Do predators perceive eyespots as eyes?

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Contents

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed. [redacted]..

Date: 30/10/2023..

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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Abstract

Eyespots are a widespread conspicuous animal colour pattern that can be effective anti-predator trait. Eyespots consist of concentric circles that resemble eyes, hence the name. This resemblance led to the theory that eyespots are effective because they mimic eyes which increases the effectiveness of the antipredator mechanism, for example causing predator hesitation and wariness to attack prey with eyespots. However, this theory is controversial, with some evidence suggesting that eyespots simply function as conspicuous antipredator signals without involving eye mimicry. In this study I will be testing these two theories by manipulating the gaze direction of eyespots. Based on the sensitivity of predators to gaze direction of real eyes, I predict that if eyespots are perceived as eyes, when the gaze of eyespots is shifted towards the approaching predator then the eyespots will be more effective than when gaze is shifted away from the approaching predator. Predator behaviour in response to artificial prey with different gaze directions and predator approach directions were manipulated in two experiments. The first experiment took place at bird feeders in gardens and measured attack latency and approach-retreat behaviour, while the second experiment took place in woodland and measured prey survival. Overall, my results support the eye mimicry theory; approach-retreat behaviour was exhibited significantly more towards targets with eyespots with gaze shifted towards the approaching bird, indicating that the birds perceive the eyespots as ‘looking’ at them and therefore hesitating to attack. Gaze direction relative to approach direction did not affect attack latency, but this was significantly greater when eyespots were present compared to controls. The second experiment found outward facing eyespots were advantageous for prey on right hand corners but disadvantageous on left hand corners compared to inward facing eyespots. My results show that eyespots work due to mimicking eyes and just due to conspicuousness and that predators are gaze sensitive to eyespots and react accordingly through hesitation and retreat behaviour. These findings are important for our understand of how eyespots work and highlights that how they work is complex due to eye mimicry.

Introduction

Eyespots are a type of animal colour pattern widely considered a defence mechanism against predation (Stevens, 2005). Eyespot markings at minimum consist of a circular shape that is usually darker than the rest of the body and conspicuous. Many examples of eyespots consist of a more complex series of concentric circular rings, giving eyespots a resemblance to eyes, hence the name (Blest, 1957). The resemblance to real eyes formed the basis of a textbook example of evolution by natural selection whereby prey possessing eyespots gained protection from predation by mimicking the appearance of their predator's predator or much larger animal, dissuading them from attacking a potentially dangerous target (Poulton, 1940). However, this eye mimicry hypothesis was based on intuition and what eyespot patterns look like to humans. In recent years, multiple behavioural studies have tested predator responses to eyespots to try and determine how they work to provide protection. Currently, the eye mimicry hypothesis remains controversial with several studies rejecting it and highlight, proposing that eyespots work just by being conspicuous (Stevens *et al,* 2009). In this thesis I aim to perform new experiments to resolve the question of whether eyespots mimic eyes.

Eyespots are found in a wide variety of species in many different taxonomic groups (Figure 1a-i). Lepidoptera frequently possess eyespots on their wings (French and Brakefield, 2004), such as the small Emperor moth *Saturna pavonia* and the peacock butterfly *Aglais io*. Some species of lepidoptera such as hawkmoths are also known to produce larvae that have large eyespots on thickened anterior abdominal segments, which are thought to mimic snake heads (Hossie and Sherratt, 2014). Other taxonomic groups that have eyespots include coral reef fish (Hemingson *et al*, 2020), such as certain butterfly fish (*Chaetodontidae*) and pufferfish (*Tetradontidae*). Some species of reptiles and amphibians also possess eyespots, including the peacock day gecko.

Figure 1: A collage of images of animals with eyespots from different taxonomic groups. (a) Peacock butterfly eyespot, Franz van Duns, CC BY-SA 4.0; b) Giant peacock moth, Vegard Nossum, CC BY-SA 4.0; c) Red Admiral butterfly, Tony Wills, CC BY-SA 3.0; d) Oleander Hawkmoth butterfly caterpillar, Rajeev B, CC BY-SA 4.0; e) Eyed ladybird; © Entomart (http://www.entomart.be/contact.html); f) Long lure frogfish , Carole C. Baldwin - http://biogeodb.stri.si.edu/caribbean/en/thefishes/species/3178, Public Domain; g) Four eye butterflyfish, Chris Huss, Florida Keys National Marine Sanctuary - http://www.photolib.noaa.gov/htmls/reef2581.htm, Public Domain; h) Indian peafowl, Aprisonsan, CC BY-SA 4.0; i) Peacock Day gecko, Charles J. Sharp, CC BY-SA 4.0. All Images found on Wikimedia Commons.

Eyespot evolution and development

Lepidopteran eyespot development has four stages, firstly, eyespot centres differentiate in the middle of the eyespot-bearing wing. Next, in early pupal wings, cells in the eyespot centre organise and provide information to surrounding cells about where they will be in terms of distance from the centre to form concentric circles/colour rings. The next two steps involve the cells at the eyespot centre being sources or sinks of signals that activate different transcription factors that then activate different pigments to produce the different colours of the eyespot (Beldade and Monteiro, 2021).

The genetic origin of eyespots in butterflies is thought to be the ancestral appendage gene-regulatory network (GRN) (Murugesan *et al*, 2022), with the genes essential to eyespot development sharing similar connections to the GRN for antennae. The GRN for eyespots is ancient and was likely previously utilized in the development of legs and wings as well. Eyespots form in lepidoptera through the activity of inductive organizers or foci which are likely the source of the morphogens which ultimately determine the colour of butterfly wing scales (Brunetti *et al*, 2001). A wing pattern mutant, which forms different colour rings, is the likely cause of the regulatory step towards eyespot development. The plasticity of these regulatory interactions has likely contributed to the high frequency of eyespot occurred evolution.

Thus, concentric circles eyespots are relatively easy patterns to produce from an evolutionary and developmental perspective. This is likely one of the reasons why eyespots have diverse functions and could also explain resemblance to eyes as an incidental consequence of underlying pattern formation processes (Stevens & Ruxton, 2014).

Eyespot function

Most species investigated have been found to use eyespots as an antipredator adaptation. Lönnstedt *et al.* (2013) found Ambon damselfish (*Pomacentrus amboinensis)* eyespot size increases over time with increased predator exposure and that predators are less likely to attack animals with eyespots, increasing survival rate and fitness. The adult peacock butterfly (*Inachi io*) keeps its wings closed when at rest, hiding its eyespots, but when disturbed it will open its wings to show their eyespots. Research has shown that this startles and deters predators and that birds delay return to the butterfly and are more vigilant compared to a non-eyespot butterfly (Olofsson *et al*, 2012). Vallin *et al*, (2005) showed peacock butterflies with eyespots survived significantly better when exposed to bluetit predators compared to ones that had their eyespots covered. The effectiveness of eyespots as anti-predator markings has also been demonstrated in domestic cows. Radford *et al*, 2020 painted eyespots onto the rumps of cattle to test the anti-predatory effectiveness of them against large mammalian carnivores such as lions and leopards. Cows with eyespots were significantly more likely to survive compared to cows with no eyespots.

Eyespots are also known to have other functions. Several species of Felidae have eyespots on the back of their ears which are usually white and are used for intraspecific communication to signal the mothers whereabouts to her cubs (Jaroš, 2012). Eyespots are also used in courtship behaviour, such as the display feathers of the Indian peafowl (*Pavo cristatus*). Research has found that iridescence of the eyespots is crucial in how peahens choose their mates (Loyau *et al*, 2007). Eyespots may have been selected as a sexual signal due to female bias towards eye-like structures when choosing a mate (Sun *et al*, 2014). In summary, eyespots can have diverse functions, though they are principally considered an antipredator adaption. I focus on this function in this dissertation.

Eyespot antipredator mechanisms

There are currently three different antipredator mechanisms that explain how eyespots work to protect the animal that possesses them and reduce predation; aposematism, deimatism and deflection. All three of these mechanisms could work either via eye mimicry or simply through being conspicuous markings without supposing eye mimicry (Stevens and Ruxton, 2014).

Aposematic eyespots warn the predator (either honestly or dishonestly) that the prey is defended, and attack has potential to be costly. Under eye mimicry theory, eyespot markings may be an especially effective aposematic signal because a predator would be expected to be less likely to approach or attack its own predator due to the risk of injury or death (De Bona *et al,* 2015). This theory is intuitively appealing due to the close resemblance of the markings of some lepidoptera, for example owl butterflies (*Caligo*) that have eyespots that resemble that of an owl, a predator of many small birds and mammals that prey on moths. Alternatively, the conspicuous signal theory would argue eyespots are effective aposematic signals simply because they are highly conspicuous to predator visual systems. Kjernsmo and Merilaita (2017) tested the hypothesis that eyespots mimic the eyes of a predator's predator as part of an aposematic signal. They achieved this by utilising the lateral position of eyes in fish. Three-spined stickleback (*Gasterosteus aculeatus)* were used as the predator and perch as their enemy that the eyespots would mimic. Three-spine sticklebacks predated on artificial prey with four different patterns, one with no additional markings, one with a non-eye-like rectangle, one which reproduced the lateral view of a (predatory) perch's eye and in a typical eyespot pattern, and a fourth which reproduced the frontal view of a perch's eye involving two 'eyespots. Results showed that the sticklebacks took significantly longer to attack both targets with eye-like markings compared to the no marking target and the target with a conspicuous rectangle. This supports the theory that eyespots are effective because of eye mimicry, specifically of their own enemy's eyes. Evidence for the conspicuous signal theory for aposematic eyespots is found and supported in three different studies by Stevens and other authors. All three had similar experimental designs conducted in the field with wild birds as predators and involved the use of artificial moth-like prey which are manipulated in different ways to test eyespot function and effectiveness. Stevens *et al* (2007) tested conspicuousness by using targets with different contrasts and spot shapes. Results found that targets with higher contrast had a better survivability, which supports aposematic conspicuousness. In Stevens *et al,* (2008) paper moths had 'eyespots' manipulated in different ways, such as size, shape, number and inner spot configuration all experienced similar attack rates. Conspicuousness was supported when paired eyespots were attacked more and eyespots with inner eyes were also attacked as often. Finally in Stevens *et al* (2009) the targets had eyespots manipulated to be either horizontal (eye-like) or vertical (non-eyelike) which different colours like red, blue and yellow added. There was no significant difference in attack rates between these eyespot manipulations either, showing no support for the eye mimicry theory. Other studies have also supported the conspicuousness hypothesis with Kjernsmo *et al, (*2019) showing that more contrast between eyespots and the rest of the body had a greater effect on predation levels. However, all of these studies conducted by Stevens *et al* are inconclusive and it is hard to determine the mechanism of eyespot function from these studies, especially with the use of artificial stimuli. The inconclusiveness of these studies that support only conspicuousness is why further studies into the eye mimicry hypothesis need to be conducted.

The second way that eyespots can work is as a component of deimatic behaviour. Deimatic behaviour or 'startle behaviours' involves confusing or frightening predators into delaying or aborting attacks, with striking markings displayed as part of an antipredator behaviour (Drinkwater *et al,* 2022). The conspicuousness hypothesis proposed that eyespots are effective deimatic signals because of how striking they are and assumes that predators are startled by the eyespots because they stand out from the rest of the animal and its environment. Alternately, deimatic display may be linked to the eye mimicry hypothesis because eyes can be especially frightening, especially when they appear like those of a threat and the signal includes a behavioural component. Hossie *et al,* 2013 examined the defensive behaviour exhibited by two species of *Eumorpha* caterpillars that possess eyespots and raise their false ‘head’ in response to threat. When threatened these caterpillars will palpitate their eyespot markings, producing an effect that is similar to blinking. This effect is likely used to draw attention towards the eyespots to startle and intimidate the potential predator. How effective this behaviour is in terms of protection against predators is yet to be tested, and while it may enhance eye mimicry, it could also just work to make the signal more conspicuous (Stevens & Ruxton 2014).

The third potential antipredator mechanism is deflection, which proposes eyespots deflect predator attacks away from vital body parts of the prey towards peripheral regions such as wing margins or tail tips that can be damaged by predator attack with only minimal consequence for prey mortality, improving survival and increasing the chance of escape. This mechanism is suggested by the location of eyespots on non-vital body parts, such as wings, especially the wing periphery in many lepidoptera species. In support of this hypothesis, Prudic *et al,* (2015) used the squinting bush brown butterfly (*Bicyclus anynana*) a species that possesses eyespots but has different wet and dry season colouration, allowing their eyespots to be more or less conspicuous depending on the time of year. In the wet season, their eyespots were brighter and bigger, whilst in the dry season they were duller. Praying mantis were used as predators that would attack the butterflies. Results showed that wet season individuals were easier to detect but the butterflies were harder to attack. The wet season butterflies had a greater survival rate compared to the dry season butterflies and were more likely to escape the attack from the mantis. This was because the mantids were more likely to attack the wing margins of the wet season butterflies rather than the vital body parts. Other studies do not support the deflection hypothesis. Vlieger and Brakefield, (2007) investigated how eyespots on the same species work in deflecting the attacks of insectivorous green anole lizards (*Anolis carolinensis*). Conspicuous wet and less conspicuous dry season butterflies were offered to the lizards with results showing lizard attacks were uninfluenced by wing pattern, rejecting the deflection hypothesis. The deflection hypothesis may also involve eye mimicry since some predators preferentially attack vulnerable or key body parts such as eyes (Langkilde *et al,* 2004), and therefore deflection might work better if predator perceive eyespots as eyes.

From reviewing these various studies on eyespots, it can be determined that eyespots can work as effective anti-predatory markings through aposematism, deimatism or deflection. Whether these antipredator mechanisms are enhanced due to eye mimicry is still controversial, since some studies give support for the hypothesis and some do not and instead highlight the importance of signal conspicuousness, with eyespots simply being an effective (and developmentally easy to produce) conspicuous signal. To progress towards resolving this debate this thesis tests whether predators perceive eyespots as eyes, in order to try and understand if eyespots are effective due to mimicking eyes or if they are just conspicuous.

Until recently, the strongest of evidence favouring the eye mimicry hypothesis is Blut *et al, (*2012), which looked at the effectiveness of the ‘sparkle’ present in 53% of lepidoptera eyespots that exceed 1mm in diameter. The pupils of real eyes tend to ‘sparkle’ typically caused by light reflection. The ‘sparkle’ tends to appear at the top of real eyes and also the eyespots ‘pupil’ when lepidoptera are resting possibly helping to create the illusion of a spherical eye. The ‘sparkle’ is white and also UV reflective, something that birds are sensitive to. The importance of eyespot ‘sparkle’ was tested using three different lepidoptera dummies, one with the ‘sparkle’ in a natural position, one with the ‘sparkle’ in an unnatural position and one with no eyespots. Targets were then placed on tree trunks with dead mealworms on them to be predated on by wild predators. Results found that eyespots with the ‘sparkle’ in a natural position survived significantly better compared to the dummies with the ‘sparkle’ in an unnatural position. This highlights that the ‘sparkle’ is an important factor in the protective effectiveness of eyespots and support the eye mimicry theory because the natural and unnatural position treatments likely had equal conspicuousness. It has also been shown that pairedness of eyespots is a key element in how effective they are as anti-predatory markings (Mukherjee and Kodandaramaiah, 2015) which is another characteristic of eyes.

Following on from these studies, which aim to test the hypotheses by exploiting what is ‘special’ about eyes, this study develops the approach and methodology of recent experiment by Skelhorn and Rowland (2022). The goal of their paper was to determine if captive poultry chicks (*Gallus gallus*) would react to eyespots with differing gaze directions. For many real eyes, a change in gaze can be seen by other individuals since structures in the eye such as the iris and pupil shift with changing gaze. Gaze perception and sensitivity is an important cue/signal in social interactions between both con- and heterospecifics (Emery, 2000). Gaze sensitivity is an important cue in predator avoidance, with prey perceiving less threat from predators they detect as looking away from them and vice-versa (Davidson *et al*, 2014). This is why utilising gaze sensitivity is an effective way of testing the eye mimicry theory in eyespots. Birds are gaze sensitive and so should react to being ‘viewed’ by eyespots that has their gaze shifted towards them (Goumas *et al,* 2019).

Skelhorn & Rowland’s hypothesis was that if eye mimicry is taking place and predators are perceiving eyespots as the eyes of potential predators, then chicks would have a slower attack latency and exhibit more approach-retreat behaviour towards paper moths that had eyespots shifted to look at them when approaching. For example, if a chick approached from the left and the eyespots gaze was looking right, they should exhibit more caution, because the eyespot would be looking towards them. Results from this study found that when the eyespot configuration was shifted towards the direction of predator approach, the predators took longer to attack and exhibited more ‘approach-retreat’ behaviour, compared to eyespots shifted to ‘look away’. This paper highlights the relevancy of gaze sensitivity in how eyespots may work. As mentioned previously gaze is an important component of eyes and birds respond to the gaze of eyes. Since this paper highlights that birds are also gaze sensitive to false eyes, it supports the eye mimicry hypothesis, since differing gazes can increase the effectiveness of eyespots. This also shows that the mechanism of eyespots is more complicated than just being conspicuous (Davidson and Clayton, 2015).

Skelhorn and Rowland found that gaze direction can affect how effective eyespots are as antipredator markings against domestic chick predators. However, this experiment took place in a controlled environment with naïve predators and therefore has a lack of ecological validity compared to an experiment conducted in a natural setting with wild birds. I aim to replicate the basic experimental manipulation of prey gaze direction and predator approach direction in a ‘real-world’ setting with wild birds.

My research involves two experiments. The first takes place in gardens using bird feeding stations with constant monitoring of predation and accurate recording of attack latency times. Alongside this, approach-retreat behaviour (APRB) can be recorded, which is any exhibition of a bird flying towards the target and then flying away or hesitating without attacking the target. This experiment allows control over the approach direction of the birds. Additionally, it incorporates a ‘non-eyespot’ paper moth control to establish whether the eyespots in our experimental treatments are effective antipredator stimuli. The second experiment will further increase the ecological validity by taking place in woodland utilising wild birds as predators. Approach direction will be manipulated by placing prey on right- and left-hand corners formed naturally by tree branches. Crucially, wild predators are likely to have prior exposure to prey with and without eyespots. This difference is critical to testing eye mimicry theory (Speed, 2000). Experience may mean that wild birds are better able to discriminate real and false eyes. How birds respond to eye-like stimuli has been tested (Scaife, 1976) and found that birds have more fear responses to eye-like stimuli, especially if they resemble a predator like a hawk.

In accordance with previous evidence that eyespots are effective antipredator colouration, I predict that birds will take longer to attack moths with eyespots on them. If the eye mimicry hypothesis is supported, I predict predators will take longer to attack moths with eyespots that are gazing towards the direction of approach by the birds compared to those gazing away from the direction of approach. I also predict predators will exhibit more approach-retreat behaviour towards eyespots gazing at the direction of approach. Approach-retreat behaviour has been studied in ravens (*Corvus corax*) in relation to exploring new objects, indicating that the birds are hesitant to approach unfamiliar objects and the latency to approach gives the birds time to work out if the new object is a threat or not (Stöwe and Kotrschal, 2007). So, if birds exhibit approach-retreat behaviour in the presence of eyespots with false gaze directed toward the predator compared to away from the predator it could indicate the birds perceive the eyespot as a potential eye that is potentially threatening, especially if it is gazing toward the prey (Barnett *et al,* 2012). However, if eye mimicry is not occurring and eyespots are just conspicuous, then I predict there will be no differences in attack latency or APRB in response to changing gaze, but eyespots alone will have an increased attack latency and APRB compared to non-eyespot targets.

Methodology

Experiment One: Feeding station experiment

Experimental design:

This experiment tested whether attack latency and the amount of approach retreat behaviour exhibited by birds is affected by changes in gaze direction of eyespots on paper ‘moths.’ Eyespots on paper moth prey had their gaze manipulated to either look towards or away from approaching avian predators, with approach direction constrained by the design of the experimental apparatus. Attack latency and approach-retreat behaviour were assessed in response to a control paper moth with the same overall colour but lacking eyespots.

The experiment took place in five gardens in England and Wales with substantial bird communities that were already being fed by established bird feeders. Gardens were separated by at least 1 km to ensure that different communities of birds were being exposed to the experiment in each garden. Exposing the same community of birds to the experiment too often could lead to order effects due to predator learning, affecting results (Slagsvold and Wiebe, 2011). A minimum of four days prior to the first day of data collection, the bird tables

(Nature’s Market Premium Bird Table with Built-In Feeder, model number BF009WF) on which the experimental apparatus would be mounted were placed into each garden (Figure 2). This was to ensure that the birds would get used to the table’s presence (Cowie and Hinsley, 1988). The tables were 117 cm high and the covered tabletop measured 46 x 46 cm. Dried mealworms were added to the bird tables to encourage birds to visit it and allow insectivorous birds to associate the table with food.

Figure 2: the bird table with experimental apparatus attached.

Apparatus design

The apparatus design was influenced by Skelhorn and Rowland with the aim of physically restricting predator approach direction. This was achieved by constructing a ‘M’ shaped arena, with the sides of the ‘M’ preventing approach from the side, and the angle of the ‘M’ forming the background on which prey was attached facing either left or right. This structure restricted approach direction to either a left or right side. The arena was constructed using translucent white Correx. This material was chosen because it is sturdy and weatherproof so suitable for use outdoors for an extended period of time. Correx can also withstand avian attacks and manipulation.

It was also straightforward to pin the paper moths to it. For each bird table, three rectangles of Correx were cut, two being the same width and length to cover the sides (24x15cm) and one that was angled to force the birds to approach the paper moths from either the moth’s left or right side (36x16cm). The rectangle of Correx was split down the middle to achieve this (Figure 3). Separate apparatus was constructed for each of the five gardens.

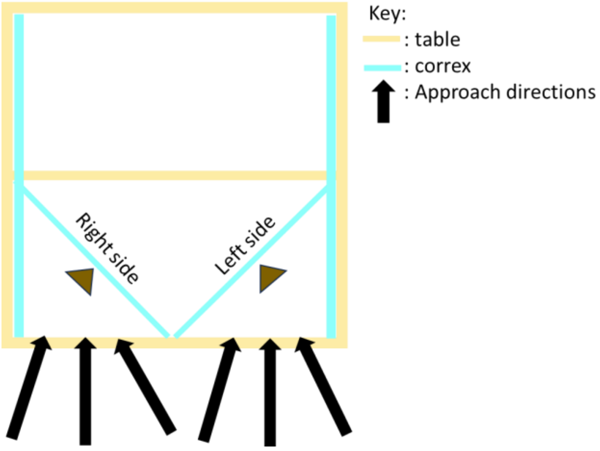


Figure 3: Apparatus design for the bird table experiment with measurements and key.

<u>Paper moth design</u>

Paper moths (L: 40mm, H: 25mm) were designed to have an overall brown colour typical of many cryptic moths. Eyespots consisted of two yellow circles (diameter 8 mm) that both contained a smaller black circle 'pupil' (diameter 2 mm). The position of the black circle in the eye was manipulated to alter gaze direction. The left-shifted gaze treatment had the pupil positioned on the left side of the yellow circle (from the predator's perspective) so it was 'looking' towards objects approaching from the right (from the prey's perspective). The right-shifted gaze treatment had the pupil positioned on the right side of the yellow circle, and so looked towards predators approaching from the left (Figure 4).

The control treatment had eyespots rearranged into a camouflage pattern consisting of small yellow and black squares. The total area for the yellow squares was equal to the total area of the yellow and black circles of the eyespot treatments so overall the treatments had the same average colour and lightness.

Figure 4: The three treatments used in the garden experiment a) left-shifted, b) right-shifted c) control.

In order to attract the birds to attack the paper moths and for them to get an edible reward for doing so, dried mealworms were pinned to the centre of the paper moths. Mealworms were chosen because they were easily available in large quantities and they are an appropriate food source for insectivorous birds. Dress-making pins (25mm) were used to pin the mealworms to the paper moths and then the paper moths onto the Correx.

Procedure

Data collection at 3/5 locations took place over three days. Due to time constraints and availability, I only managed to test over 2 days in one garden and therefore did 4 days in another garden to make up for this.

At each garden, 50 paper targets from each treatment were placed into a container and thoroughly mixed. At the start of each trial one target was chosen at random before being placed (Higgins *et al*, 2019). Initially, targets were placed alternately on the right then left approach direction. Towards the end of a day's data collection, targets were placed on the right and left side non-randomly so there were the same number of samples in each treatment combination. The sample size at the end of the whole experiment was 360 individual samples, 60 of each treatment.

The experiment began at 8am every morning at each location and continued until 7pm. Both attack latency and approach-retreat behaviour were measured for each trial. Attack latency was a timed measurement. This was timed with a stopwatch from the moment the paper moth and mealworm were placed on the apparatus until predation of the mealworm occurred. Predation is when the bird attacked and consumed the mealworm. The time was recorded in minutes and seconds. Approach-retreat behaviour was recorded in tallies as any instance of any bird visibly approaching the table and then flying away or retreating from the target before attacking. This also included any retreats that occurred after landing on the table.

I recorded the weather, categorized as sun, cloud, wind and rain since weather could have an impact on how the birds predate. For every trial, I recorded the species that attacked the target as well as any potential disturbances that occurred. Disturbances included the presence of any birds of prey, presence of cats or dogs in the garden or in a neighbour's garden, presence of neighbours in their gardens and any vehicles causing excessive noise in the surrounding area (e.g., a refuse truck). This was recorded as a binary result of yes or no.

Data Analysis

As attack latencies are positively skewed, a square root transform was applied to better approximate a normal distribution and facilitate data visualization (Lo and Andrews, 2015). The data was analysed with linear mixed models (LMM) using the package lme4 (Bates *et al*, 2015). To test significance of individual predictors I ran ANOVA tests of model fit between models with and without fixed effects. To test the key hypothesis that stimulus gaze direction relative to approach direction affected attack latency and approach-retreat behaviours, I tested the interaction between gaze direction and approach direction. Approach direction and gaze direction were also tested as main effects. All models included the random effect's location and day, and the fixed effects attack species (categorical) and disturbance (binary). To visualize results, attack latency was plotted against approach direction and gaze direction. Attack latency was also plotted against location, disturbance and attack species. Analysis of attack latency was repeated for approach retreat behaviour. This included visualising with plots and modelling.

To check assumptions of LMMs I used the modelDiagnostics function in the R package JWileymisc v1.4.0, examining Q-Q plots to confirm residuals were approximately normally distributed.

Experiment Two: Clyne Valley country park

Experimental Design

The second experiment was similar in design to the garden experiment, except that the control condition was not tested in order to maximise the sample size for the two critical treatments. The left/right approach direction manipulation was achieved by pinning targets to trees with left and right-hand corners between branches, under the assumption that the branch provided the same physical barrier to approaching prey from one direction as the sides of the 'M' shaped Correx (Figure 5). Instead of observing attack latency directly, predation on targets was checked after 4, 8 and 24 hours of being placed.

Figure 5: a) a diagram showing a top-down view of a tree with suitable branches, b) a tree with a target placed on a left side approach direction branch with a left-gaze paper moth pinned to it (inward looking). The red lines visual the angle of the branch.

Location

The experiment took place in Clyne Valley Country Park, Swansea, Wales (51.6042° N, 4.0014° W). Before data collection began, I located three areas of the park with at least 32 trees that had branches that grew at a right angle parallel to the ground within a 30-minute walk of each other so that it would not take too long to place the all the moths and do all the necessary checks.

Procedure

Based on pilot work to determine overall predation intensity, it was determined that doing checks 4 hours, 8 hours, and 24 hours after being placed allowed enough time for a suitable attack rate on the paper moths. In each block at each area, a total of 32 moths were placed, 16 right-shifted, 16 left-shifted. Half of these were placed on left hand corners and half on right hand corners. Thus, the experiment was a 2x2 factorial design. Prior to placing, all targets had a code written on the back (e.g.: L12, R45 etc.) to make it easier to track and re-find targets in the field by noting each target code on a sketched map at each location.

Like the garden experiment, mealworms were pinned with dress-making pins to the moths to attract birds to attack them. They were then pinned to either branch that created the 90° angle between each other. For each trial, the moths would be chosen randomly to once again avoid bias. Which side of the branch to pin to was also chosen at random, except towards the end of placing when it would be chosen non-randomly to ensure the same number of each treatment was done.

Targets were placed at 8am and then checked at 12pm, 4pm and then 8am the next day. Any form of attack was noted. If the whole mealworm had been taken, then avian predation was assumed. If the mealworm was partially eaten, for example the inside had only been eaten or if there were small bite marks on the mealworm, then non-avian predation was assumed and instead consumption by an arthropod was noted. If the target was lost then I carefully checked the surrounding area for it or any injured birds, including the ground and other parts of the tree it could have fallen on to. If I was certain, it was lost then I made a note of which code was lost.

Data Analysis

Targets that were eaten by non-avian predators or that survived to the end of the experiment were censored. The survival time of prey eaten by birds was recorded.

Survival analysis via a Cox proportional hazards regression model (Stel *et al*, 2011) using the coxph function in the survival package (Therneau *et al*, 2023) for R tested the interaction between gaze direction and approach direction. Other predictors included in the model were weather and day (block). Location was added as a cluster term. A hazard ratio plot was made to show the effect on predation of each variable. The frequency of predation was also visualized on bar plots. I tested the proportional hazards assumption using the cox.zph function and checked residual plots for linearity between log hazard and the predictors. Assumptions were met for models presented.

Results

Garden Experiment

Attack Latency

The interaction between gaze direction and approach direction had no significant effect on attack latency (estimate= -0.917, Std error= 3.695, p= 0.174). The hypothesis that attack latencies should be longer when the gaze direction is different to approach direction (so eyespots were looking outwards, towards the oncoming predator) was not supported. (Figure 6).

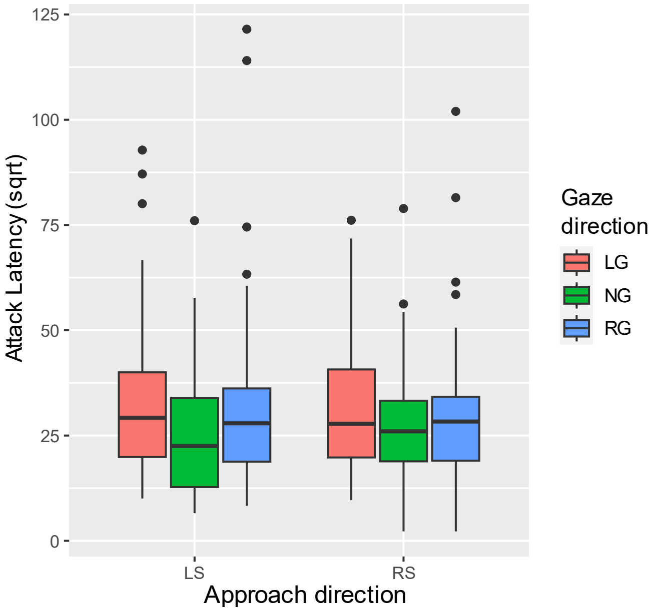


Figure 6: Attack latencies for each approach direction and gaze direction (LS=Left-Side, RS=Right-side; LG=Left-gaze, RG=Right-gaze, NG=No gaze).

Treatment did have a significant effect on attack latency (β =-9.490, SE = 2.605, p= 1.056e-14, Figure 6). Targets without eyespots had shorter attack latencies compared to targets with eyespots. The presence of eyespots had a positive impact on how long the target would survive an attack, since the birds attacked non-eyespot stimuli significantly faster. Approach direction also had a significant effect on attack latency (estimate= -2.128, SE= 2.577, p=9.148e-13); attack latencies were faster on the left side approach direction.

To investigate results further I explored differences in attack latencies on each treatment between the different gardens (Figure 7). To simplify the visualisation, this figure and those that follow use ‘in’, ‘out’ and ‘no gaze’ instead of a combination of gaze direction and approach direction as in the previous plot. There was a significant difference in attack latencies between each garden (Std Dev= 6.185, p= 1.404e-5). As might be expected, some gardens have a greater average attack latencies compared to other gardens. There was also a significant difference in attack latency between each day (Std Dev= 6.553, p=-3.26e-8). Figure 8 shows that attack latencies were much longer on day 1 compared to later days.

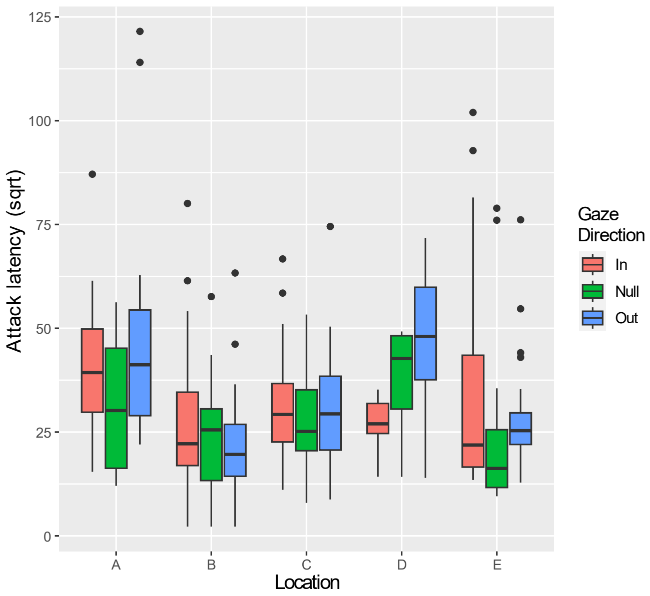


Figure 7: Attack latency differences between locations with each gaze direction type. (Null= No eyespot).

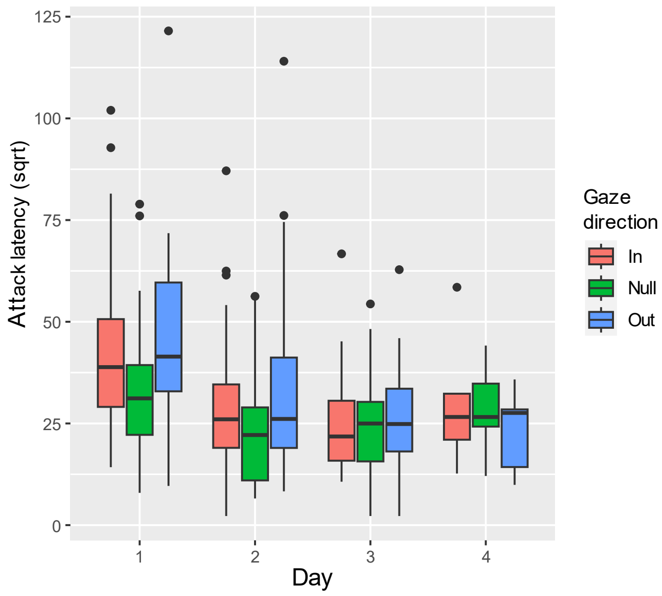


Figure 8: Attack latency differences between each day of testing with each gaze direction type

Figure 7 also suggests that eyespots were more effective at some locations compared to others (A&E vs. B&C), and that outward facing eyespots may have been more effective at location D only. To explore whether there was an aspect of location D that could have influenced the effect of gaze direction, I investigated the role of disturbance on responses. Disturbance was found to significantly increase attack latency ($\beta = 12.805$, SE= 1.698, p=1.591e-14, Figure 9). Disturbance increased attack latencies in all the locations except C, with the effect of disturbance being strongest at location D (Figure 9).

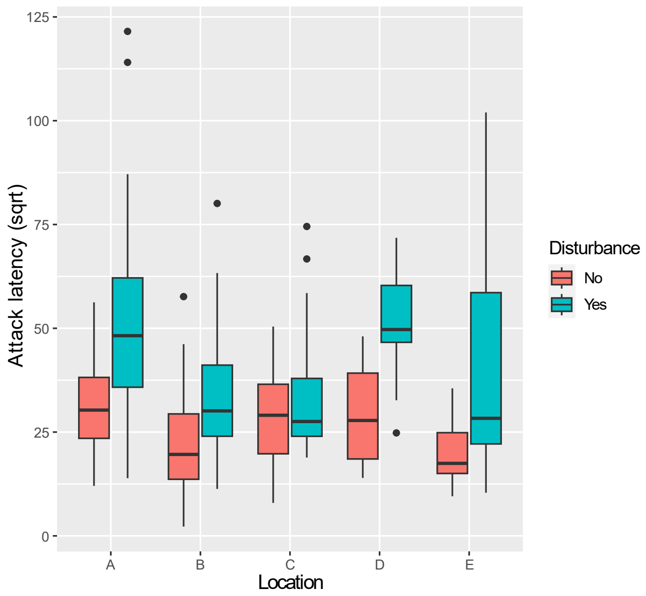


Figure 9: Boxplot showing how disturbance affected attack latency in the different locations.

To investigate further why treatments seemed to perform differently and disturbance had a different effect at locations, I investigated differences in predator species in each location. Figure 8a shows that the gardens had different groups of species attacking the targets, for example location at a and b the majority of attacks were by starlings, whereas at location e, most attacks were by sparrows. Overall, predator species had no significant effect on attack latency (β = -1.388, SE=5.507, p = 0.160). Figure 10a and 10b indicates that there may be some predator species differences in sensitivity to eyespot treatment, with magpies and sparrows only having longer latencies to attack outward gazing stimuli. In a post-hoc analysis to investigate the potential for these species being sensitive to gaze direction, I tested the gaze * approach direction interaction only for trials where sparrows or magpies were the predators, but this was not significant (β = 0.939, SE= 2.768, p= 0.824).

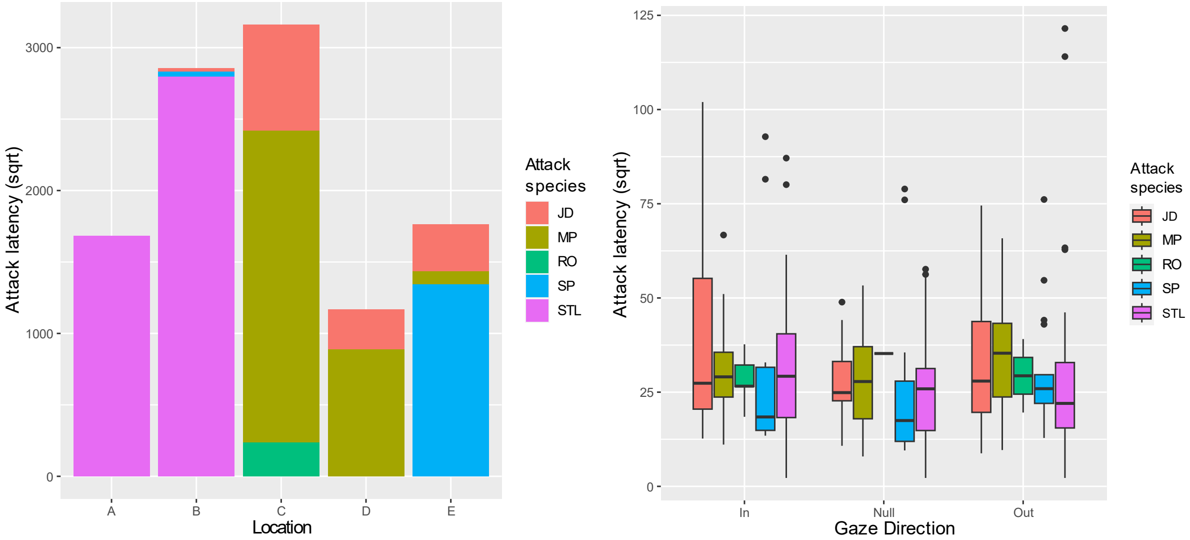


Figure 10a and 10b: a) a stacked bar plot showing the proportion of attacks performed by each species in each location (JD=Jackdaw, MP= Magpie, RO=Robin, SP= Sparrow, STL= Starling), b) Boxplot showing how the bird species affected attack latency in different gaze treatments.

Approach-retreat behaviour (APRB)

There was a significant interaction between gaze direction and approach direction in the amount of APRB exhibited (β =-1.309, SE=0.601, p= 0.035 Figure 11). The hypothesis that more approach-retreat behaviour would be exhibited for treatments with a different gaze direction to approach direction was supported. The plot shows that more APRB was exhibited when gaze direction was different to approach direction i.e., when the eyespots were looking out towards the approaching predator.

There was a significant difference in the amount of APRB between prey treatment (β =-2.411, SE= 0.426, p=1.376e-15) (Figure 11). More APRB was exhibited for treatments with eyespots. Approach direction alone did not have a significant effect (β = 0.169, SE= 0.419, p=0.056).

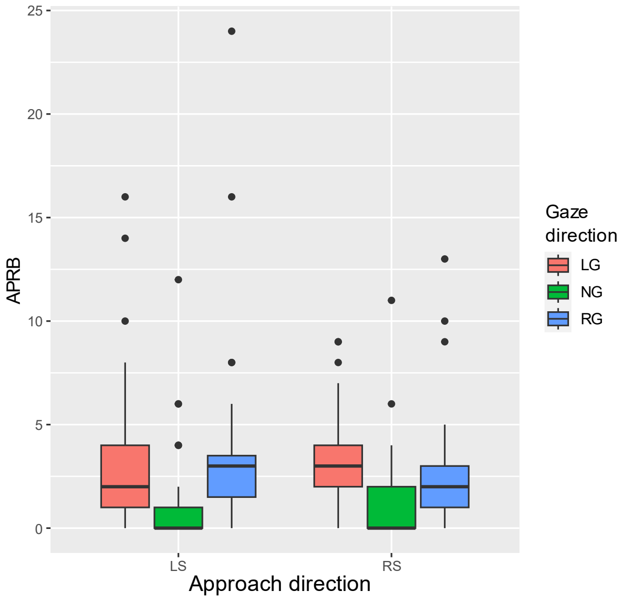


Figure 11: A boxplot showing the amount of approach-retreat behaviour that was recorded for each type of treatment.

Next, I investigated further by analysing other variables. APRB is visualised against the different locations in Figure 12. however, there was a significant difference in the amount of APRB exhibited between different days (Std deviance= 1.035, deviance= 1642.2, p= 1.7e-07). Figure 13 shows that more APRB was exhibited on the first day.

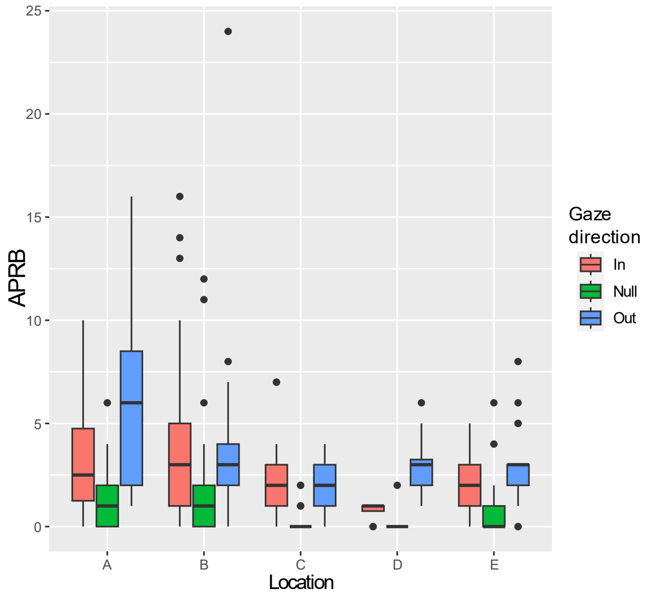


Figure 12: A boxplot showing the amount of approach retreat behaviour exhibited in each garden for each type of treatment.

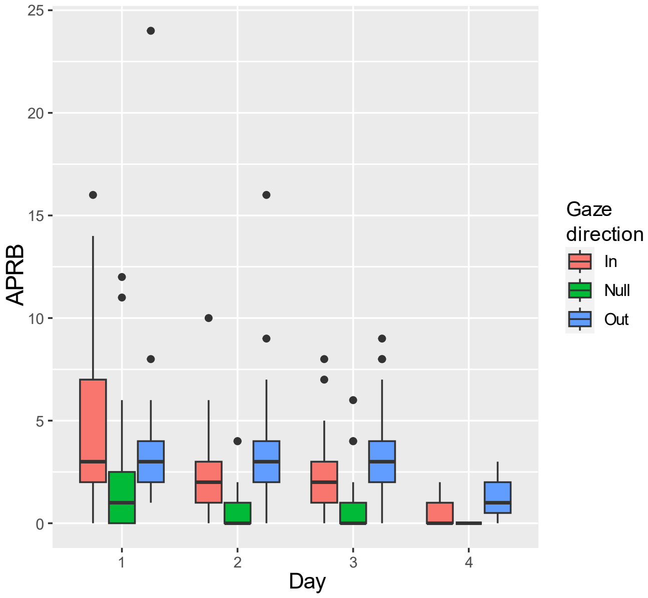


Figure 13: the amount of APRB exhibited on each day of testing with each type of gaze direction

Analysis found that disturbance does cause a significant difference in the amount of APRB that is exhibited (β = 1.160, SE=0.275, p=2.141e-04). If disturbance occurred during a trial, then birds would exhibit more APRB (Figure 14). The plot shows that disturbance impacted APRB in all locations.

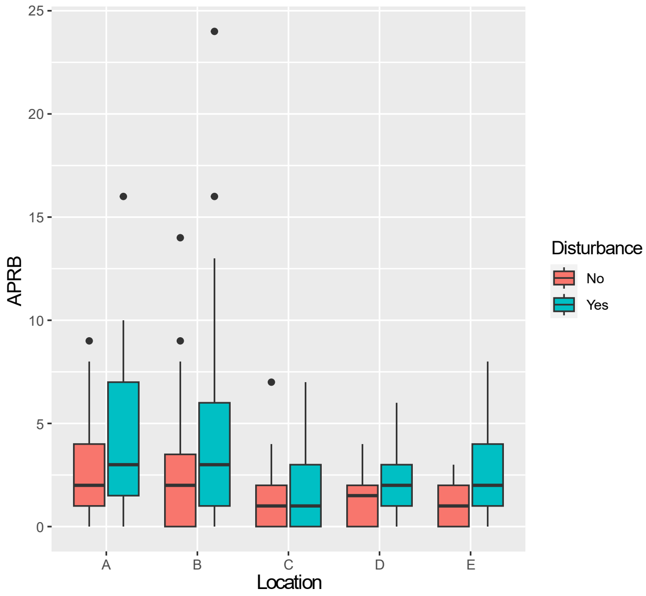


Figure 14: Boxplot showing how disturbance affected the amount of approach retreat behaviour exhibited for each treatment in all locations.

Finally, how the predator species affected the amount of APRB exhibited was analysed. This had a significant effect (estimate=1.987, Std error=0.429, p=0.001). Some attack species exhibited more or less APRB towards the targets (Figure 15). On figure 15 it is shown that starlings (STL) exhibited more APRB across all three treatments, compared to other attack species (Figure 15b).

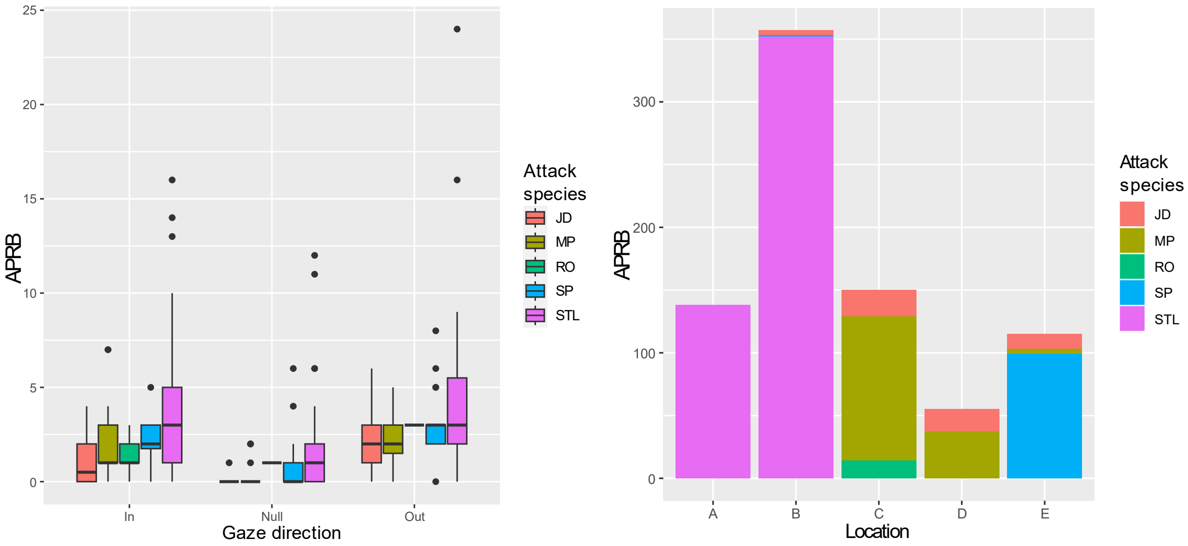
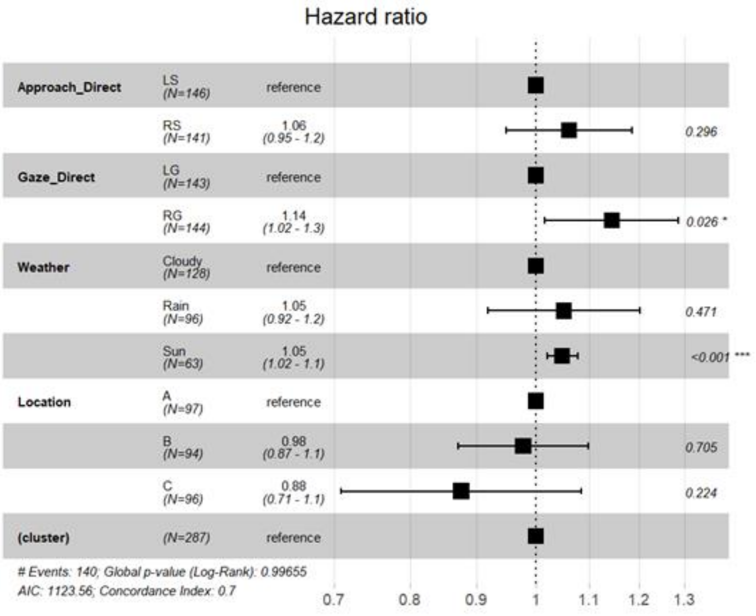


Figure 15: a) Boxplot showing the amount of approach retreat behaviour exhibited by each attack species for each treatment, b) a stacked bar plot showing the frequency of APRB exhibited by each attack species in each location.

Woodland Experiment Results

Table 1: A hazard ratio plot for each variable in the results. The significant interaction term is not shown.



The interaction between gaze and approach direction was significant; for left side approach direction, as predicted left gazing targets survived significantly better than right gazing targets (exp(coef) = 1.13, se = 0.24, $p < 0.001$; Figure 16). However, on right side approach directions, right gazing targets survived worse (exp(coef) = 0.95, se = 0.24, $p < 0.001$). This analysis shows that the interaction between gaze direction and approach direction had an effect on survival rate, although not in the way predicted. As can be seen, where gaze direction and approach direction were the same, the frequency of attack was similar at 50% predation. However, where gaze direction and approach direction are different (eyespots were looking outwards), the frequency of attack is reduced (44%) when left gaze targets are on a right-hand corner but increased (54%) when right gaze targets are on a left-hand corner. These results do not support the prediction that moths with eyespots looking outwards towards the approaching predator should have a better survival rate compared to inward looking eyespots.

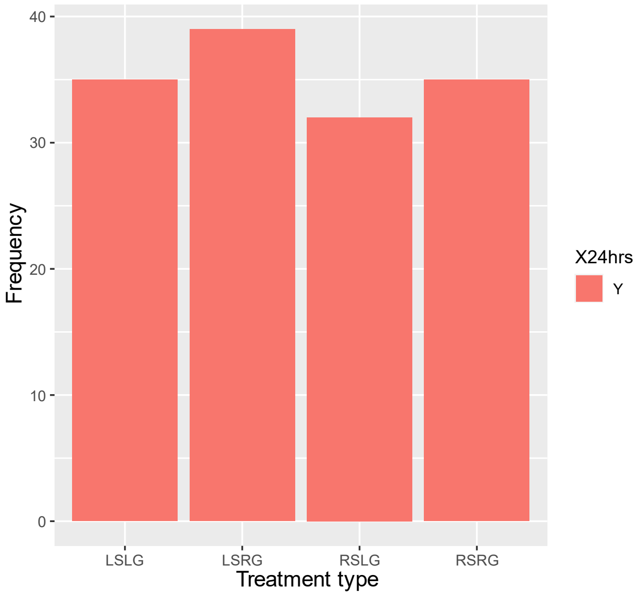


Figure 16: A bar graph showing the frequency of predation (Y) for each treatment after 24 hours. (LSLG=Left-side + Left gaze, LSRG=Left-side + right gaze, RSLG=Right-side + left gaze, RSRG=Right-side + right gaze).

Other variables were also tested for their effect on survival. It was found that all treatments were significantly more likely to survive if the weather was sunny compared to cloudy or rainy conditions (sun results: exp(coef)= 1.048, se(coef)= 0.218, p =0.001, rain results: exp(coef)= 1.051, se(coef)= 0.236, p=0.471) with all treatments 1.05x more likely to be predated on in sunny weather (Figure 17). The three locations did not have a significantly different survival rate (location b: exp(coef): 0.978, se(coef)= 0.215, p= 0.705 | location c: exp(coef): 0.876, se(coef)= 0.234, p= 0.224). The frequency of predation depending on location and day is visualised below (Figure 18a and 18b).

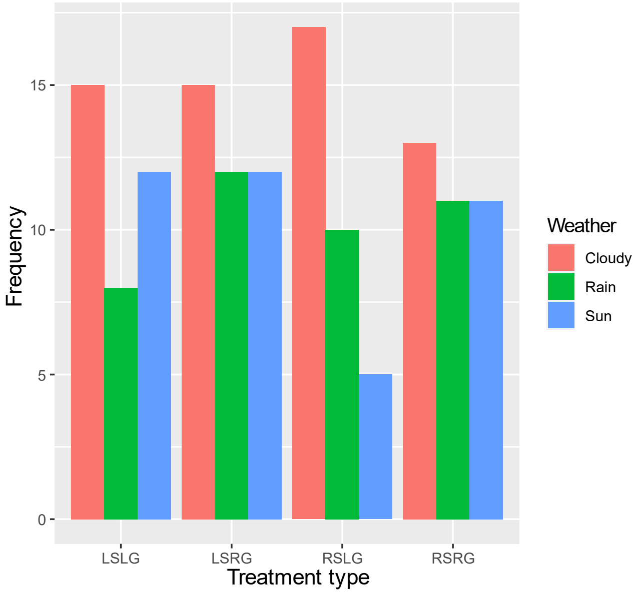


Figure 17: Barplot showing the frequency of predation for each treatment for each type of weather

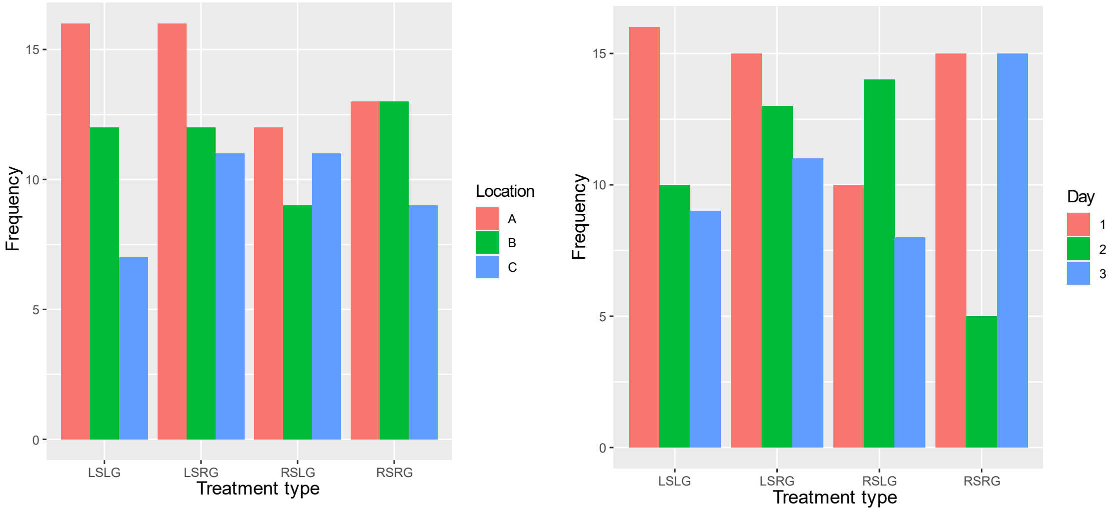


Figure 18: Barplots showing a) the frequency of predation for each treatment at each location, b) the frequency of predation for each treatment during each day.

Discussion

Results demonstrated that eyespots worked as an effective anti-predator pattern in the garden experiment, since attack latency and APRB was significantly greater in the presence of eyespots (Halali *et al*, 2019). This result confirms many previous studies that demonstrate eyespots are an effective anti-predatory mechanism (Stevens, 2007).

The results from the garden experiment support the prediction made by the eye mimicry hypothesis that the gaze direction of eyespots, whether towards or away from an approaching predator, affects how a predator responds to prey (Postema, 2022). While there was no effect on attack latency, I observed significantly more APRB when eyespots gazed towards the predator approach direction. The woodland experiment found a significant interaction between gaze direction and approach direction affecting survival rate of prey, indicating that predator decisions were influenced by gaze direction relative to approach direction, however the pattern was not fully consistent with my prediction. On the presumed right-side approach direction, eyespots were more effective if their gaze shifted to look towards the oncoming predator, as predicted. However, on the presumed left-side approach direction, eyespots were less effective if they 'looked' at the approaching predator.

The eye mimicry hypothesis but not the conspicuousness hypothesis predicts that if the eyespots looked towards the oncoming predator, then the predator would display more approach-retreat behaviour as they would be more wary and vigilant due to gaze sensitivity. The garden experiment results found that this did occur, replicating a key result of Skelhorn and Rowland (2022) in a natural, 'real-world' setting with wild birds. In comparison the conspicuous theory predicted there only being a significant increase in attack latency and APRB towards targets with eyespots, regardless of gaze manipulation. This only occurred with the attack latency results, not the APRB results, so eyespots only being conspicuous is not supported from my results.

The eye mimicry hypothesis predicts that if eyespots were perceived as eyes, then predators should also take longer to attack the targets with eyespots that had their 'gaze' shifted to 'look' towards the approaching predator. Longer attack latency indicates the predator is more apprehensive of attacking the prey and is sometimes seen as decision time by the predator (Gamberale-Stille, 2000). However, unlike Skelhorn and Rowland, I found predators had the same attack latency on average towards eyespots that had their gaze shifted away or towards the oncoming predator. This may be due to noise in the attack latency measure due to daily feeding behaviour of wild predators. During the garden experiment I observed that birds were more actively foraging, and therefore attacking the targets, in the morning and evenings. Dawn and dusk foraging are a common behaviour in birds (Kacelnik, 1979). Therefore, there were sometimes long periods of time in the middle of the day where there were no birds present in gardens, which would have increased the 'attack latency' of some trials even though no birds were near the apparatus or reacting to the targets. A solution to try and avoid this in repeat experiments could involve only observing and timing trials in the morning and evenings rather than the whole day. Approach retreat behaviour, which by definition is only recorded when birds are foraging, is likely a more effective way of determining the effectiveness of eyespots as anti-predatory markings. Another variable that affected attack latency that did not occur in Skelhorn and Rowland's study is the effects of disturbance. In the garden experiment there were various uncontrollable disturbances that had a significant effect on both attack latency and APRB, for example domestic cats and dogs, birds of prey and the presence of humans in surrounding gardens. If there was an instance of disturbance during a trial then attack latency was longer and more APRB was exhibited (Foresman *et al,* 2010).

For the woodland experiment I predicted that moths with eyespots shifted towards the presumed direction of approach by the predator would have a greater survival compared to moths that had eyespots with gaze shifted away from the approaching predator. This did occur but only on the presumed right side approach direction. On the left side approach direction, the opposite occurred - survival rate was better when the eyespots had gaze shifted away from approaching predators. Why the influence of gaze direction was opposite for right- and left-hand corners is hard to determine, especially because there was no constant observation of the targets in the woodland experiment. There are some possible explanations for this result. In each location there were areas that had more dense foliage compared to other areas such as pathways and clearings. Birds have been known to use paths and clearings as faster flightpaths compared to areas with more trees (Bolger *et al,* 2001) so targets that were placed on trees near pathways might have been visited more frequently and therefore attacked more. The targets and approach directions were chosen at random but nevertheless one approach side may have been chosen for branches near pathways more so than the other side, or topology and light levels may have influenced the approach direction of predators on right- and left-hand corners differently. There is also a possibility of perceptual or behavioural bias is occurring so birds are preferring to attack eyespots looking in different directions in different ways (Nakamura *et al,* 2011). Although all these potential explanations are speculative, that there was an effect of target gaze direction relative to whether the target was placed on a left- or right-hand corner, suggests that eyespot direction influences predator responses to prey, and this cannot be easily explained by the conspicuousness theory.

The support for the eye mimicry theory from my results is important and advances the overall understanding of how eyespots work. With this experiment showing that eyespots likely are effective because they mimic eyes it can hopefully lead to other experiments that test the eye mimicry theory.

My experiment utilized the predator-prey relationship between lepidoptera and birds, and the eyesight of birds is different to our own, so how they interpret eyespots is hard to determine from a single behavioural study. Gaze sensitivity was the key aspect of vision that I tested to determine if eyespots successfully mimic eyes. Studies have found that birds such as European starlings (*Sturnus vulgaris*), a species which was present in our garden experiment, are sensitive to the gaze direction of their predators (Carter *et al,* 2018). So, sensitivity to the gaze direction of eyespots was expected and has occurred in the garden experiment. However, the eyesight and colour vision of birds is different to our own. Most notably birds as tetrachromats can see 2-8 times the number of colours compared to us (Kelber, 2019), and this diverse colour vision is mainly used to discriminate food and mates from the surrounding environment. The predators observed in the garden experiment are all thought to be sensitive to ultraviolet light (Kevan *et al,* 2001). The stimuli used in my experiment were simple, with simple colours and eyespot designs to make the gaze shift easier to manipulate. I did not measure the colour of my prey and compare it to the colour of backgrounds, predator eyes, or lepidopteran eyespots. It is therefore unknown what resemblance prey had to species of Lepidoptera that possess eyespots (Delhey *et al,* 2015). The birds could have been sensitive to this dissimilarity and therefore reacted differently compared to eyespots on actual moths.

Resolving this issue could involve using more realistic stimuli, maybe completely replicating a lepidoptera species that is known to possess eyespots in avian perceptual space.

Another thing to consider adding to the garden experiment methodology is testing with another target type that has central gazing eyespots, to observe gaze sensitivity even more. It would be interesting to look at the different behaviours that birds could exhibit towards eyespots facing forward compared to eyespots looking away (Bateman and Fleming, 2011).

One limitation of both experiments was that I could not control what birds were approaching and attacking the targets. In the garden experiment it was observed that many attacks at each location were made by the same individual bird leading to non-independence in data. This could have affected the results because of a bird's ability to learn (West *et al*, 2003). If the same individual continued to approach the table after a certain number of trials, the bird would eventually get used to the presence of each paper moth, regardless of treatment. The bird would also likely learn that the paper moths pose no threat and that attacking it would result in food. Altering the methodology in order to try and prevent this from occurring in repeat experiments could involve identifying each individual bird that makes an attack and determining how many attacks are made by the same individual and then only analysing attacks made by different birds (Ferreira *et al*, 2020). There is also a possibility that this occurred in the woodland experiment as well since the same three locations were used each with their own community of birds. However, individual predators were not recorded so determining this is not possible and something to consider if the experiment is repeated.

A design decision and limitation in the garden experiment that may help explain null attack latency results was the colour of Correx used. The only translucent Correx available to us was white, meanwhile the paper moths were brown to replicate wild moth species. Typically, moths would be camouflaged within their environment which they actively choose in some instances (Kang *et al*, 2012), and this camouflage would also help the conspicuousness of their eyespots. However, in our garden experiment the moths were not camouflaged against the environment and perhaps the predators picked up on this difference. This is also important when it comes to the aposematic mechanism of eyespots as defence markings. Also, in relation to eye mimicry, the predator's predator would also be camouflaged within its environment, for example Tawny owls (*Strix aluco*) have patterned plumage that helps them camouflage amongst tree bark (Solonen, 2021). The paper moths being placed on a white background that is not the same as their body colour leads to reduced similarity to their predator's predator and would be expected to reduce the effectiveness of eye mimicry but not conspicuousness. In repeat experiments, using material that better camouflages the target body might lead to more realistic results.

An issue for the Woodland experiment design was that targets were left for 24 hours with no constant observation, so how the birds behaved whilst approaching and attacking the targets is unknown. No approach-retreat behaviour was able to be recorded or any other reactions to the moths, which might have helped answer my main research question. Also what species were making the attacks could not be recorded either. The bird species that were making the attacks could be a similar make up to the garden experiment, or be very different, there being differences is more likely since it is a different environment. Solving these issues might involve the use of camera traps to video the approach and attack, although this would be difficult to do at scale (Randler and Kalb, 2018).

Another limitation with this experiment was influencing the direction of approach by the birds was a lot harder compared to the garden experiment. Branches that were at a right angle that was parallel to the ground were used to try and influence approach direction however this may not have been effective. It is possible that the birds approached from the opposite direction or from above/below the target therefore negating the original gaze direction of the target. These possible differences in approach direction may give an explanation as to why my prediction of results only occurred on one presumed approach direction side (the right side). Resolving this could involve observing how birds typically react and interact with the targets either prior to the experiment in a pilot study or during the study using camera traps to video some of the targets.

Finally, something to consider is whether my results can generalise to a wider diversity of eyespots. Firstly, as previously mentioned, my experiment is specific to eyespots that are for antipredator defence, and while results are not inconsistent with the hypothesis that eyespots used as sexual signals involve eye mimicry, testing this would require new experiments in mating contexts. This is especially prevalent when we consider how important the eyespot marking is in terms of mate choice for the female, for example in the Indian peafowl (Loyau *et al*, 2007). Secondly, results may not generalise to eyespots with different forms. Some species possess eyespots that are more complex and resemble eyes more so than others. For example, owl butterflies have eyespots that are quite detailed and mimic the eyes of avian predators very well. In comparison, a frogfish has eyespots that are merely a large conspicuous circle (Mikhail and Said, 2017) on a flap near the tail. The eyespots shape used was very simple, so it is likely results would generalise to eyespots with more eye-like forms. Whether simpler forms such as a contrasting spot could involve eye mimicry is less likely, but still merits investigation. Many species such as the velvet ant *Hoplomutilla spinosa* feature pairs of spots as antipredator signals. Against predators with low acuity visual systems (arthropods, small tetrapods) it is plausible that these are potentially misclassified as potential eyes as well. Thirdly, it is possible results do not generalise to other predator-prey systems, my experiment utilised the predator-prey relationship between moths and passerine birds. But, other predator-prey relationships can differ from each other such as in behaviour of both the predator and prey animals, certain prey species will manipulate their eyespots to deter predators in different ways, e.g. the spicebush swallowtail caterpillar will move in a particular way to mimic snake behaviour. Another difference between predator-prey systems is predator vision. Eyesight is very diverse between species and how certain animals see the world can differ in terms of colour, focus and what type of wavelengths are picked up by different eyes. Considering differences in predator vision can be considered in different experiments for testing eyespot eye mimicry. My experiment focused on eyespots on moths and the targets were designed to reflect this. Therefore, it could be argued that my results are only relevant for showing eye mimicry occurring in eyespots of Lepidoptera, but determining this in other animals with eyespots requires further research.

Conclusion

Overall, my results support eye mimicry being a reason for the eyespots presented in these two experiments being effective antipredator traits. In the garden experiment, despite attack latency not being a significant different for eyespots gazing towards or away from approaching predators, approach retreat behaviour was, a result that cannot be explained by conspicuousness theory alone as apart from the position of the black 'pupil' the stimuli are identical. This also highlights that measuring displays of APRB might be a more effective way of determining hesitancy and apprehension to attack instead of attack latency. In the woodland experiment, while results are not fully consistent with the predictions of the eye mimicry hypothesis, they show that the effectiveness of eyespots can be manipulated by gaze and approach direction, indicating that predators are sensitive to the position of eyespot pupils in a way that cannot be explained by the conspicuousness hypothesis. Despite the differences between my experiment and Skelhorn and Rowlands such as, the environment and shifting the experiment into a natural, 'real-world' setting with predators that are likely exposed to prey with eyespots prior to the experiment, we both found similar results.

My results do not preclude a role for conspicuousness in eyespot effectiveness, as has been demonstrated previously, but they have given clear support that eyespots are effective not just due to conspicuousness but also because they mimic eyes and are perceived as such by wild predators. The significant effect that gaze direction had on approach retreat behaviour demonstrates that birds that predate on moths with eyespots are wary and hesitate to attack targets with eyespots shifted to look towards them.

Due to the support for the eye mimicry hypothesis from the results, what I have found is important for the wider understanding of eyespots and their effectiveness as warning signals. The increased support for the eye mimicry theory highlights that how eyespots work is more complex compared to if they were just conspicuous. Mimicking the eyes of your predator's predator is unique and an ingenious mechanism. Moving forward from my study, a lot can still be tested in relation to the eye mimicry theory, eyes are a complex sensory organ that have many characteristics that can be manipulated and tested with eyespots to further support the eye mimicry hypothesis. Reviews of previous studies that look into the eye mimicry theory conclude that research on this hypothesis is in its infancy, and this remains the case (Stevens and Ruxton, 2014).

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Appendix

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: [redacted]

Date: 30/10/2023…...

Supervisor: [redacted]

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s). Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed: [redacted]

Date: 30/10/2023 ....

Supervisor: [redacted]

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for electronic sharing after expiry of a bar on access approved by the Swansea University.

Signed: [redacted]

Date: 30/10/2023 ....

Supervisor: [redacted]

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signed: [redacted]..

Date: 30/10/2024...

Statement of Expenditure:

Equipment:

Nature’s Market Premium Bird Table with Built-In Feeder x6 = £240

2kg Dried Mealworms = £14.99

Translucent Correx sheets = £7.99

Dress-making pins x600 = £3.95

Transport:

Return train ticket Swansea to Banbury = £119.80

Conference:

WEEN (Wales Ecology and Evolution Network) = £40

Risk Assessment:

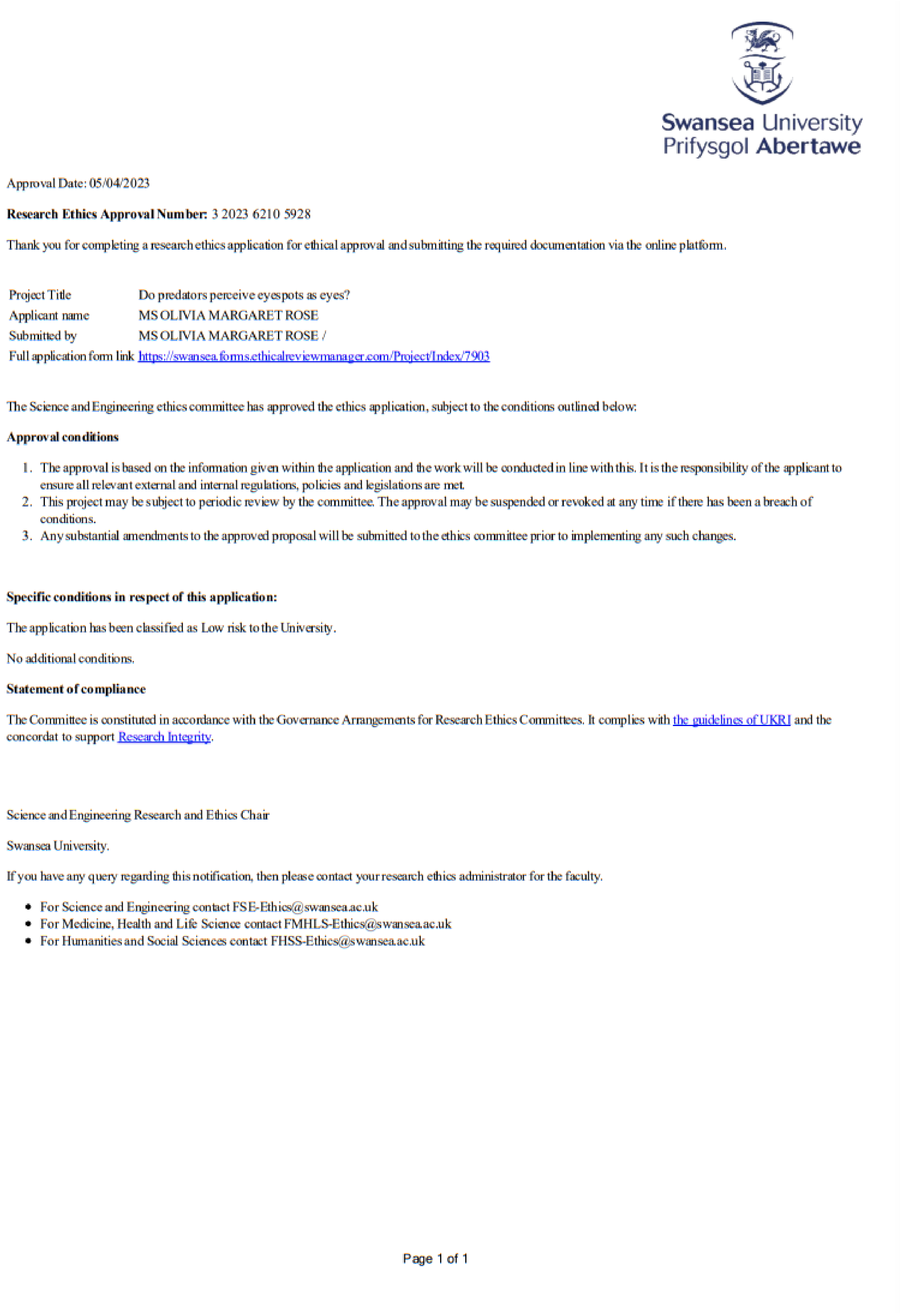
Risk Assessment			
College/PSU	Science and Engineering	**Assessment Date**	July 2023
Location	Clyne Valley Country Park	**Assessor**	Dr Will Allen
Activity	Data collection	**Review Date (if applicable)**	
Associated documents	●	●	

Part 1: Risk Assessment

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?	Action by whom?	Action by when?	Done Yes/No
Cycling	Me	Falling off bike	Wearing a bike helmet	Take care on roads and paths. Not cycling in the dark.	Myself	Travelling to location	Yes
Sun	Me	Sunburn, Heatstroke Dehydration	Wearing suncream and staying hydrated	Finding shade when needed	Myself	At all times	Yes
Rain, wind cold weather	Me	Hyperthermia Exposure	Wearing enough layers and a sufficient waterproof coat	Avoiding fieldwork if weather is too severe.	Myself	At all times	Yes

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?	Action by whom?	Action by when?	Done Yes/No
Uneven terrain	Me	Falling	Wearing appropriate footwear and taking care	Using appropriate footpaths when available. Not working in the dark	Myself	At all times	Yes
Dogs	Me	Dog bites	Avoiding dogs and respecting dog walkers		Myself	At all times	Yes
Ticks	Me	Lyme disease	Wearing long sleeves and tucking trousers into socks	Checking skin for ticks after fieldwork	Myself	At all times	Yes

Ethics Approval Letter



Swansea University
Prifysgol Abertawe

Approval Date: 05/04/2023

Research Ethics Approval Number: 3 2023 6210 5928

Thank you for completing a research ethics application for ethical approval and submitting the required documentation via the online platform.

Project Title	Do predators perceive eyespots as eyes?
Applicant name	MS OLIVIA MARGARET ROSE
Submitted by	MS OLIVIA MARGARET ROSE /
Full application form link	https://swansea.forms.ethicalreviewmanager.com/Project/Index/7903

The Science and Engineering ethics committee has approved the ethics application, subject to the conditions outlined below:

Approval conditions

1. The approval is based on the information given within the application and the work will be conducted in line with this. It is the responsibility of the applicant to ensure all relevant external and internal regulations, policies and legislations are met.
2. This project may be subject to periodic review by the committee. The approval may be suspended or revoked at any time if there has been a breach of conditions.
3. Any substantial amendments to the approved proposal will be submitted to the ethics committee prior to implementing any such changes.

Specific conditions in respect of this application:

The application has been classified as Low risk to the University.

No additional conditions.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees. It complies with the guidelines of UKRI and the concordat to support Research Integrity.

Science and Engineering Research and Ethics Chair

Swansea University.

If you have any query regarding this notification, then please contact your research ethics administrator for the faculty.

- For Science and Engineering contact FSE-Ethics@swansea.ac.uk
- For Medicine, Health and Life Science contact FMHLS-Ethics@swansea.ac.uk
- For Humanities and Social Sciences contact FHSS-Ethics@swansea.ac.uk

List of Tables and Illustrations:

Abbreviations:

APRB: Approach-retreat behaviour.

Statement of Contributions:

Contributor Role	Persons involved
Conceptualisation	WA
Data curation	OR
Formal analysis	OR, WA
Funding acquisition	N/A
Investigation	OR
Methodology	OR, WA
Project administration	WA, OR
Resources	WA, OR
Software	OR
Supervision	WA
Validation	N/A
Visualisation	OR, WA
Writing – original draft preparation	OR
Writing – review & editing	OR, WA

OR = Olivia Rose (Author)
WA = Dr Will Allen (Primary supervisor)