisions that have fitness consequences (reviewed in Jones & Ratterman 2009; Fernández-Juricic 2012; Stevens & Ruxton 2012). In visually oriented organisms, for instance, determining the specific components of male signals that females use can help understand the evolution of mating strategies through sexual selection (Ronald, Fernández-Juricic & Lucas 2012; Yorzinski *et al.* 2013), as well as the mechanisms of reproductive isolation in closely related species living in sympatry (e.g. Santana, Lynch Alfaro & Alfaro 2012). Additionally, establishing the visual cues animals use to detect food items in complex visual backgrounds can provide novel insights into the evolution of camouflage (Troscianko *et al.* 2011).

Vertebrates use their retina to gather information from their environment, but not all regions of the retina gather information of the same quality. This can make it challenging to estimate what components of a visual image an animal pays attention to. There are retinal areas that provide higher spatial visual resolution (i.e. centres of acute vision, such as foveae) than others (i.e. retinal periphery; Hughes 1977). The centres of acute vision have been established as the centres of visual attention during object-oriented tasks in some vertebrates (Bisley 2011). Because the centres of acute vision occupy a very small portion of the visual field, their projection and movement patterns can provide information about how animals visually explore their environment and investigate objects of interest.

Accurately tracking gaze (i.e. the projection of the centre of acute vision into visual space) can facilitate our understanding of multiple visually guided behaviours such as foraging, predator detection, flight control and mate choice and can also shed light into more fundamental questions as to how the animals divide their visual attention (e.g. detecting predators while foraging; Dukas & Kamil 2000). Eye-tracking studies are now relatively common in vertebrates with frontally placed eyes (e.g. Machado & Nelson 2011; Hu *et al.* 2013; Dixson *et al.* 2014). Yet, tracking gaze is more difficult in vertebrates with laterally placed eyes because of hardware and software constraints. In species with laterally placed eyes, the video capture for each

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eye cannot be projected into a single binocular image because the eyes have a lower degree of overlap than in species with frontally placed eyes. Additionally, some eye-tracking software assumes that the centre of acute vision is close to the centre of the eye (e.g. humans). However, the position of the centre of acute vision varies substantially between different species of vertebrates (Collin 1999; Moore *et al.* 2012).

There has been some previous development of eye-tracking and gaze-tracking systems for use in species with laterally placed eyes. Kjærsgaard *et al.* (2008) proposed the use of head-mounted infrared laser modules to track a subject bird's gaze by projecting a laser onto the visual scene. Unfortunately, the mass and size of this laser system restricts its use to larger species and tracks only head movement while assuming little or no eye movement (Kjærsgaard *et al.* 2008). However, many bird species actually have a large degree of eye movement (Martin, White & Butler 2008; Moore *et al.* 2013; Tyrrell *et al.* 2013). Voss & Bischof (2009) reported eye-movement behaviour in restrained zebra finches (*Taeniopygia guttata*) using two eye cameras (i.e. one camera recording each eye) and a single overhead camera to record the scene around the subject. While this method is ideal for recording eye movements, it does not determine the finches' gaze direction. Yorzinski *et al.* (2013) developed a system for tracking eye movements and gaze into visual space in freely moving peafowl (*Pavo cristatus*), but this system only records one eye and is limited to use in large birds that can support a 25 g headpiece and 345 g of hardware attached to the back of the animal. Finally, Wallace *et al.* (2013) and Schwarz, Sridharan & Knudsen (2013) each developed an eye-tracking system for freely moving rats and chickens, respectively, that consist of hardware attached to the head that record the movements of each eye. While some, but not all, small birds could support these more lightweight systems, the hardware is attached to a head plate that is surgically implanted, which is not ideal for studying vertebrates that are captured but later released in the wild.

We developed a non-invasive eye-tracking system that can accurately map the gaze of both eyes onto a scene image and can be used in a wide variety of contexts using species with laterally placed eyes, including small birds that cannot support the mass of heavier systems (e.g. Yorzinski *et al.* 2013). Here, we describe the system, the gaze-tracking parameters that can be measured quantitatively, the type of visual stimuli animals responded to most and the limitations of the system. Additionally, we discuss potential applications for evolutionary ecology research.

## Materials and methods

### SUBJECTS

We used twelve adult European starlings (*Sturnus vulgaris*) that were captured from wild populations in Tippecanoe County, Indiana and had been used in previous behavioural experiments. Birds were housed in groups of 2–4 per cage (0·6 m × 0·6 m × 0·75 m) indoors on a 14:10 light:dark cycle and were fed dry cat food and poultry feed *ad libitum*. The Purdue Institutional Animal Care and Use Committee (protocol 111200398) approved all procedures.

### EYE-TRACKING

Generally, there are two categories of eye-tracking devices classified as either remote (i.e. camera(s) placed far away) or head mounted. Remote eye trackers capture the eye/head in a limited volume of free space dictated by eye tracker's field of view and extract eye position(s) with respect to a particular plane such as a computer screen. Head-mounted eye trackers are designed so that the eye camera(s) and camera(s) observing the 'scene' are fixed together and move with the head. Even though we used very small micro-lens cameras, they were still too large to be worn by small animals. Therefore, we designed a new eye-tracking system around the stationary non-invasive restraining methods used for measuring the visual fields of wild birds (Martin 1986) that does not require the animal to support any weight from the eye-tracking cameras or electronics. We used a foam cradle with a torso-sized groove carved into a soft foam block to restrain the bird by wrapping it and the cradle with straps to restrict wing mobility. We placed the bird on a platform supported by a tripod and restrained the head by taping the bill to a suspended wire bracket (Fig. 1a). The wire bracket was then adjusted such that the subject's head was in a horizontal position, the projection of the bill was perpendicular to the frontal display screen (Fig. 1c), and both eyes were in the same elevational plane. We raised or lowered the platform until the eyes were level with the centre of the surrounding display screens and the subject was positioned such that the mid-point of a horizontal line, which connected the focal points of the two eyes, was at the centre of the experimental arena.

The bi-ocular eye-tracking system was developed by Positive Science, LLC, New York, NY, USA (\http://www.positivescience.com/). Two infrared-sensitive eye cameras, one for each eye, recorded the subject's eye movements. Each eye camera was paired with an 850 nm infrared-emitting diode (IRED). The infrared light provides controlled illumination for the eye camera to achieve optimal image processing conditions, but it is not visible to the subject (Duchowski 2007). Both eye cameras were mounted to flexible armatures secured to the metal platform with magnetized base plates, making their positions fully adjustable to potentially accommodate multiple vertebrate taxa.

For each trial, we positioned the eye cameras on each side of the subject's head at *c.* 3 cm from the eye and slightly caudal with respect to the optic axis of the eye to avoid obstructing the foveal gaze (Fig. 1a). A horizontal rod suspended two scene cameras above the subject's head. We positioned the scene cameras directly over the right and left eyes of the subject and rotated the cameras to view the right and left display screens (Fig. 1a), effectively simulating the image available to the subject's right and left retinae. The recordings of all four cameras (two eye cameras and two scene cameras) were synchronized and multiplexed into a single video file using a DVR (LOREX Vantage ECO2, Baltimore, MD, USA) and an H-264 video converter box (Blackmagic H.264 Pro Recorder, Melbourne, Vic., Australia).

The bi-ocular eye-tracking system is designed for both dark pupil and corneal reflection tracking. We used PSBinocularTracker eye-tracking software (Positive Science, LLC, New York, NY, USA) to track the pupil centroid and corneal reflection centroid, the latter of which was generated by the IRED mounted to the side of each eye camera. For each left and right eye video frame, the software identifies the pupil coordinates as the centroid of the darkest ellipse (Fig. 1b). Similarly, the corneal reflection is identified as the centroid of the brightest pixel region (with a maximum diameter limit) (Fig. 1b). Because the IRED is physically attached to eye camera, the resulting corneal reflec-

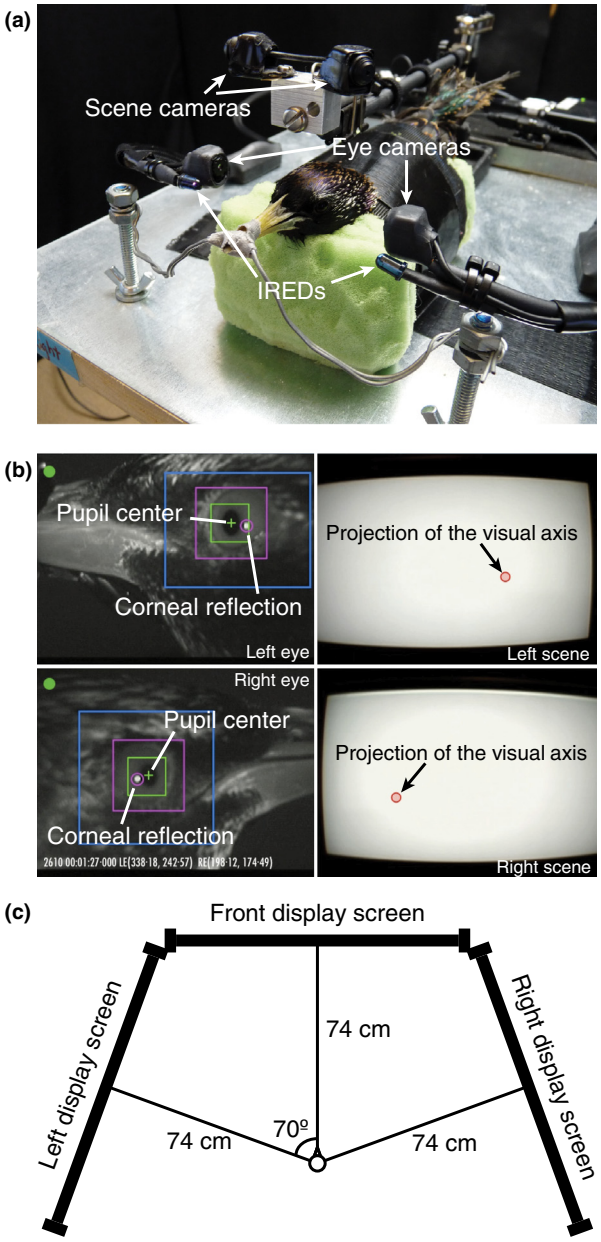


**Fig. 1.** (a) Image of the eye-tracker. The subject is restrained at the center of the experimental arena. A scene camera is positioned over each eye to allow the eye tracker to see what the bird can see. An eye camera with a corresponding infrared-emitting diode (IRED) is directed at each eye to record the movements of the eyes. (b) Image of a recording with PSBinocularTracker eye-tracking software implemented. The green crosshairs track the center of the pupil, the magenta circle tracks the position of the corneal reflection, and the red dot on the scene image displays the gaze position of each eye. (c) Schematic of experimental arena. The subject was restrained at the center of the arena with its beak pointing towards the center of the front display screen. Two additional display screens flanked the subject.

tion in the eye video frame only moves with respect to the eye camera itself. Tracking the pupil–corneal reflection relationship is important because the corneal reflection provides a point of reference with respect to the pupil centre. By comparing the relationship between the pupil centre and the corneal reflection, rather than analysing only the position of the pupil, the system was more robust to small changes in head position.

## CALIBRATION

To track the subject's gaze, each video must be calibrated to establish a relationship between the 'raw' position of the eye and its corresponding position in the scene. Once calibration is complete, the software can superimpose a marker indicating the estimated foveal gaze over the scene image. PSBinocularTracker was implemented after trials were completed and the subject was returned to its housing enclosure. Offline implementation of PSBinocularTracker software allows the researcher to display calibration stimuli after experimental stimuli (but while the subject is still restrained); thus, responses to experimental stimuli are not biased by first exposing the animal to calibration procedures. To increase the reliability of our data, we calibrated videos using two different methods: (1) a behavioural calibration and (2) an oculometric calibration. We thus conducted two independent calibrations per trial, which allowed us to verify their accuracy.

In the behavioural calibration, we input the position of the pupil and corneal reflection when the subject was fixating on known points throughout the scene image. We presented a series of randomly located mealworms on the display screens to elicit fixations to known points in the scene image. We only used fixations for calibration where the subject made an eye movement greater 7° in the direction of the stimulus. We used small calibration stimuli (4° of visual space) to provide a low-noise calibration. Stimuli should be of sufficient interest to attract the subject's attention and generate eye movements that would align the retinal centre of acute vision with the stimulus. Ideal calibration stimuli are expected to vary between species. For example, an image of a fly may be suitable for an insectivorous species, but would likely elicit fewer fixations from a granivorous species, thereby reducing the accuracy of the calibrations. We used six to ten calibration points for each eye, choosing points that were well-spaced (i.e. avoiding central clustering) over the maximum range of possible eye positions (estimated based on Martin 1986).

The oculometric calibration method determines the projection of the optic axis (i.e. the axis through the centre of the cornea and lens). To calibrate using the oculometic method, we used a handheld infrared flashlight (850 nm; Lilly Electronics, Wuhan, China) to generate a second corneal reflection on the eye (Fig. 2a). When this reflection is at the centre of the pupil, the optic axis is aligned with the light source (Hamada 1984). We moved the light source to multiple positions in the scene image and elicited eye movements with noises and hand gestures to acquire six to ten well-spaced calibration points per eye. The light source was held as close to the plane of the display screens as possible to minimize the parallax error that would occur in the vertical dimension because the scene camera was positioned above the eye (3·5 cm), rather than the exact position of the eye (Maurer 1975) (Fig. 2). The length of the handheld flashlight (13 cm) created a parallax error that was corrected for by shifting calibration points up by 3·2°.

Additionally, the gaze estimate from the oculometric calibration was consistently too far forward as a result of the eye camera not being aligned with the ever-changing position of the optic axis as the eye moves. However, this shift was compensated for by applying a correction coefficient (Appendix S1), with which we shifted the calibration point 0·32 degrees caudally per degree of offset between the optic axis and the eye camera.

One potential error with the oculometric technique is that it calibrates to the optic axis, rather than the visual axis (i.e. the projection of the centre of acute vision into the visual field). Optic axes produce the highest quality of optical images for the retina. Therefore, optic axes normally approximate visual axes, which increase the clarity of optics at centres of acute vision. For example, the offset between the optic axis

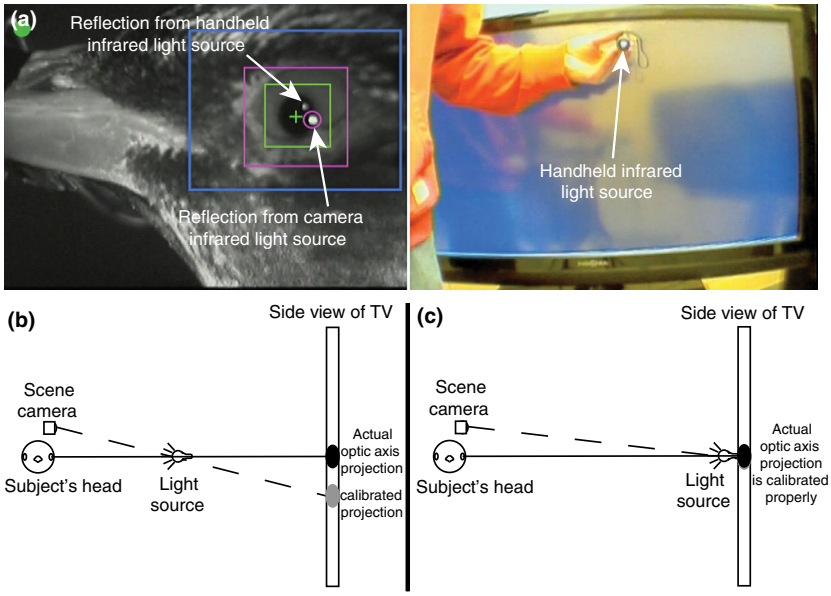


**Fig. 2.** (a) An oculometric calibration point is created when the reflection from a handheld light source falls on the center of the pupil (green crosshairs). (b) A scene camera above the subject's head creates a parallax error when the light source used to calibrate the eye-tracker is not at the same distance as the display screen. The parallax error is in the vertical dimension because the camera is vertically displaced from the subject's eye. (c) Parallax error is reduced when the light source is held at a similar depth as the display screen.

and visual axis (i.e. angle *alpha*: Fig. 3a) is <6° in humans (Nagamatsu *et al.* 2008) and pigeons (McFadden & Reymond 1985). However, that is not always the case. The binocular visual axis in kingfishers has been found to be offset from the optic axis by 40° (Pettigrew 1986). Therefore, we decided to apply a correction for angle *alpha* by determining the difference in the projections of the optic and visual axes. The projection of optic axis can be directly measured by mounting a visible light source onto the side of an opthalmoscope and aligning Purkinje images corresponding to the anterior and posterior edges of the cornea and of the lens (for detailed methods see Martin 1984). The projection of the visual axis can be inferred by measuring the location of the centre of acute vision (e.g. fovea) on a retinal topographic map (Moore *et al.* 2012) and projecting the retina into the known limits of the visual field (following Tyrrell *et al.* 2013). We determined that the angle *alpha* for the European starling was 1° (Fig. 3b), based on previously published information on its optic axis projection (57° from the beak axis; Martin 1986) and calculating the visual axis projection (56° from the beak axis) from its published retinal configuration (Dolan & Fernández-Juricic 2010) and visual field dimensions (Martin 1986).

To test the accuracy of each calibration technique, we displayed a second set of calibration stimuli that were not used to apply calibration points. We determined the difference between the calibrated gaze and the stimulus location in cases where the subject made a saccade (>7°) towards the stimulus. Additionally, we measured the similarity of the behavioural and oculometric calibration methods by comparing the difference in calibrated gaze positions at every frame for the duration of the experimental trials. Calibration accuracy and similarity of the two methods is presented in the results section.

## EXPERIMENTAL ARENA AND STIMULI

We performed an experiment that exposed starlings to different types of video stimuli to quantify their visual response. A single individual was placed in a foam holder at the centre of an arena with walls covered in black fabric and a roof of white fabric to homogenize the overhead lighting. We arranged three LCD televisions (Insignia 42″ 120 Hz, Model NS-42L260A13; Richfield, MN, USA) to the left, front and right of the animal such that the centre of each display screen was 74 cm from the centre of the arena and the left and right screens were rotated 70° inward from the frontal screen (Fig. 1c).

Each starling was exposed to twelve treatments (six treatments per eye) in a single trial using only the lateral screens. The six stimuli were videos of the following: (1) a stationary black dot, (2) a moving black dot, (3) a stationary image of a mealworm, (4) a moving mealworm, (5) a stationary image of a Harris's hawk and (6) a Harris's hawk that rises out of the grass and flies towards the subject. The frontal screen dis-

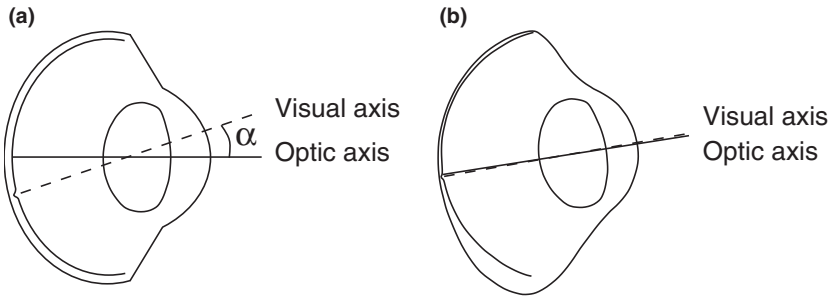


**Fig. 3.** (a) Schematic of a hypothetical bird's eye. The optic axis (solid line) is the axis passing through the center of the cornea and lens. The visual axis (dashed line) is the axis aligned with the fovea on the retina. Alpha is the angular difference between the optic and visual axes. (b) Schematic of a European starling's eye (adapted from Martin 1986). Just 1° separates the starling optic axis and visual axis (Martin 1986; Dolan & Fernández-Juricic 2010).

played a light grey background but no visual stimuli. Dots were 4 × 4 degrees and occupied 12·6 degrees$^2$ of visual space, and mealworms were 7 × 1·8 degrees and occupied 12·6 degrees$^2$ of visual space. The moving dot stimulus moved 20·1 degrees s$^{-1}$ and moved randomly within 10 degrees of its starting position. The moving mealworm stimuli were videos of a live mealworm moving 4·6 degrees s$^{-1}$ along a winding but primarily unidirectional path. The moving hawk moved 15·4 degrees s$^{-1}$ tangentially and loomed. Motion was not included as an independent factor because the properties of motion (e.g. speed and looming) were not identical across treatment types. The dots and mealworms were displayed on a light grey background and the hawk was displayed with a natural grassland backdrop. All stimulus videos were presented with similar average brightness across the whole display screen (hawk treatments = 1559·8 ± 6·3 lux, mealworm treatments = 1520·3 ± 5·7 lux, dot treatments = 1511·9 ± 2·3 lux). Treatment order was randomized and the interval between treatments was 30–60 s. Each treatment video was 12 s long, consisting of a 4 s *before phase* where the background was displayed with no stimulus, a 4 s *during phase* where the stimulus was presented, and a 4 s *after phase* where the background was again displayed with no stimulus.

### DATA OUTPUT

After applying a behavioural calibration, we used PSBinocularTracker software to output a file that contained the Cartesian coordinates for the pupil position, corneal reflection, and point of regard (i.e. the gaze position on the scene image) for both eyes at every frame of the recorded trial. The tracking of eye features was interrupted when the subject blinked the nictitating membrane, and therefore, we filtered the data to exclude points of regard when the pupil was momentarily occluded, which resulted in points of regard orders of magnitude outside the feasible range of values. An observer (SRB) manually recorded the first and last frames of every saccade during each treatment. Saccades were defined as any movement of the eye within the orbit and confirmed by the visible incidence of extraocular muscular contractions. We define a fixation as the interval of time between each saccade. We considered a fixation on a stimulus to be a subclass of fixations that were within 5° of the stimulus (Yorzinski & Platt 2014). Fixations are often associated with overt attention to gain detailed information about the target (Land & Tatler 2009). Means ± standard errors are presented throughout.

### STATISTICAL ANALYSES

To assess the probability of starlings fixating on different stimuli, we used a generalized linear model (proc Glimmix, sas v. 9·2; SAS Institute, Cary, NC, USA) with a binomial error distribution, logit link function and subject added as a random effect to account for the non-independence of within-subject measurements. We back-transformed least squares means of the model to probabilities of fixating on each type of stimulus. Treatment order was included to test for potential habituation to repeated stimulus presentation. To assess the variation in fixation duration across treatments in different intervals (before, during, and after the stimulus), we used a general linear model (proc Mixed, sas v. 9·2) repeated on subject, with treatment and the interaction between treatment and interval included as an independent factor. We used a general linear model (using the same options as our first model) to analyse the number of fixations the bird made during each stimulus (4 s stimulus duration). When an independent variable had a significant effect on our dependent variable, we ran pairwise comparisons to determine which levels differed significantly from each other.

## Results

The eye tracker reliably tracked the gaze of European starlings. The accuracy of the behavioural calibration method was 4·4° ± 0·3°, and the accuracy of the oculometric calibration method was 4·3° ± 0·8°. We found the two calibration methods to produce similar interpretations of gaze position, differing by only 4·1° ± 0·4°.

An example of the system is presented as a supplementary video with the moving mealworm treatment (Video S1). In the video, the projection of the centre of acute vision is illustrated with a moving red dot. There is a delay until the subject fixates on the hawk, which is likely to be associated with detection. As the mealworm moves, the subject tracks it by making additional saccades to keep the fovea directed at the mealworm.

The order of visual stimuli presentation did not have a significant effect on the probability of fixating on a stimulus ($F_{1,98}$ = 0·82, $P$ = 0·369). However, treatment stimulus identity did have a significant effect on the probability of fixation ($F_{5,48}$ = 3·19, $P$ = 0·014) (Fig. 4a). Starlings were significantly more likely to fixate on moving mealworms than stationary mealworms ($t_{48}$ = 2·11, $P$ = 0·040) (Fig. 4a). Additionally, starlings were more likely to fixate on moving and stationary hawks than (1) moving dots ($t_{48}$ = 2·44, $P$ = 0·018; $t_{48}$ = 2·12, $P$ = 0·040, respectively), (2) stationary dots ($t_{48}$ = 2·57, $P$ = 0·014; $t_{48}$ = 2·21, $P$ = 0·032, respectively) or (3) stationary mealworms ($t_{48}$ = 2·74, $P$ = 0·009; $t_{48}$ = 2·39, $P$ = 0·021, respectively). However, there was no significant difference between the probability of fixation on moving mealworms and moving hawks ($t_{48}$ = 0·62, $P$ = 0·536) or stationary hawks

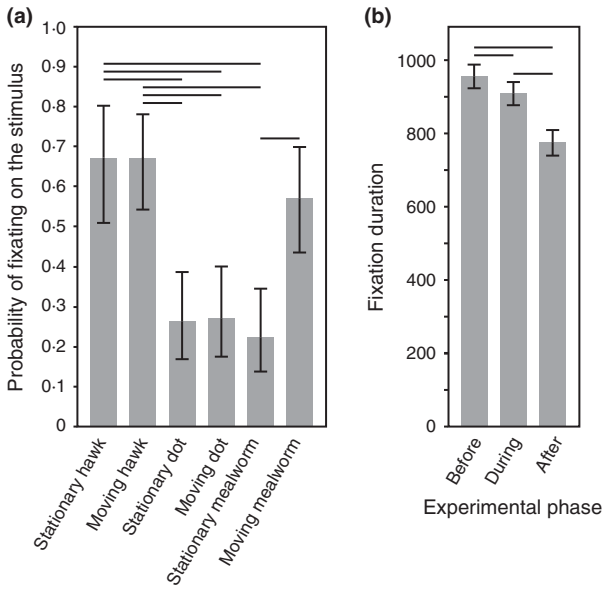


**Fig. 4.** (a) Least squares means (±SE) for the probability of fixating on each of the six stimulus types. All pairwise comparisons were made. Horizontal bars between stimuli indicate significant pairwise comparisons. (b) Least squares means (±SE) for the duration of all fixations 4 s before a stimulus appeared, during stimulus presentation, and after a stimulus disappeared. Horizontal bars indicate significant pairwise comparisons.

($t_{48}$ = 0·53, $P$ = 0·596) (Fig. 4a). We found no effect of sex ($F_{1,10}$ = 1·31, $P$ = 0·279) or eye (i.e. left vs. right; $F_{1,11}$ = 0·24, $P$ = 0·634) on the probability of fixating on a stimulus.

Fixation duration varied significantly with experimental phase ($F_{2,46}$ = 8·77, $P$ < 0·001). Fixation duration was significantly shorter after stimulus disappearance (773·1 ± 34·9 ms) than before stimulus appearance (954·6 ± 32·3 ms, $t_{46}$ = 4·07, $P$ < 0·001) or during stimulus presentation (907·1 ± 31·6 ms, $t_{46}$ = 3·06, $P$ = 0·004) (Fig. 4b). Fixation duration did not significantly differ between the during stimulus presentation phase and the before stimulus appearance phase ($t_{46}$ = 1·13, $P$ = 0·265).

For stimuli that were fixated on, we found a significant difference for the number of fixations per stimulus ($F_{5,22}$ = 3·05, $P$ = 0·031), with the moving hawk (2·58 ± 0·26) being fixated on more times than stationary hawks (1·68 ± 0·32, $t_{22}$ = 2·87, $P$ = 0·009), stationary mealworms (1·74 ± 0·40, $t_{22}$ = 2·09, $P$ = 0·049), stationary dots (1·60 ± 0·36, $t_{22}$ = 2·74, $P$ = 0·012) and moving dots (1·79 ± 0·36, $t_{22}$ = 2·13, $P$ = 0·044). No other pairwise comparisons were significant ($P$ > 0·05).

## Discussion

The eye-tracking system presented here is the first of its kind that can be used to simultaneously track the gaze of both eyes in small vertebrates with laterally placed eyes. We were able to successfully track the gaze of European starlings with an accuracy of 4·4° and elicit fixations when presenting realistic stimuli (predator and food).

We did not find a significant effect of treatment order on the probability of fixating on a stimulus, suggesting habituation was not an issue for the starlings used in this study. We did, however, find that starlings had shorter fixation durations after stimulus disappearance than before or during stimulus presentation. Because birds use saccadic eye movements to shift gaze (Walls 1942), shorter fixation durations (more saccades per unit time) increase the amount of visual space viewed by the centre of acute vision. Shorter average fixation durations after stimulus disappearance were also found in peafowl (Yorzinski & Platt 2014) and suggest birds increase visual exploratory behaviour (e.g. visual search) upon the sudden disappearance of a stimulus from the visual field. Future eye-tracking studies should have inter-stimulus intervals that are long enough for subjects to return to baseline levels of visual search (*c.* 10–30 s for starlings).

Starlings were more likely to fixate on both predator treatments (stationary and moving) and the moving mealworm than the other stimuli. Additionally, starlings made more saccades to track the moving hawk than any of the other stimuli. Simple moving and static dots were not sufficient for eliciting fixations, even though dots do elicit fixations in humans (Duchowski 2007). Similarly, a recent eye-tracking study found that humans and rhesus monkeys (*Macaca mulatta*) have different fixation patterns (Hu *et al.* 2013). Taken together, these results suggest that the visual system of non-human vertebrates may be responsive to different types of visual stimuli.

Although the dot stimuli used in this study did not consistently elicit fixations, it has been found that jumping spiders only display a turning response to dots of a certain size and speed (Zurek *et al.* 2010). Given that we did not test all combinations of dot size and speed, we cannot make final conclusions about the efficacy of dots in general for birds. We do, however, suggest future eye-tracking studies should select stimuli that are relevant to the study species (e.g. predators, conspecifics, and food), and with some degree of motion to attract attention (Abrams & Christ 2003). Careful stimulus selection before running an experiment will increase the reliability of statistical tests. For instance, the response variable detection latency would have less noise if the stimulus used consistently elicits a fixation, because the likelihood of a false positive with a random latency is reduced.

Calibration is a critical step in eye-tracking that can be particularly challenging with some species (Hamada 1984). We developed two calibration methods that are able to achieve a gaze accuracy for starlings that was similar to the accuracy of systems used to track the gaze of humans (Duchowski 2007) and peahens (Yorzinski *et al.* 2013). Both calibration methods are valid, but each has its own limitations and advantages. The oculometric calibration method does not require the same extent of cooperation from the subject (i.e. the subject is expected to make saccades towards multiple calibration stimuli in the behavioural method). However, the oculometric method is more accurate with knowledge of the retinal topography and visual field dimensions of the study species to apply the appropriate correction factors. We suggest using retinal topography and visual field data for each study species because this information is readily available for a large number of taxa (Collin 2008; Troscianko *et al.* 2012; Martin 2014) and because the position of centres of acute vision is highly variable among vertebrates (Moore *et al.* 2012).

This eye-tracking system has at least two additional limitations. First, in the present version of this eye tracker, the subject is not able to move its body and head to interact freely with its visual environment. This could partially account for the lack of success in eliciting a response with some static stimuli. While tracking the gaze of a free-moving subject is ideal, the technology is not yet available for small animals. For example, present day wireless video transmitters still weigh more than many small vertebrates used in evolutionary ecology studies. Secondly, we used video playback for our visual stimuli, which is known to have several limitations particularly for species with wider colour space like birds (i.e. commercially available television screens cannot display in the UV). Nevertheless, video playbacks have been shown to be a valid method of stimulus presentation in studies of animal behaviour (D'Eath 1998; Oliveira *et al.* 2000). Additionally, we do not foresee any major problems using real stimuli (e.g. conspecifics) instead of the TV screens as long as the distance between the subject and the stimulus allows for calibration and parallax errors are corrected (Fig. 2).

Despite the limitations, new methods to measure where an animal directs its attention provide the opportunity to answer long-standing questions in evolutionary ecology related to

mate choice, foraging, sexual selection, speciation and antipredator behaviour and could also have management implications for how animals inspect anthropogenic objects, such as bird collisions with wind turbines. It is also important to have the technology to track gaze for the first time in many of the classic model species in evolutionary ecology, which in many cases have their eyes laterally placed (e.g. Grant & Grant 2008). The eye-tracking system described in this study has the potential to assess how gaze varies in between left and right lateral visual fields, allowing us address another set of fundamental questions about the coordination and patterns of eye movement, laterality, functions of binocular vision and the functional importance of the centres of acute vision. The flexibility of this eye-tracking system to accommodate animals of various sizes also opens doors for comparative studies. By making the details of a bi-ocular eye tracker available for use, we hope to encourage the use of gaze-tracking in future studies of animal behaviour.

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## Data accessibility

Data deposited in the Dryad repository: \http://datadryad.org/resource/doi:10·5061/dryad.84hc0 (Tyrrell *et al*. 2014).

## References

Abrams, R.A. & Christ, S.E. (2003) Motion onset captures attention. *Psychological Science*, **14**, 427–432.

Bisley, J.W. (2011) The neural basis of visual attention. *The Journal of Physiology*, **589**, 49–57.

Collin, S.P. (1999). Behavioural ecology and retinal cell topography. *Adaptive Mechanisms in the Ecology of Vision* (eds S.N. Archer, M.B.A. Djamgoz, E.R. Loew, J.C. Partridge & S. Vallerga), pp. 509–535. Kluwer Academic, Dordrecht.

Collin, S.P. (2008) A web-based archive for topographic maps of retinal cell distribution in vertebrates. *Clinical and Experimental Optometry*, **91**, 85–95.

D'Eath, R.B. (1998) Can video images imitate real stimuli in animal behaviour experiments? *Biological Reviews*, **73**, 267–292.

Dixson, B.J., Grimshaw, G.M., Ormsby, D.K. & Dixson, A.F. (2014) Eye-tracking women's preferences for men's somatotypes. *Evolution and Human Behavior*, **35**, 73–79.

Dolan, T. & Fernández-Juricic, E. (2010) Retinal ganglion cell topography of five species of ground-foraging birds. *Brain, Behavior and Evolution*, **75**, 111–121.

Duchowski, A.T. (2007) *Eye Tracking Methodology: Theory and Practice*, 2nd edn. Springer-Verlag, London.

Dukas, R. & Kamil, A.C. (2000) The cost of limited attention in blue jays. *Behavioral Ecology*, **11**, 502–506.

Fernández-Juricic, E. (2012) Sensory basis of vigilance behavior in birds: synthesis and future prospects. *Behavioural Processes*, **89**, 143–152.

Grant, P.R. & Grant, B.R. (2008) *How and why Species Multiply: The Radiation of Darwin's Finches*. Princeton University Press, Princeton, New Jersey, USA.

Hamada, T. (1984) A method for calibrating the gain of the electro-oculogram (EOG) using the optical properties of the eye. *Journal of Neuroscience Methods*, **10**, 259–265.

Hu, Y.-Z., Jiang, H.-H., Liu, C.-R., Wang, J.-H., Yu, C.-Y., Carlson, S. *et al*. (2013) What interests them in the pictures? Differences in eye-tracking between rhesus monkeys and humans. *Neuroscience Bulletin*, **29**, 553–564.

Hughes, A. (1977). The topography of vision in mammals of contrasting life style: comparative optics and retinal organisation. *Handbook of Sensory Physiology* (ed. F. Crescittelli), pp. 613–756. Springer, New York.

Jones, A.G. & Ratterman, N.L. (2009) Mate choice and sexual selection: what have we learned since Darwin? *Proceedings of the National Academy of Sciences*, **106**, 10001–10008.

Kjærsgaard, A., Pertoldi, C., Loeschcke, V. & Hansen, D.W. (2008) Tracking the gaze of birds. *Journal of Avian Biology*, **39**, 466–469.

Land, M. & Tatler, B. (2009) *Looking and Acting: Vision and eye Movements in Natural Behaviour*. Oxford University Press, Oxford.

Machado, C.J. & Nelson, E.E. (2011) Eye-tracking with nonhuman primates is now more accessible than ever before. *American Journal of Primatology*, **73**, 562–569.

Martin, G.R. (1984) The visual fields of the tawny owl, *Strix aluco* L. *Vision Research*, **24**, 1739–1751.

Martin, G.R. (1986) The eye of a passeriform bird, the European starling (*Sturnus vulgaris*): eye movement amplitude, visual fields and schematic optics. *Journal of Comparative Physiology A*, **159**, 545–557.

Martin, G.R. (2014) The subtlety of simple eyes: the tuning of visual fields to perceptual challenges in birds. *Philosophical Transactions of the Royal Society B*, **369**, 20130040.

Martin, G.R., White, C.R. & Butler, P.J. (2008) Vision and the foraging technique of Great Cormorants *Phalacrocorax carbo*: pursuit or close-quarter foraging? *Ibis*, **150**, 485–494.

Maurer, D. (1975) Infant visual perception: methods of study. *Infant Perception: From Sensation to Cognition vol 1. Basic Visual Processes* (eds L.B. Cohen & P. Salapatek), pp. 1–76. Academic Press, New York.

McFadden, S.A. & Reymond, L. (1985) A further look at the binocular visual field of the pigeon. *Vision Research*, **25**, 1741–1746.

Moore, B.A., Kamilar, J.M., Collin, S.P., Dominy, N.J., Hall, M.I., Heesy, C.P. *et al*. (2012) A novel method for comparative analysis of retinal specialization traits from topographic maps. *Journal of Vision*, **12**, 1–24.

Moore, B.A., Doppler, M., Young, J.E. & Fernández-Juricic, E. (2013) Interspecific differences in the visual system and scanning behavior of three forest passerines that form heterospecific flocks. *Journal of Comparative Physiology A*, **199**, 263–277.

Nagamatsu, T., Kamahara, J., Iko, T. & Tanaka, N. (2008) One-point calibration gaze tracking based on eyeball kinematics using stereo cameras. *Proceedings of the 2008 Symposium on eye Tracking Research Applications*, pp. 95–98. ACM, Savannah, Georgia, USA.

Oliveira, R.F., McGregor, P.K., Schlupp, I. & Rosenthal, G.G. (2000) Video playback techniques in behavioural research. *Acta Ethologica*, **3**, 1.

Pettigrew, J.D. (1986) The evolution of binocular vision. *Visual Neuroscience* (eds J.D. Pettigrew, K.J. Sanderson & W.R. Levick), pp. 208–222. Cambridge University Press, London.

Ronald, K.L., Fernández-Juricic, E. & Lucas, J.R. (2012) Taking the sensory approach: how individual differences in sensory perception can influence mate choice. *Animal Behaviour*, **84**, 1283–1294.

Santana, S.E., Lynch Alfaro, J. & Alfaro, M.E. (2012) Adaptive evolution of facial colour patterns in Neotropical primates. *Proceedings of the Royal Society of London B*, **279**, 2204–2211.

Schwarz, J.S., Sridharan, D. & Knudsen, E.I. (2013) Magnetic tracking of eye position in freely behaving chickens. *Frontiers in Systems Neuroscience*, **7**, 91.

Stevens, M. (2013) *Sensory Ecology, Behaviour, and Evolution*. Oxford University Press, Oxford.

Stevens, M. & Ruxton, G.D. (2012) Linking the evolution and form of warning coloration in nature. *Proceedings of the Royal Society of London B*, **279**, 417–426.

Troscianko, T., Benton, C.P., Lovell, P.G., Tolhurst, D.J. & Pizlo, Z. (2011) Camouflage and visual perception. *Animal Camouflage: Mechanisms and Function* (eds M. Stevens & S. Merilaita), pp. 118–144. Cambridge University Press, Cambridge.

Troscianko, J., von Bayern, A.M.P., Chappell, J., Rutz, C. & Martin, G.R. (2012) Extreme binocular vision and a straight bill facilitate tool use in New Caledonian crows. *Nature Communications*, **3**, 1110.

Tyrrell, L.P., Moore, B.A., Loftis, C. & Fernández-Juricic, E. (2013) Looking above the prairie: localized and upward acute vision in a native grassland bird. *Scientific Reports*, **3**, 3231.

Tyrrell, L.P., Butler, S.R., Yorzinski, J.L. & Fernández-Juricic, E. (2014) Data from: a novel system for bi-ocular eye-tracking in vertebrates with laterally placed eyes. *Dryad Digital Repository*, doi: 10.5061/dryad.84hc0.

Voss, J. & Bischof, H.-J. (2009) Eye movements of laterally eyed birds are not independent. *The Journal of Experimental Biology*, **212**, 1568–1575.

Wallace, D.J., Greenberg, D.S., Sawinski, J., Rulla, S., Notaro, G. & Kerr, J.N.D. (2013) Rats maintain an overhead binocular field at the expense of constant fusion. *Nature*, **498**, 65–69.

Walls, G.L. (1942) *The Vertebrate Eye and its Adaptive Radiation*. Hafner, New York.

Yorzinski, J.L. & Platt, M.L. (2014) Selective attention in peacocks during predator detection. *Animal Cognition*, **17**, 767–777.

Yorzinski, J.L., Patricelli, G.L., Babcock, J.S., Pearson, J.M. & Platt, M.L. (2013) Through their eyes: selective attention in peahens during courtship. *The Journal of Experimental Biology*, **216**, 3035–3046.

Zurek, D.B., Taylor, A.J., Evans, C.S. & Nelson, X.J. (2010) The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *The Journal of Experimental Biology*, **213**, 2372–2378.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Correction coefficient for the oculometric calibration method.

**Appendix S2.** Workflow for eye-tracker video acquisition and calibration.
**Video S1.** Supplementary video.