TOCing with birds: Touchscreen-equipped operant chambers as flexible tools for avian behavioral experiments

Sandra Winters[1,2*], George R.A. Hancock[1,3], Theo Brown[1], Ossi Nokelainen[4,5], Sanni Silvasti[6], Janne Valkonen[4], Johanna Mappes[1*]

[1]Department of Organismal and Evolutionary Biology, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

[2]BioFrontiers Institute, University of Colorado Boulder, Boulder, Colorado, USA

[3]Centre for Ecology & Conservation, University of Exeter, Penryn, UK

[4]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

[5]Open Science Centre, University of Jyväskylä, Jyväskylä, Finland

[6]School of Natural Sciences, Macquarie University, Sydney, Australia

[*]Corresponding authors:

Sandra Winters
Jennie Smoly Caruthers Biotechnology Building
3415 Colorado Avenue
Boulder, CO 80303
United States of America
sandra.winters@colorado.edu

Johanna Mappes
University of Helsinki
Viikinkaari 1
00790 Helsinki
Finland
johanna.mappes@helsinki.fi

Abstract

1. Understanding how animals respond to visual stimuli is a key goal of behavioral and evolutionary ecology. Experiments documenting the behavioral responses of receivers to different stimuli are an essential explanatory tool, yet presenting stimuli that engage in complex but controlled animal behaviors – such as naturalistic movement – to biologically relevant receivers remains a significant logistical challenge.

2. We describe a touchscreen-equipped operant chamber (TOC) for documenting the reactions of wild birds to visual stimuli; the setup includes a touchscreen display designed to work with avian anatomy and visual systems. We give a comprehensive overview of the TOC and experimental protocols, provide

practical advice for generating stimuli and training birds, discuss the advantages and disadvantages of TOCs, and highlight its potential to advance research in ecology and evolution.
3. TOCs are a versatile and valuable component of the experimental toolkit in ecology and evolution. By facilitating controlled experiments with biologically relevant dynamic stimuli, TOCs allow investigations into fundamental ecological and evolutionary processes. Here, we emphasize the utility of this tool for understanding predator-prey dynamics and protective coloration in prey avoiding avian predators, but the TOC is also broadly applicable to answering a wide range of questions in ecology and evolution.

Keywords: touchscreen-equipped operant chambers; bird behavior; experimental methods; predator prey interactions

1. Introduction

In experimental behavioral ecology, psychology, neurobiology, and related fields, the paramount objective of conducting experiments is to create controlled and repeatable experimental conditions that enable researchers to dissect the underlying mechanisms governing animal behavior. Many experiments have successfully utilized videos and images to study how animals react to various stimuli, including responses to conspecifics and predators (Cuthill et al., 2000; D'eath, 1998; Fleishman et al., 1998). This approach has proven highly effective at elucidating phenomena such as social learning (e.g., (Guillette & Healy, 2017; Hämäläinen et al., 2017)), problem-solving abilities (e.g., Chow et al., 2017; Horner et al., 2013), sexual selection (e.g., Dubuc et al., 2016; Ord et al., 2002; Rosenthal, 1999; Rowland et al., 1995), and species recognition (e.g., Macedonia & Stamps, 1994; Roberts & Mendelson, 2021; Winters et al., 2020) in animals. With advancements in image and video manipulation, coupled with the ever-increasing computing power of modern computers, researchers now have access to almost limitless possibilities for creating and manipulating visual stimuli.

One critical element missing from many image and video-based studies, particularly in contexts involving behavioral interactions such as predator-prey dynamics, is interactivity. Logistical challenges, including the suitability of using touchscreens designed for humans with animal subjects and the complexity of designing dynamically responding stimuli, have posed obstacles to integrating interactive elements into experimental setups. Yet many research questions are difficult to address without stimuli that can be directed to respond in carefully controlled ways to subject animal behaviors. Predation, for example, often involves a sequence of behavioral interactions between predators and prey (Ruxton et al., 2019). Predator attack and prey survival strategies differ across the sequence, influencing the outcome and resulting in strong selection on the individuals involved. Experimentally evaluating anti-predator strategies, particularly those that involve behavioral responses to predators, often requires presenting stimulus animals that behave and respond in carefully controlled ways, necessitating interactive experimental elements.

Birds, as vital visually hunting predators of numerous insect species, exert substantial selective pressure on insect phenotypes, including their patterns and coloration (e.g., Medina et al., 2025). Despite the pivotal role of birds as predators, technical and logistical challenges have impeded their utilization in touchscreen and video-based experimental setups. Consequently, previous research has often turned to using humans as surrogate predators to address fundamental questions, such as the optimization of camouflage (e.g., Barnett et al., 2021; Troscianko et al., 2017, 2021) or the mechanisms underlying mimicry in prey species (e.g., McGuire et al., 2006; Taylor et al., 2017). While employing humans as model predators offers certain advantages, such as the ease of obtaining large sample sizes and the ability to train and instruct participants, it fails to account for crucial differences in cognition and visual systems between humans and other animals. Hence, there is a pressing need to develop interactive experimental systems that can effectively incorporate wild animals, such as birds, which play pivotal roles as predators in various ecosystems, particularly in interactions with insects.

Here, we provide a comprehensive overview of the practical application of touchscreen-equipped operant chambers (TOCs) to testing the behavioral responses of wild birds to visual stimuli, and discuss the limitations and potential pitfalls that researchers may encounter during their implementation. TOCs have long been used in cognitive psychology and neuroscience experiments involving a variety of animal species (e.g., birds: Aust & Huber, 2010; Bond & Kamil, 1998, 2002; Cook, 1992; Guigueno et al., 2015; rats: Cook et al., 2004; Dumont et al., 2021; dogs: Range et al., 2008; Zeagler et al., 2016; primates: Fagot & Paleressompoulle, 2009; Schmitt, 2018; tortoises: Mueller-Paul et al., 2014; bears: Perdue, 2016; Vonk & Beran, 2012; squirrels: Chow et al., 2017), and previous authors have provided technical descriptions of various TOC setups (e.g., Gibson et al., 2004; O'Leary et al., 2018; Pineño, 2014; Seitz et al., 2021; Steurer et al., 2012). Yet this technology has rarely been utilized in ecology and evolutionary biology, despite a clear need for tools facilitating interactive experiments. Critically, most previous studies have ignored the sensory and perceptual systems of birds, which differ from those of humans in important ways and are often key considerations in experiments in ecology and evolutionary biology, especially in research on predator prey interactions. Most previous research has also used captive birds or wild-born model species such as pigeons. We provide technical specifications and procedural considerations for running experiments using a TOC designed for use with wild-caught passerine birds (and tested with blue tits and great tits, commonly used species in visual ecology). By offering insights into common challenges and strategies to avoid them, we aim to equip researchers with the knowledge necessary to navigate the complexities of experimental design and implementation effectively.

We begin by outlining the key behavioral and perceptual characteristics of birds that are relevant to designing and implementing interactive experiments. Next, we present a detailed examination of our TOC apparatus, highlighting design considerations and technical specifications essential for its functionality. Throughout this discussion, we aim to provide researchers with insights into the practicalities of setting up and using TOCs effectively. We then discuss experimental protocols relevant to running trials with birds using TOCs, including the generation of stimuli tailored to avian visual systems and training procedures devised to acclimate wild birds to the TOC setup. We emphasize the potential for TOCs to provide controlled and repeatable experimental conditions, while also addressing the challenges associated with adapting laboratory equipment for use with wild animal subjects. Through a balanced exploration of its capabilities and challenges, we aim to facilitate informed decision-making and enhance the utility of TOC as a valuable tool in behavioral research in ecology and evolutionary biology.

2. Materials and Methods

2.1 The challenge: interactive screen experiments with birds

Experiments in which avian subjects interact with stimuli on screens are an exciting approach that could address numerous questions in ecology and evolution, yet involve a unique set of challenges. Many aspects of avian behavior and visual perception are particularly critical to consider when designing and implementing interactive screen experiments.

Birds, and especially wild birds, are not always ideal experimental participants. Much of the research that uses interactive computer games to make inferences about evolutionary processes has used humans as the target species (e.g., Bae et al., 2019; Barnett et al., 2018; Hughes et al., 2014; Rowe et al., 2024; Stevens et al., 2013; Troscianko et al., 2017) at least in part because of the ease of implementation: human experimental participants can be given precise instructions, will generally attend to the task at hand, and can ask questions if they are confused. Bird subjects, on the other hand, must be precisely trained to engage in even the simplest experimental tasks, are often inattentive, are difficult to behaviorally control, and cannot report their subjective experiences. When wild animals are used in laboratory experiments, their reactions are further complicated by the need to habituate to unfamiliar environments, adding an additional layer of complexity when interpreting their behavioral responses.

The avian visual system also differs from that of humans in a few key ways. Most birds are tetrachromats: their visual system has four classes of photoreceptor cones that detect color, which have peak absorption in the red, green, blue, and violet/ultraviolet range of the light spectrum (Kelber, 2019). Luminance (dark to light) perception is achieved through separate double cones. The precise spectral sensitivities of these cones vary across species; in particular, diurnal birds differ in the extent to which they can perceive ultraviolet light, based on the spectral tuning of the shortest wavelength cone, which can be either violet sensitive (VS, less ultraviolet perception) or ultraviolet sensitive (UVS, more ultraviolet perception). The spacing of the long and medium wavelength cones (which respond to red and green light, respectively) also differs substantially between birds and mammals, which influences color perception. So birds both see colors imperceptible to humans and differ in their sensitivity to the colors that we can see. Like humans, birds also have good color constancy (Olsson et al., 2016), and at least some species may perceive colors categorically (Caves, Green, et al., 2018). The details of avian color perception and visual modeling have been extensively described in previous publications (e.g., Endler & Mielke, 2005; Kelber, 2019; Kelber et al., 2003; Ödeen & Håstad, 2013).

In addition to color perception, birds also vary in visual acuity. Spatial acuity refers to the level of detail that can be resolved by a visual system, and is typically measured in cycles per degree (CPD, the number of contrasting lines that can be resolved within one degree of visual arc). Higher CPD values indicate the ability to resolve smaller lines, and most literature on acuity reports the peak CPD, which is the highest CPD value the animal can detect and thus the smallest spacing of lines. The majority of birds have lower peak spatial acuities than humans (i.e., they can perceive fewer details in a scene), although some raptors have comparable or, in the case of large Old World vultures and eagles, higher peak spatial acuities (Caves, Brandley, et al., 2018; Mitkus et al., 2018). Irrespective of this, the brightness contrast required for birds to detect features across spatial scales is almost an order of magnitude greater than that of mammals (Harmening et al., 2009). This difference in contrast sensitivity means that stimuli with similar colors are more likely to blend together for birds compared to humans. So, objects or patterns that are easy to detect for humans, despite only being detected by narrow edges or subtle differences in brightness, may actually be highly cryptic to birds.

Birds also differ from humans in their temporal acuity. This refers to the speed at which a visual system can process changes in the visual scene (i.e., the 'frame rate' of the eye), and is typically measured using the critical flicker fusion threshold, which identifies the frequency at which a flickering stimulus is perceived as steady (reported in hertz, Hz). Humans see at about 50-90 Hz in most circumstances (Davis et al., 2015). In birds, there is wide variation across species that have been tested, from around 40-50 Hz in some owls to very high temporal acuities of over 130 Hz in some passerines (Ault & House, 1987; Boström et al., 2016; Lafitte et al., 2022; Porciatti et al., 1989). It is important to account for the differences between human and avian visual perception when designing any visually based experiment with birds, even when color and pattern are not the focus of the experiment. For instance, a numerosity test is not valid if the subject's visual system cannot sufficiently resolve the objects meant to be counted.

Choosing appropriate technology and experimental protocols is key to meeting these challenges and running successful interactive screen experiments in a TOC. We discuss the equipment needed to present digital stimuli to avian visual systems in section 2.2, and give advice for designing interactive stimuli for birds in section 2.3. Advice on experimental procedures and training regimes suitable for birds is provided in sections 2.4 and 2.5, respectively.

2.2 The touchscreen-equipped operant chamber

Touchscreen-equipped operant chambers (TOCs) are widely customizable, but typically contain three main components: (1) a digital display that responds to animal touches, (2) a chamber in which the subject is contained, and (3) a reward system to reinforce subject behavior. Each component should be designed with the behavior and physiology of the target species in mind. Here, we provide an overview of design considerations and describe the TOCs we have successfully used with blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*) (Figure 1), although many design principles are relevant for any type of screen-based experiment.

Display

The TOC display presents stimuli and records subject responses. Touchscreen devices for humans typically use capacitive screens, which respond to changes in electrical charge, such as those created by contact with human fingers. Such screens can also be used by some other species, such as primates (e.g., Hopper et al., 2019; Leinwand et al., 2020). Bird beaks, however, do not provide electrical feedback, and so birds cannot interact with capacitive screens by pecking. An alternative technology is an infrared frame, which registers any object that pierces a grid of infrared beams projected within the frame region; this approach has previously been used with a variety of species (e.g., birds: Seitz et al., 2021; dogs: Dale et al., 2019; tortoises: Mueller-Paul et al., 2014). Infrared light is outside the perceptible range of avian visual systems, rendering the infrared grid invisible to birds (and humans). When placed in front of a computer screen, an infrared frame effectively functions as a mouse, registering "clicks" whenever the grid is disrupted. This setup can therefore record bird pecks on the screen, although it is also important to note that any disruption to the infrared grid will be treated as a "click"; the system does not differentiate, for instance, between a directed peck with the beak and an incidental brush from the tail. We currently use GreenTouch Multi-Points Infrared Touch Overlay frames in our TOC setup. An acrylic protective panel can be placed between the screen and the infrared frame to protect the screen from scratches and defecation. Note that infrared frames are versatile tools with many experimental uses, such as tracking the 2D position of animals.

Within the TOC, stimuli are typically presented on a computer screen, placed directly behind the infrared frame. When choosing a screen, it is important to consider both the visual system of the relevant subjects and the requirements of the experiment. Birds have excellent color vision (Kelber, 2019), and if accurate color representation is important for the study, then it is imperative to verify that the screen can display colors relevant to the experiment. Most high-quality screens have a large color gamut and can display >99% of sRGB color space. A key caveat of the vast majority of computer screens is their inability to display UV light, which is perceptible to many species, including birds. However, this limitation can be mitigated by designing experiments that do not rely on UV reflectance or by avoiding color altogether. A variety of tools are available for quantifying colors as perceived by birds, including pavo (Maia et al., 2018) and patternize (Van Belleghem et al., 2017) in R and the MICA toolbox (Troscianko & Stevens, 2015; van den Berg et al., 2020) in ImageJ, and methods of visualizing the influence of contrast sensitivity are becoming increasingly available (Troscianko & Osorio, 2023).

Visual acuity is also an important consideration. Birds vary substantially in spatial visual acuity (i.e., the resolution of the perceived scene, Caves, Brandley, et al., 2018), and the resolution of the screen should be sufficiently high that stimuli will not appear pixelated at relevant viewing distances. For most avian species this is unlikely to be a concern (and raptors with very high spatial acuity may be unmanageable in relatively small TOC setups), but the resolution of the screen should always be compared to that of the target visual systems under relevant viewing distances; calculations can be run using existing software (e.g., AcuityView in R, Caves & Johnsen, 2018 and MICA in ImageJ, Troscianko & Stevens, 2015; van den Berg et al., 2020).

Perhaps the most critical consideration when selecting a screen is the temporal acuity (i.e., the framerate of perceived motion) of the target visual system. Some birds have very high temporal acuity (e.g., average critical flicker fusion threshold for blue tits = 131 Hz, collared flycatchers = 141 Hz, pied flycatchers = 146 Hz; Boström et al., 2016). Because of this elevated temporal acuity, standard framerate monitors (60 Hz) may appear to flicker if the screen is not flicker-free (i.e., the screen flashes on and off at 60hz). For moving stimuli, low frame rates can produce temporal aliasing artifacts, e.g., the wagon wheel effect, and will lack the motion blur that would be generated under natural conditions (Andrews & Purves, 2005; Santon et al., 2025; Umeton et al., 2017). The smaller and/or faster the object moving is, the greater the issue will be. This is especially problematic for experiments dealing with the influences of movement on perception and tracking (Hughes et al., 2014). For static stimuli, the screen should be flicker-free. Whereas, for moving stimuli, the screen should have a refresh rate (Hz) that is greater than or equal to the critical flicker fusion of the target species. Additionally, the stimuli used should be displayed using software capable of high frame rates (GPU-enabled) and pre-rendered to allow for motion smoothing with frame interpolation, also known as subframe sampling (Andrews & Purves, 2005; Santon et al., 2025). This involves rendering stimuli at a much higher frame rate, e.g., 1000 Hz, then blending frames using Gaussian filters of a sigma that would collapse a square wave blinking at the same rate as the contrast sensitivity of the observer.

Our TOCs are equipped with Republic of Gamers Swift PG329Q 32'' screens, which can display 160% of sRGB color space, have 2560x1440 pixel resolution (i.e., about 3.6 pixels per mm, suitable for visual systems of up to around 30 CPD when viewed from up to 90 cm away), and a screen refresh rate of 175 Hz. These screens work well for blue tits (spatial acuity: approximately 6 CPD, based on closely related species, Martin, 2017; temporal acuity 131 Hz in bright lighting, Boström et al., 2016). When selecting a computer to run the TOC display, it is also important to consider the computational requirements of the screen and video playback at relevant speeds; the most important component is the GPU, which should be sufficiently powerful to achieve the necessary framerate in video playback. Our computers have NVIDIA GeForce RTX 3070 GPUs.

TOC screens should be calibrated to ensure the reliable display of stimuli in experiments. Color calibration can be achieved by comparing produced colors to underlying pixel values, then correcting for any disparity by updating the ICC profile in the display settings of the computer. This can be accomplished easily using screen calibration devices such as the Calibrite ColorChecker Studio. Many high-quality screens come pre-calibrated for color, but color calibration should ideally be re-run each time the screen is moved and periodically throughout experiments (e.g., Winters et al., 2020 calibrated experimental screens daily, but monthly calibration is likely adequate). The spatial and temporal display systems of the screen should also be verified, particularly when the desired settings are outside of the human perceptible range. The spatial resolution of modern screens is unlikely to be a concern and the relatively high spatial acuity of the human visual system (Caves, Brandley, et al., 2018) means experimenters are likely to detect aberrations, unless the target species has very high spatial acuity. The temporal resolution of the display is another matter, at least for species where the temporal acuity of the visual system exceeds that of humans (about 60 CPD, Cronin et al., 2014). A variety of tools are freely available to measure screen refresh rates and video playback frames per second. Some screens may reset to default settings on startup, so regular checking is important. If, for instance, a screen resets to a 60 Hz refresh rate, this would not be perceptible to human experimenters during playback, but the screen would appear to flicker to blue tit subjects.

In addition to the display itself, for some species it may be necessary to provide additional structures to assist in accessing the full screen area. Species that do not naturally flutter (sustained hovering) may struggle to peck targets in midair; providing strategically placed perches may be critical in these cases. We have used a wire grid or horizontal acrylic bars placed in front of the screen, which allows birds to access any screen location by hopping along the structures. The placement of any assistive structures should be carefully considered, as this can also influence interaction with the infrared frame. For instance, closer perches facilitate easier pecking of the screen, but also make incidental tail "clicks" more common; testing is likely required to optimize the design and placement of assistive structures for different species and tasks.

Reward system

Any operant chamber requires a system for delivering rewards for 'correct' responses. There are many possible reward delivery systems in a TOC; the key considerations are that reward delivery should be minimally threatening and easy to habituate in new subjects. For instance, birds that were not hand-reared are likely to respond with fear to hand-delivered rewards, so this is not ideal. We use a 'magic box' – essentially a box with a remote-controlled lid – to reward subjects. The box is filled with an appropriate reward (e.g., seeds, mealworms, etc.); the lid is remotely opened for correct responses, the subject takes a reward (we find that birds typically select a single reward item per peck into the box), and the lid is remotely closed. The magic box is a novel environment that requires a brief habitation period (see Bird training section below), but most birds accept it quickly. Trained birds also learn to associate the slight mechanical sound of the box opening with the reward, which provides immediate auditory feedback for correct responses. Alternative reward delivery systems include (but are not limited to) placing individual rewards into mechanical delivery mechanisms (e.g., a tube or a sliding tray) in the side of the box. We rely on manual control of the reward system, as this is important in sensitive phases of subject training (see section 2.5 below), but automated reward delivery is likely feasible in at least some portions of experiments. For experiments that require multiple trials per subject, rewards will ideally be small to facilitate sustained motivation. We have used small pieces of sunflower seeds, peanuts, and mealworms as rewards for blue and great tits.

Chamber

The TOC chamber should contain all necessary animal welfare items and be designed with experimental convenience in mind. Most birds are stressed by observation by humans, so an opaque chamber minimizes distress and distractions. A small viewing window opposite the screen facilitates observations; a sliding cover allows dynamic control of the window size. Some birds are noticeably disturbed by even very small viewing windows, therefore the interior of the chamber can also be monitored via a tablet affixed to the outside of the box, with the rear camera positioned over a hole. Trials can be recorded using the tablet or a camera attached to the inside of the chamber. We recommend using internal cameras that can be wirelessly controlled via an app installed on the exterior tablet (e.g. GoPros) to facilitate easy control of video acquisition without disturbing subjects. Fisheye camera settings can record virtually the entire chamber with a single camera, although it is noteworthy that linear measurements will not be possible from resulting videos. The interior of the chamber should be lit by a neutrally colored bulb or light strip. Most bird species should be provided with at least one perch; we position a perch in the approximate middle of the box, from which the subject should be able to see the whole display. Additional access panels can be added to provide access to the internal portion of the chamber. When designing these additions, important considerations include the potential need to access the entire floor and display (for cleaning and to remove any discarded food items), as well as any internal equipment or other items (e.g., magic box, cameras, food/water bowls, etc.) with minimal disruption to a bird in the chamber. For instance, we find that a hinged panel along the bottom of the box is useful for cleaning, adding and removing food and water dishes, and raking out discarded food items (small telescopic back-scratchers work well for the latter); access panels on the side of the TOC (e.g., a hole with a sliding cover) are useful for adjusting other internal components. We line the bottom of the chamber with paper to facilitate easy cleaning. Many birds will use any available surfaces within the chamber for perching, including cameras, lights, air vents, etc. Minimizing access to these objects (e.g., with coverings that prevent perching) and providing appropriate perches will help to control subject locations. One wall of the TOC chamber is hinged to facilitate thorough cleaning and reorganization. We transfer birds into and out of the TOC chamber by hand (see below); when catching a bird, a net or cloth sheet can be draped over the open portion of the box to help prevent escape. Our TOC chambers are approximately 79 (length) x 90 (height) x 90 (depth) cm; this size allows for naturalistic bird movement and fits a relatively large touchscreen display.

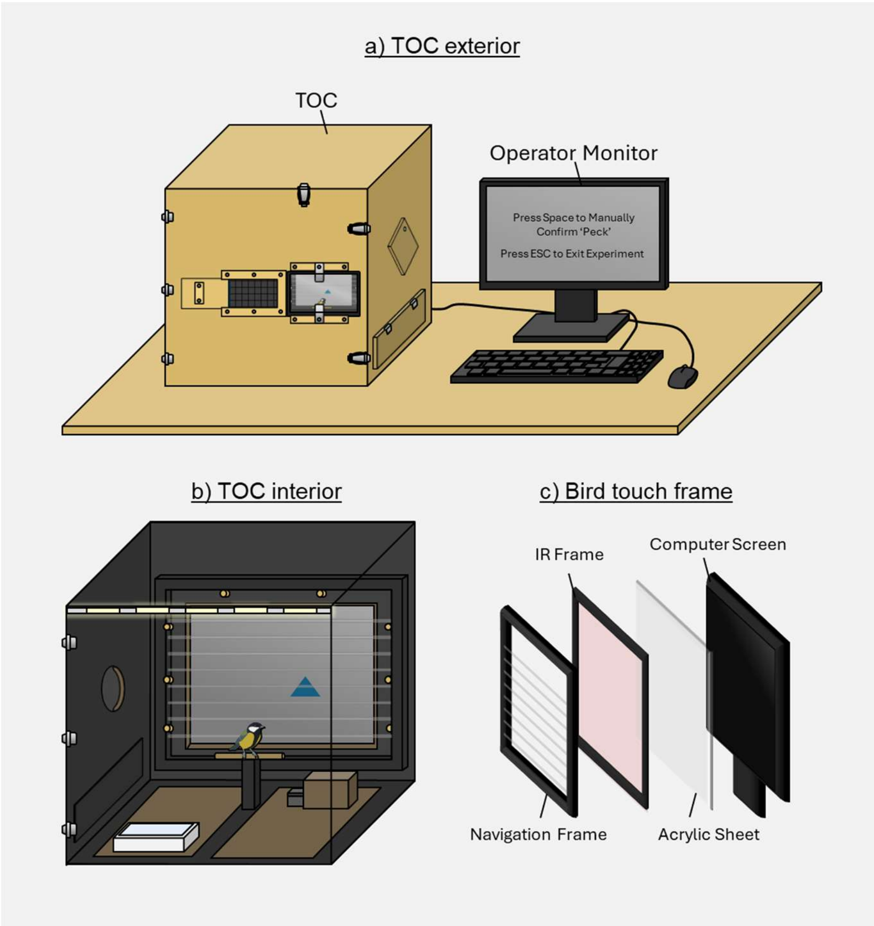

Figure 1: The TOC. (a) exterior view of the TOC setup. The interior of the TOC is visible through a gauze mesh (viewing slot), a camera hole for a tablet, and a bluetooth connected internal camera, e.g., a GoPro. (b) interior view of the TOC with example triangle stimuli. (c) touch frame tailored for use by a bird. A frame with horizontal acrylic bars for navigation is placed in front of the infrared (IR) touch frame, and a thin 2-3 mm thick acrylic sheet is placed behind the frame to protect the computer screen used to display stimuli behind it.

2.3 Designing stimuli

A wide range of stimuli can be presented via the TOC screen, the nature of which will vary based on the experimental question. Stimuli should be designed with bird vision in mind, accounting for the color, acuity, and motion perception of the subject's visual system (see reviews: Caves, Brandley, et al., 2018; Donner, 2021; Kelber, 2019). UV colors are best avoided, unless the TOC screen is capable of displaying them (virtually all screens cannot). Simply using the human-visible (i.e., red, green, and blue) portion of an object or scene can also be problematic, since the lack of UV reflectance can alter perception within the avian visual system. For instance, an object that has equally high reflectance across all visible wavelengths – thereby appearing white – would look different to birds (although not to humans) without the UV component of the color; this would be akin to removing all blue reflectance from scenes presented to humans (Figure 2). Therefore, it is best to avoid depicting objects that naturally reflect UV light, as they may appear strange to avian subjects on a UV-dark screen. Some animals and many natural scenes reflect minimal UV (e.g., foliage, Arnold et al., 2010; Grant et al., 2003; Willink et al., 2014; but see Tedore & Nilsson, 2019), so despite this limitation, there are many options for generating naturalistic stimuli. The spatial and temporal resolution of the scene should also be suitable for the target species, based on the parameters discussed above in sections 2.1 and 2.2. The appearance of a given scene to a particular visual system can be modeled using existing software (e.g., Troscianko & Stevens, 2015; Maia et al., 2018; Caves & Johnsen, 2018).

Figure 2: An original image (left) and the same image with the blue channel set to zero (right). This is akin to presenting UV-reflective scenes to birds without the UV channel. Note that despite there being few blue colors in the original image, the loss of the blue channel creates substantial differences in color. White and purple are particularly distorted because they both include blue light. Purple is a non-spectral color that results from stimulating SW (short wavelength, i.e. blue) and LW (long wavelength, i.e. red) reflectance; birds can perceive multiple nonspectral colors that include the UV channel (e.g., UV-red, UV-green, UV-yellow; Stoddard et al., 2020). Yellow color discrimination is also impaired, because it is the opponent to blue in human color perception. These color anomalies highlight the importance of considering the color composition of stimuli presented to UV-sensitive birds on a UV-dark display.

A wide variety of experimental designs can be implemented using a TOC, including preference tests and reaction time experiments. Depending on the task, peck location ([x, y] coordinates), peck accuracy (linear distance to target region), or peck latency (time to peck) may be relevant response variables. Peck location and accuracy should be straightforward to save based on the absolute and relative location of the peck, respectively. But when peck accuracy is a critical variable, it is important to verify that the birds are capable of the type of responses required by the task. For instance, preliminary testing has found that blue tits are only able to actively pursue a stimulus moving at up to 8-10 cm/s due to limitations related to their need for assistive structures as they move across the screen; this means that trials measuring the accuracy of pecks at faster-moving stimuli may not be suitable for this species in the current TOC setup (Silvasti et al., 2024). For peck latency, it is important to consider the time at which to start the “clock”. This could be at stimulus presentation, although in this case it is critical to control for differences in subject attention at the start of the trial. Another option is to integrate the “stopwatch” into the trial design, for instance by having a first peck trigger a change in stimuli and the start of the clock, and a second peck for which latency is recorded (as implemented by Brown et al. in prep). In experimental designs that include interactive elements – such as a peck in a certain location triggering a change in stimuli – it is important to consider the potential for non-intentional pecks (e.g., from the tail, see section 2.2) to trigger these events and to prepare appropriate experimental protocols to deal with this eventuality. In trials that include moving stimuli, it is often useful for downstream analysis to extract and save the stimulus appearance (e.g., the relevant video frame) at the time of the peck for potential downstream processing, or at least the relevant data to reconstruct the frame exactly as it was.

Birds will need training to use the TOC (see section 2.5 below), necessitating the use of training stimuli. It is critical that these be carefully selected to minimize any confounding effects in experimental trials. For instance, if two colored targets are used in experimental trials, then training stimuli that include targets intermediate between these two colors (in bird color space) may be suitable. All training stimulus characteristics, which may also include target shape, patterning, movement patterns, etc., should be carefully considered to prevent biasing subjects toward or away from any of the experimental stimuli. Training stimuli should also be designed to familiarize subjects with using the TOC in whatever ways are necessary for the experimental trials. Some trials, for instance, require birds to interact with targets at different locations across the screen, but we have observed that many birds have initial preferences that influence this interaction (e.g., avoidance of corners is common). Designing training stimuli that habituate subjects to general screen use is therefore critical. Setting training criteria, such as requiring a specific number of successful training trials that meet various conditions (e.g., a subject must successfully peck a target in each corner, must accept particular colors/shapes/speeds, etc.), can ensure that subjects are adequately trained to use the TOC before experimental trials commence.

Stimuli can be prepared and displayed using a wide variety of programs and coding environments. A key choice is between pre-generated and live rendered stimuli. The former allows for precise control and verification, but can be unwieldy and require substantial disk space for larger experiments. When stimuli are live rendered, it is important to save all critical data for each stimulus so as to not lose information that may be important in downstream processing. Live rendering also limits the ability to control for temporal aliasing artefacts. As motion blur generated by whole body movement requires predetermined flight paths. Any image, video, or animation editing program would be suitable for generating stimuli. When stimuli are presented, the key consideration is that the software used is capable of the spatial and temporal resolutions required for the relevant avian visual system (e.g. a high video frame rate; see sections 2.1 and 2.2). Psychtoolbox-3 (Brainard, 1997) in MATLAB is a good option for presenting stimuli at high frame rates and can be used with both pre-generated and live rendered trials.

2.4 Experimental Procedures

Working with wild birds in the Touchscreen Operant Chamber (TOC) requires attention to practical considerations and careful handling to ensure accurate, ecologically relevant results. The use of wild-caught birds can introduce unique challenges, as they may be easily distracted, lose motivation, or respond negatively to unintended stimuli. In this section, we outline best practices to help maximize training efficiency, minimize distractions, and prioritize animal welfare.

Reducing distractions and maintaining bird focus is important in TOC experiments. Wild birds are highly sensitive to external stimuli, which can affect their responsiveness within the TOC. To minimize distractions, we recommend conducting experiments in a fully darkened, sound-isolated room, with only minimal lighting necessary for running the apparatus. External noises, such as doors slamming, and light or shadows near the viewing slot, should be carefully controlled to avoid influencing the birds' responses, especially when measuring traits such as delay of attack or attention to stimuli. Additionally, experimenters should avoid giving subtle cues, such as leaning forward or making noise as birds approach the screen, as these may inadvertently condition the bird to associate cues with pecking the stimuli.

It is also critical to promote and monitor bird motivation across trials. To ensure that birds are motivated to engage with the TOC, food deprivation for a brief period before testing can be effective in increasing the value of food rewards. This period varies across individuals and species but typically ranges between 30 and 120 minutes for blue and great tits. It is important to clear all food residues from the box before testing to avoid distractions that could reduce motivation. Throughout the trial, monitoring bird motivation is essential. Signs of reduced motivation include sitting on a perch without engaging with the stimuli or ignoring rewards. In such cases, a short break (20-30 minutes) often helps the bird regain focus. Conversely, signs of extreme hunger, such as puffed feathers, ground perching, and immobility, should be watched closely, especially if the bird experiences consecutive unsuccessful trials.

Careful organization of the interior of the TOC can help minimize disruptions and facilitate smoothly running trials. It is helpful to place physical stimuli or the reward box close to the side access slots for easy refilling or adjustment with minimal disruption to the bird’s focus. After accessing the TOC or adjusting its setup, a short 2-3 minute rest period is beneficial, allowing the bird to reorient and regain focus before resuming the trial.

As with all animal research, appropriate permits, licensing, and ethical approvals are important, in accordance with the specific regulations of the research location. Whenever possible, wild experimental birds should have an identification ring to prevent unintentional reuse of the same individual in multiple trials, and bird ringing requires additional licensing in some areas. In our protocol, each bird receives both a standard metal ring and a color plastic ring to facilitate identification.

These practical guidelines can help researchers maximize the effectiveness of TOC sessions, prioritize animal welfare, and ensure reliable, ecologically relevant data from studies with wild birds.

2.5 Bird Training

The design and implementation of training protocols must be tailored to the specific objectives and requirements of each study to ensure that birds are adequately prepared for the tasks they will encounter. Fundamentally, the goal is to train birds to associate a chosen stimulus with a reward. Bird training can be divided into three distinct stages: pre-training, association training, and digital stimulus training.

Pre-training: Familiarization with the stimulus and reward system

The purpose of pre-training is to build the bird’s confidence in approaching and pecking at the training stimulus while independently taking a reward from the designated reward system (e.g., the ‘magic box’). To begin, birds are introduced to physical training stimuli (such as colored disks or shapes) with seeds attached to or hidden beneath them to encourage interaction. Simultaneously, the reward system is left fixed open and filled with seeds to allow the bird to become accustomed to it. Repetition of these steps is beneficial, as it helps create a strong foundation for further training.

Association training: Linking the physical stimulus to the reward

This stage is the most sensitive part of the training process and requires careful handling to avoid accidentally reinforcing unwanted behaviors. Here, the aim is for birds to associate pecking the physical stimulus with receiving an immediate reward from the system. This is achieved by placing the stimulus close to the reward system and opening it upon a successful peck. Gradually, the stimulus is moved closer to the touchscreen or designated experimental area until the bird confidently pecks the stimulus in different positions. This step-by-step association prepares the bird to respond to a digital stimulus later on.

Digital stimulus training: Transitioning to the experimental stimulus

In the final stage, birds are trained to peck at a digital stimulus on the TOC screen and then receive a reward. Birds generally transition well from a physical to a digital stimulus, particularly when the digital version closely resembles the initial training stimulus in color and shape. Depending on the experiment's needs, further digital training steps may be added to solidify the bird's confidence and ensure they consistently associate the digital stimulus with a reward.

Troubleshooting common challenges in training wild birds

Training wild birds in the TOC presents specific challenges, including potential hesitation to peck at the stimulus or distraction by the reward system. Early in training, it can be helpful to reward "near misses," where the bird approaches but does not quite peck the stimulus, as this reduces frustration. However, as training progresses, only precise pecks should be rewarded. The sound of the reward system opening may initially deter birds but typically becomes a positive reinforcement as they associate it with a reward. Ensuring that the reward system conceals the reward entirely when closed is critical, as visible rewards can distract the bird from engaging with the stimulus.

In experiments we have conducted, the training process for blue tits and great tits has taken around 1-7 days per bird in experiments we have conducted (Brown et al., In prep.; Hancock et al., in press; (Silvasti et al., 2024)).

3. Concluding Remarks

TOCs present a versatile research tool for investigating the behavior, psychology and visual systems of animals, due to their interactivity and automatic acquisition of data. While we focus on their utilization for predator-prey interaction experiments with wild birds, they can be used for a wide range of species, particularly when using an IR touch frame, and ecological contexts.

Acknowledgements

We thank the staff at Lammi and Konnevesi Biological Stations, especially John Loehr, Hanna Rosti, Esa-Pekka Tuominen, and Joni Uusitalo for help with research logistics and construction. Special thanks to the many student interns who spent long days in dark, cold bird rooms during the dark, cold Finnish winter. This research was supported by the Wild Animal Phenotyping Research Infrastructure (grant no. 1353545), the Research Council of Finland (awards 345091, 355995, 1355995 to JM; award 349613 to SW), and the Finnish Society of Sciences and Letters (to JM and SW).

References

Andrews, T., & Purves, D. (2005). The wagon-wheel illusion in continuous light. *Trends in Cognitive Sciences*, *9*(6), 261–263. https://doi.org/10.1016/j.tics.2005.04.004

Arnold, S. E. J., Faruq, S., Savolainen, V., McOwan, P. W., & Chittka, L. (2010). FReD: The Floral Reflectance Database — A Web Portal for Analyses of Flower Colour. *PLoS ONE*, *5*(12), e14287. https://doi.org/10.1371/journal.pone.0014287

Ault, S. J., & House, E. W. (1987). Electroretinographic Responses of the Great Horned Owl (bubo Virginianus). *Journal of Raptor Research*, *21*(4), 10.

Aust, U., & Huber, L. (2010). Representational insight in pigeons: Comparing subjects with and without real-life experience. *Animal Cognition*, *13*(2), 207–218. https://doi.org/10.1007/s10071-009-0258-

4

Bae, S., Kim, D., Sherratt, T. N., Caro, T., & Kang, C. (2019). How size and conspicuousness affect the efficacy of flash coloration. *Behavioral Ecology*, *30*(3), 697–702. https://doi.org/10.1093/beheco/arz006

Barnett, J. B., Michalis, C., Scott-Samuel, N. E., & Cuthill, I. C. (2018). Data from: Distance-dependent defensive coloration in the poison frog Dendrobates tinctorius, Dendrobatidae. *Proceedings of the National Academy of Sciences*, *115*, 6416–6421. https://doi.org/10.5061/dryad.3kd4134

Barnett, J. B., Michalis, C., Scott-Samuel, N. E., & Cuthill, I. C. (2021). Colour pattern variation forms local background matching camouflage in a leaf-mimicking toad. *Journal of Evolutionary Biology*, *34*(10), 1531–1540. https://doi.org/10.1111/jeb.13923

Bond, A. B., & Kamil, A. C. (1998). Apostatic selection by blue jays produces balanced polymorphism in virtual prey. *Nature*, *395*(6702), 594–596. https://doi.org/10.1038/26961

Bond, A. B., & Kamil, A. C. (2002). Visual predators select for crypticity and polymorphism in virtual prey. *Nature*, *415*(6872), 609–613. https://doi.org/10.1038/415609a

Boström, J. E., Dimitrova, M., Canton, C., Håstad, O., Qvarnström, A., & Ödeen, A. (2016). Ultra-Rapid Vision in Birds. *PLOS ONE*, *11*(3), e0151099. https://doi.org/10.1371/journal.pone.0151099

Brainard, D. H. (1997). The psychophysics toolbox. *Spatial Vision*, *10*(4), 433–436.

Brown, T., Winters, S., Nokelainen, O., Tammaru, T., & Mappes, J. (In prep.). Testing flash coloration in wild avian predators. *Unpublished Manuscript*.

Caves, E. M., Brandley, N. C., & Johnsen, S. (2018). Visual Acuity and the Evolution of Signals. *Trends in Ecology & Evolution*, *33*(5), 358–372. https://doi.org/10.1016/j.tree.2018.03.001

Caves, E. M., Green, P. A., Zipple, M. N., Peters, S., Johnsen, S., & Nowicki, S. (2018). Categorical perception of colour signals in a songbird. *Nature*, *560*(7718), 365–367. https://doi.org/10.1038/s41586-018-0377-7

Caves, E. M., & Johnsen, S. (2018). AcuityView: An R package for portraying the effects of visual acuity on scenes observed by an animal. *Methods in Ecology and Evolution*, *9*, 793–797. https://doi.org/10.1111/2041-210X.12911

Chow, P. K. Y., Leaver, L. A., Wang, M., & Lea, S. E. G. (2017). Touch screen assays of behavioural flexibility and error characteristics in Eastern grey squirrels (Sciurus carolinensis). *Animal Cognition*, *20*(3), 459–471. https://doi.org/10.1007/s10071-017-1072-z

Cook, R. G. (1992). Acquisition and transfer of visual texture discriminations by pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, *18*(4), 341–353. https://doi.org/10.1037/0097-7403.18.4.341

Cook, R. G., Geller, A. I., Zhang, G.-R., & Gowda, R. (2004). Touchscreen-enhanced visual learning in rats. *Behavior Research Methods, Instruments, & Computers*, *36*(1), 101–106. https://doi.org/10.3758/BF03195555

Cronin, T. W., Johnsen, S., Marshall, N. J., & Warrant, E. J. (2014). *Visual ecology*. Princeton University Press.

Cuthill, I. C., Hart, N. S., Partridge, J. C., Bennett, A. T. D., Hunt, S., & Church, S. C. (2000). Avian colour vision and avian video playback experiments. *Acta Ethologica*, *3*(1), 29–37. https://doi.org/10.1007/s102110000027

Dale, R., Palma-Jacinto, S., Marshall-Pescini, S., & Range, F. (2019). Wolves, but not dogs, are prosocial in a touch screen task. *PLOS ONE*, *14*(5), e0215444. https://doi.org/10.1371/journal.pone.0215444

Davis, J., Hsieh, Y.-H., & Lee, H.-C. (2015). Humans perceive flicker artifacts at 500 Hz. *Scientific Reports*, *5*(1), 7861. https://doi.org/10.1038/srep07861

D’eath, R. B. (1998). Can video images imitate real stimuli in animal behaviour experiments? *Biological Reviews*, *73*(3), 267–292. https://doi.org/10.1017/S0006323198005179

Donner, K. (2021). Temporal vision: Measures, mechanisms and meaning. *Journal of Experimental Biology*, *224*(15), jeb222679. https://doi.org/10.1242/jeb.222679

Dubuc, C., Allen, W. L., Cascio, J., Lee, D. S., Maestripieri, D., Petersdorf, M., Winters, S., & Higham, J. P. (2016). Who cares? Experimental attention biases provide new insights into a mammalian sexual signal. *Behavioral Ecology*, *27*, 68–74. https://doi.org/10.1093/beheco/arv117

Dumont, J. R., Salewski, R., & Beraldo, F. (2021). Critical mass: The rise of a touchscreen technology community for rodent cognitive testing. *Genes, Brain and Behavior*, *20*(1), e12650. https://doi.org/10.1111/gbb.12650

Endler, J. A., & Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*, *86*(4), 405–431.

Fagot, J., & Paleressompoulle, D. (2009). Automatic testing of cognitive performance in baboons maintained in social groups. *Behavior Research Methods*, *41*(2), 396–404. https://doi.org/10.3758/BRM.41.2.396

Fleishman, L. J., McClintock, W. J., D'Eath, R. B., Brainard, D. H., & Endler, J. A. (1998). Colour perception and the use of video playback experiments in animal behaviour. *Animal Behaviour*, *56*(4), 1035–1040. https://doi.org/10.1006/anbe.1998.0894

Gibson, B. M., Wasserman, E. A., Frei, L., & Miller, K. (2004). Recent advances in operant conditioning technology: A versatile and affordable computerized touchscreen system. *Behavior Research Methods, Instruments, & Computers*, *36*(2), 355–362. https://doi.org/10.3758/BF03195582

Grant, R. H., Heisler, G. M., Gao, W., & Jenks, M. (2003). Ultraviolet leaf reflectance of common urban trees and the prediction of reflectance from leaf surface characteristics. *Agricultural and Forest Meteorology, UV Radiation and Its Impacts on Agriculture and Forests*, *120*(1), 127–139. https://doi.org/10.1016/j.agrformet.2003.08.025

Guigueno, M. F., MacDougall-Shackleton, S. A., & Sherry, D. F. (2015). Sex Differences in Spatial Memory in Brown-Headed Cowbirds: Males Outperform Females on a Touchscreen Task. *PLOS ONE*, *10*(6), e0128302. https://doi.org/10.1371/journal.pone.0128302

Guillette, L. M., & Healy, S. D. (2017). The roles of vocal and visual interactions in social learning zebra finches: A video playback experiment. *Behavioural Processes, CO3: In Honor of Karen Hollis*, *139*, 43–49. https://doi.org/10.1016/j.beproc.2016.12.009

Hämäläinen, L., Rowland, H. M., Mappes, J., & Thorogood, R. (2017). Can video playback provide social information for foraging blue tits? *PeerJ*, *5*, e3062. https://doi.org/10.7717/peerj.3062

Hancock, G. R. A., Lehtonen, H., Brown, T., Ejjite, A., Nokelainen, O., Mappes, J., & Winters, S. (In prep.). Biologically inspired warning patterns deter birds from wind turbines. *Unpublished Manuscript*.

Harmening, W. M., Nikolay, P., Orlowski, J., & Wagner, H. (2009). Spatial contrast sensitivity and grating acuity of barn owls. *Journal of Vision*, *9*(7), 13. https://doi.org/10.1167/9.7.13

Hopper, L. M., Egelkamp, C. L., Fidino, M., & Ross, S. R. (2019). An assessment of touchscreens for testing primate food preferences and valuations. *Behavior Research Methods*, *51*(2), 639–650. https://doi.org/10.3758/s13428-018-1065-0

Horner, A. E., Heath, C. J., Hvoslef-Eide, M., Kent, B. A., Kim, C. H., Nilsson, S. R. O., Alsiö, J., Oomen, C. A., Holmes, A., Saksida, L. M., & Bussey, T. J. (2013). The touchscreen operant platform for testing learning and memory in rats and mice. *Nature Protocols*, *8*(10), 1961–1984. https://doi.org/10.1038/nprot.2013.122

Hughes, A. E., Troscianko, J., & Stevens, M. (2014). Motion dazzle and the effects of target patterning on capture success. *BMC Evolutionary Biology*, *14*(1). https://doi.org/10.1186/s12862-014-0201-4

Kelber, A. (2019). Bird colour vision – from cones to perception. *Current Opinion in Behavioral Sciences*, *30*, 34–40. https://doi.org/10.1016/j.cobeha.2019.05.003

Kelber, A., Vorobyev, M., & Osorio, D. (2003). Animal colour vision – behavioural tests and physiological concepts. *Biological Reviews*, *78*(1), 81–118. https://doi.org/10.1017/S1464793102005985

Lafitte, A., Sordello, R., Legrand, M., Nicolas, V., Obein, G., & Reyjol, Y. (2022). A flashing light may not be that flashy: A systematic review on critical fusion frequencies. *PLOS ONE*, *17*(12), e0279718. https://doi.org/10.1371/journal.pone.0279718

Leinwand, J. G., Huskisson, S. M., Egelkamp, C. L., & Hopper, L. M. (2020). Within- and between-species variation in the responses of three primate species to a touchscreen gambling task. *Learning and Motivation*, *71*, 101635. https://doi.org/10.1016/j.lmot.2020.101635

Macedonia, J. M., & Stamps, J. A. (1994). Species Recognition in Anolis grahami (Sauria, Iguanidae): Evidence from Responses to Video Playbacks of Conspecific and Heterospecific Displays. *Ethology*, *98*(3–4), 246–264. https://doi.org/10.1111/j.1439-0310.1994.tb01074.x

Maia, R., Gruson, H., Endler, J. A., & White, T. E. (2018). *pavo 2.0: New tools for the spectral and spatial analysis of colour in R*. https://doi.org/10.1101/427658

Martin, G. R. (2017). *The Sensory Ecology of Birds*. Oxford University Press.

McGuire, L., Van Gossum, H., Beirinckx, K., & Sherratt, T. N. (2006). An empirical test of signal detection theory as it applies to Batesian mimicry. *Behavioural Processes*, *73*(3), 299–307. https://doi.org/10.1016/j.beproc.2006.07.004

Medina, I., Exnerová, A., Daňková, K., Penacchio, O., Sherratt, T. N., Albrecht, T., Baidya, S., Bosque, R. J., Brown, H., Burdfield-Steel, E., Cain, K. E., Cezário, R. R., Chiari, Y., Esquivel, C., Ferreira, R. G., Franklin, A. M., Garvey, A., Guchu, S., Hastings, B. T., … Allen, W. L. (2025). Global selection on insect antipredator coloration. *Science*, *389*(6767), 1336–1341. https://doi.org/10.1126/science.adr7368

Mitkus, M., Potier, S., Martin, G. R., Duriez, O., & Kelber, A. (2018). Raptor Vision. In M. Mitkus, S. Potier, G. R. Martin, O. Duriez, & A. Kelber, *Oxford Research Encyclopedia of Neuroscience*. Oxford University Press. https://doi.org/10.1093/acrefore/9780190264086.013.232

Mueller-Paul, J., Wilkinson, A., Aust, U., Steurer, M., Hall, G., & Huber, L. (2014). Touchscreen performance and knowledge transfer in the red-footed tortoise (*Chelonoidis carbonaria*). *Behavioural Processes*, *106*, 187–192. https://doi.org/10.1016/j.beproc.2014.06.003

Ödeen, A., & Håstad, O. (2013). The phylogenetic distribution of ultraviolet sensitivity in birds. *BMC Evolutionary Biology*, *13*(1), 36. https://doi.org/10.1186/1471-2148-13-36

O'Leary, J. D., O'Leary, O. F., Cryan, J. F., & Nolan, Y. M. (2018). A low-cost touchscreen operant chamber using a Raspberry Pi™. *Behavior Research Methods*, *50*(6), 2523–2530. https://doi.org/10.3758/s13428-018-1030-y

Olsson, P., Wilby, D., & Kelber, A. (2016). Quantitative studies of animal colour constancy: Using the chicken as model. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1830), 20160411. https://doi.org/10.1098/rspb.2016.0411

Ord, T. J., Peters, R. A., Evans, C. S., & Taylor, A. J. (2002). Digital video playback and visual communication in lizards. *Animal Behaviour*, *63*(5), 879–890. https://doi.org/10.1006/anbe.2001.1983

Perdue, B. M. (2016). The Effect of Computerized Testing on Sun Bear Behavior and Enrichment Preferences. *Behavioral Sciences*, *6*(4), 19. https://doi.org/10.3390/bs6040019

Pineño, O. (2014). ArduiPod Box: A low-cost and open-source Skinner box using an iPod Touch and an Arduino microcontroller. *Behavior Research Methods*, *46*(1), 196–205. https://doi.org/10.3758/s13428-013-0367-5

Porciatti, V., Fontanesi, G., & Bagnoli, P. (1989). The electroretinogram of the little owl (*Athene noctua*).

Vision Research, *29*(12), 1693–1698. https://doi.org/10.1016/0042-6989(89)90151-X

Range, F., Aust, U., Steurer, M., & Huber, L. (2008). Visual categorization of natural stimuli by domestic dogs. *Animal Cognition*, *11*(2), 339–347. https://doi.org/10.1007/s10071-007-0123-2

Roberts, N. S., & Mendelson, T. C. (2021). Identifying female phenotypes that promote behavioral isolation in a sexually dimorphic species of fish *Etheostoma zonale*. *Current Zoology*, *67*(2), 225–236. https://doi.org/10.1093/cz/zoaa054

Rosenthal, G. G. (1999). Using Video Playback to Study Sexual Communication. *Environmental Biology of Fishes*, *56*(3), 307–316. https://doi.org/10.1023/A:1007597604793

Rowe, Z. W., Scott-Samuel, N. E., & Cuthill, I. C. (2024). How background complexity impairs target detection. *Animal Behaviour*, *210*, 99–111. https://doi.org/10.1016/j.anbehav.2024.02.003

Rowland, W. J., Bolyard, K. J., Jenkins, J. J., & Fowler, J. (1995). Video playback experiments on stickleback mate choice: Female motivation and attentiveness to male colour cues. *Animal Behaviour*, *49*(6), 1559–1567. https://doi.org/10.1016/0003-3472(95)90077-2

Ruxton, G. D., Allen, W. L., Sherratt, T. N., & Speed, M. P. (2019). *Avoiding Attack: The Evolutionary Ecology of Crypsis, Aposematism, and Mimicry*. Oxford University Press.

Santon, M., Troscianko, J., Heatubun, C. D., & How, M. J. (2025). Stealth and deception: Adaptive motion camouflage in hunting broadclub cuttlefish. *Science Advances*, *11*(13), eadr3686. https://doi.org/10.1126/sciadv.adr3686

Schmitt, V. (2018). *Implementing new portable touchscreen-setups to enhance cognitive research and enrich zoo-housed animals* (p. 316042). bioRxiv. https://doi.org/10.1101/316042

Seitz, B. M., McCune, K., MacPherson, M., Bergeron, L., Blaisdell, A. P., & Logan, C. J. (2021). Using touchscreen equipped operant chambers to study animal cognition. Benefits, limitations, and advice. *PLOS ONE*, *16*(2), e0246446. https://doi.org/10.1371/journal.pone.0246446

Silvasti, S., Kemp, D. J., White, T. E., Nokelainen, O., Valkonen, J., & Mappes, J. (2024). The flashy escape: Support for dynamic flash coloration as anti-predator defence. *Biology Letters*, *20*(7), 20240303. https://doi.org/10.1098/rsbl.2024.0303

Steurer, M. M., Aust, U., & Huber, L. (2012). The Vienna comparative cognition technology (VCCT): An innovative operant conditioning system for various species and experimental procedures.

Behavior Research Methods, *44*(4), 909–918. https://doi.org/10.3758/s13428-012-0198-9

Stevens, M., Marshall, K. L. A., Troscianko, J., Finlay, S., Burnand, D., & Chadwick, S. L. (2013). Revealed by conspicuousness: Distractive markings reduce camouflage. *Behavioral Ecology*, *24*(1), 213–222. https://doi.org/10.1093/beheco/ars156

Stoddard, M. C., Eyster, H. N., Hogan, B. G., Morris, D. H., Soucy, E. R., & Inouye, D. W. (2020). Wild hummingbirds discriminate nonspectral colors. *Proceedings of the National Academy of Sciences*, *117*(26), 15112–15122. https://doi.org/10.1073/pnas.1919377117

Taylor, C. H., Warrin, J., Gilbert, F., & Reader, T. (2017). Which traits do observers use to distinguish Batesian mimics from their models? *Behavioral Ecology*, *28*(2), 460–470. https://doi.org/10.1093/beheco/arw166

Tedore, C., & Nilsson, D.-E. (2019). Avian UV vision enhances leaf surface contrasts in forest environments. *Nature Communications*, *10*(1), 238. https://doi.org/10.1038/s41467-018-08142-5

Troscianko, J., Nokelainen, O., Skelhorn, J., & Stevens, M. (2021). Variable crab camouflage patterns defeat search image formation. *Communications Biology*, *4*(1), 287. https://doi.org/10.1038/s42003-021-01817-8

Troscianko, J., Skelhorn, J., & Stevens, M. (2017). Quantifying camouflage: How to predict detectability from appearance. *BMC Evolutionary Biology*, *17*(1), 7. https://doi.org/10.1186/s12862-016-0854-2

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox—A free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution*, *6*(11), 1320–1331. https://doi.org/10.1111/2041-210X.12439

Van Belleghem, S. M., Papa, R., Oritz-Zuazaga, H., Hendrickx, F., Jiggins, C. D., McMillan, W. O., & Counterman, B. A. (2017). patternize: An R package for quantifying color pattern variation. *bioRxiv*. https://doi.org/10.1101/121962

van den Berg, C. P., Troscianko, J., Endler, J. A., Marshall, N. J., & Cheney, K. L. (2020). Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods in Ecology and Evolution*, *11*(2), 316–332. https://doi.org/10.1111/2041-210X.13328

Vonk, J., & Beran, M. J. (2012). Bears 'count' too: Quantity estimation and comparison in black bears, *Ursus americanus*. *Animal Behaviour*, *84*(1), 231–238. https://doi.org/10.1016/j.anbehav.2012.05.001

Willink, B., Bolaños, F., & Pröhl, H. (2014). Conspicuous displays in cryptic males of a polytypic poison-dart frog. *Behavioral Ecology and Sociobiology*, *68*(2), 249–261. https://doi.org/10.1007/s00265-013-1640-4

Winters, S., Allen, W. L., & Higham, J. P. (2020). The structure of species discrimination signals across a primate radiation. *eLife*, *9*. https://doi.org/10.7554/eLife.47428

Zeagler, C., Zuerndorfer, J., Lau, A., Freil, L., Gilliland, S., Starner, T., & Jackson, M. M. (2016). Canine computer interaction: Towards designing a touchscreen interface for working dogs. *Proceedings of the Third International Conference on Animal-Computer Interaction, ACI '16*, 1–5. https://doi.org/10.1145/2995257.2995384