DOCTOR OF PHILOSOPHY

Unravelling Map and Compass Cues in Bird Navigation

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Charlotte Griffiths

PRIFYSGOL
BANGOR
UNIVERSITY

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Declaration

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw’r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o’r blaen ar gyfer unrhyw radd, ac nid yw’n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy. Rwy'n cadarnhau fy mod yn cyflwyno'r gwaith hwn gyda chytundeb fy Ngoruchwyliwr (Goruchwylwyr).

Abstract

Over the last seventy years, homing pigeons have been used as a model for avian navigation. Experiments utilising homing pigeons have uncovered many navigational cues used in the process of homing, as well as investigating their development, interaction, and processing in the brain. However, despite work investigating the relationships between cues, no framework of cue integration theory has been applied to the model of homing pigeons. In this thesis I aim to investigate how the theories of cue integration developed in the field of human psychology can be applied to cue integration in homing pigeon navigation and see how well these theories stand up to experimental manipulation of cues. In order to achieved this, I use modern tracking technology in the form of light-weight GPS and accelerometers to monitor navigation in field experiments, as well as a controlled arena environment where behaviour can be more closely monitored. Field experiments highlight the importance of considering individual strategies when studying homing pigeons. Where homing flights may not be maximally efficient, routes may represent individual preferences, and releases from novel locations can produce a variety of strategies, suggesting that there may be no single optimum. I find that there is evidence to support the use of a maximum likelihood estimation framework when studying the integration of cues in homing pigeons. In particular, the response of clock-shifted birds to the introduced conflict between the solar compass and other navigational cues supports cue integration. Additionally, at the location of our field experiments, the homing pigeons do not appear to be relying on the magnetic compass when the solar compass is manipulated, supporting the use of visual landmark cues in familiar area navigation. This research demonstrates that the approach used to study cue integration in humans can be applied to a non-human subject and used to ask questions about important behavioural traits. Taking this approach in future research into cue use across a range of contexts when considering the interaction between the sensory system and resulting behaviours.

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Author Contributions

This section lists where other authors have made contributions to each experimental chapter of this thesis.

Richard Holland contributed to the conception of the project, planning of the fieldwork, and feedback on all chapters.

Simon Watt contributed to the conception of the human psychology cue-integration elements of the project and gave feedback on all related chapters.

Anna Gagliardo contributed to the conceptualisation and planning of the experiment in chapter 2, as well as the data analysis, proof-reading and additions to the publication.

Charles Bishop contributed to the design, production and use of the accelerometers used in chapter 5.

Ingo Schiffner and Dmitri Kishkinev contributed to the data collection for chapter 4, and Ingo Schiffner contributed data analysis to chapter 3.

Emily Price and Meghan Charnell-Hughes contributed data collection to chapter 3.

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Chapter 1

General Introduction

Literature Review

The Study of Bird Navigation

Birds display some of the most remarkable feats of navigation found in nature, from long-distance migration to local homing, abilities which have intrigued scientists for decades (Wallraff, 2005). The ability to navigate has many ecological benefits, allowing efficient foraging trips across a range of distances, for example in the Manx shearwater, where birds take long foraging trips at sea (Padget et al., 2018). Migratory birds can travel vast distances over an extended period of time, making yearly migrations to locations with favourable conditions as the seasons change, such as the migration of the arctic tern between the arctic and Antarctic summers (Egevang et al., 2010). Evolutionarily, birds which were better navigators were more likely to locate the favourable locations, and so had a better chance of survival. Therefore, there are strong evolutionary drivers to make navigation more accurate. However, the mechanisms by which navigation took place were, for a long time, a mystery. Early work focussed on developing a framework under which avian navigation could be studied, in the form of Kramer's map and compass model (Kramer, 1953), then continued to work on the sensory mechanisms for each of these components (Keeton, 1979; Papi, 1986; Able, 1995).

Homing pigeons (domesticated rock doves, *Columba livia domestica*) have been an essential research tool in the study of bird navigation, due to their ability to accurately home across great distances, and their amenability to keeping, handling, and sensory manipulation (Wiltschko and Wiltschko, 2017b). Discoveries made in homing pigeons have been found to be widely applicable in bird navigation as a whole (Wallraff, 2005; Wiltschko and Wiltschko, 2017b), and much work has also been done on migratory songbirds and sea birds (Wiltschko and Wiltschko, 2009; Deutschlander and Beason, 2014; Holland, 2014; Chernetsov, 2016).

The map and compass model is still central in modern-day studies of bird navigation (Able, 2001; Wiltschko and Wiltschko, 2003), but the way in which map cues and compass cues are integrated in the decision-making process of navigation remains unclear. This is particularly the case in familiar-area navigation, where information from many redundant cues including memorised features of the area may be integrated (Beason and Wiltschko, 2015).

Cue hierarchies have been studied in migratory birds, looking at which cues are used in preference to others under certain conditions (Able, 1993; Giunchi et al., 2015). However, no attempts have been made to model cue integration between map and compass elements. Cue integration studies are traditionally in the realm of human psychology, where highly controlled environments and

cue/sensory manipulations can be carried out to test human perception under a model of Bayesian optimal integration (Trommershauser et al., 2011). Such techniques may be applicable in homing pigeon studies, where cue-conflicts via manipulation of sun-compass perception are already widely used. The accuracy of track data necessary to study cue integration from a probabilistic approach has only been readily available in recent years, due to the increasing availability and accuracy of GPS tracking devices. Analysis of complete homing tracks has been used in the past decade to reveal much about familiar area navigation but has not yet been applied to the study of cue integration.

Aims of the Review

In this review, I will begin a conceptual overview of the problem of avian navigation, looking at the cues available, and the evidence concerning how these cues are used. This will be followed by an analysis of more recent research into familiar area navigation, the cues available, and controversies regarding the relative importance of landmark cues. Much work on familiar area navigation has used a clock-shift procedure (Chappell, 1997) to set sun-compass information in conflict with other cues such as the magnetic compass or familiar landmarks, so my review will continue to discuss these past experiments, and what they have sought to demonstrate. From here, I will consider in more detail the psychological techniques and probabilistic models that have been used to successfully study cue integration in humans, what studies into lateralisation of cue perception and processing can tell us about cue use, and how current tracking technology may allow these techniques to be applied to field studies of homing pigeons. The review will conclude with what open questions remain in the area of map and compass cue integration as a justification of my research proposals, and how these questions may be addressed.

A Conceptual Overview of the Problems in Avian Navigation

The Problem of Navigation

When navigating across any distance, birds face the problem of determining a route between their current location, and either a location they have been to before (in the case of homing pigeons returning to their loft), or a location which is unfamiliar to them (such as in the case of first-time migrants). They may be navigating from an area which they are either familiar (have visited before) or unfamiliar with (have not visited before). I will concentrate on the problems faced by birds navigating from both familiar and unfamiliar areas to a known home location.

From both familiar and unfamiliar starting points, birds face the same task: determining and taking the direction they must fly in to reach home. This has been conceptualised by Kramer (Kramer, 1953)

as a two-step process: first determining the necessary direction between the current position and home, based on the relative position of the two locations, and secondly finding this appropriate direction in reality, relative to one's own position. These are known as map and compass steps respectively. The map component is generally considered to be either a 'mosaic map' system (Wallraff, 1974), where a bird remembers a local network of cues which it uses as beacons to navigate in a familiar area, or a gradient map. In theory, a gradient map could allow navigation over much larger distances, beyond the individual experience of a bird's previous flights (Chernetsov, 2016).

Distinguishing between 'map' and 'compass' cues can be challenging – the same environmental signal may be used for both purposes, if it can provide the necessary information. However, in a behavioural context, the two components are more difficult to unravel. In theory, a bird with access to only compass information would be able to take up a particular heading, but not to find the appropriate heading for their current position. This can be seen in the displacement of first-time migrants, which take up an innate compass direction regardless of their starting point, as they have not yet had the experience to build a navigational map (Thienemann, 1931). In homing pigeons, birds deprived of map information often fly either in a trained direction (directional training, i.e. many releases in the same direction can lead to the automatic use of that compass direction (Benvenuti et al., 1973)), or in a preferred compass direction (a frequently observed bias in compass orientation independent of site, but often population-specific. Function/cause unknown, but observed in homing pigeons and many migratory bird populations (Wallraff, 1978)). Without access to compass information however, birds cannot take up a particular course, so orientation tends to be at random.

Information regarding location and direction can be gained from various external cues, and the ways in which these are thought to work are discussed in the following sections.

Available cues and evidence for their use

Information from the Earth's magnetic field

Birds have the ability to sense the Earth's magnetic field and could therefore use it for navigational purposes. Electrophysiological studies have looked into the basis of magnetic sensitivity, where two different systems are thought to be used. The first is thought to be a light-dependent photopigment (possibly cryptochrome) based system in the retina, sensitive to inclination but relatively insensitive to field strength. A second system in the trigeminal nerve thought to be based on magnetite shows sensitivity to small changes in field intensity (Beason, 2005). The neural basis for the reception and processing of this information is unknown, however.

The Earth's magnetic field is generally described by three parameters: intensity, declination, and inclination. Intensity describes the strength of the magnetic field, which varies between 0.25 and 0.65G. Declination describes the angle of magnetic North relative to true North, and inclination describes the angle that the magnetic field makes with the horizontal, when facing magnetic North. Although these are all human measurements of the magnetic field, they describe the basic properties which may provide navigation information. All three parameters vary across the globe, and therefore may provide positional map information if their variations could be learnt on a large scale, or directional information if the relative parameters between two locations could be learnt in a small-scale local map.

Evidence that birds use geomagnetic cues during navigation comes from experiments which disrupt their ability to sense it, often via the attachment of small magnets to the head or back. Early work in migratory birds showed that birds contained within Kramer cages/Emlen funnels could be induced to change the orientation of migratory restlessness by imposing a different magnetic field, and disruption of the field altogether caused a random distribution of movement (Wiltschko and Wiltschko, 1972). Targeted manipulation of magnetic field parameters revealed that European robins use magnetic inclination to orient during migration (Wiltschko and Wiltschko, 1972). The ability to manipulate magnetic field parameters on a local scale means that caged birds can undergo 'virtual displacement', where they experience the magnetic field parameters present at a different location (Chernetsov, 2016).

In homing pigeons, early experiments found that birds with a magnet glued to the back were less able to orient under overcast skies, but also that first-flight birds were affected under sunny conditions. Most birds showed some effect at longer distances (Keeton, 1971). This suggests that a) there is at least some redundancy between use of solar and magnetic cues, b) this redundancy is dependent on learnt associations between the magnetic compass and the movement of the sun, which is incomplete in inexperienced birds, and c) magnetic cues may be more suited for long-distance navigation than solar cues. However, it should be noted that repeated testing by Keeton failed to replicate these findings, with no significant difference between birds with magnets or brass bars attached to their backs, when released under overcast conditions (Moore, 1988).

It is also possible for magnetic information to provide map information, in the form of a gradient map. The idea of a gradient map can be applied if two or more cues are available which vary predictably in perpendicular (or near-perpendicular) directions, to effectively produce a spatial grid. Movement across this grid and the changes in cue value associated could provide directional information (e.g. cue (a) value increases to the north and cue (b) value increases to the East), or map information if the

absolute values could be associated with geographical location via learning processes. Evidence for a magnetic map sense has generally been lacking, but recent evidence that Reed Warblers can use magnetic declination suggests that a bicoordinate grid based on magnetic field parameters may be available to migrating birds (Chernetsov et al., 2017).

Olfactory gradients

One of the cues available to birds, which has increased in popularity within the scientific community over the last few decades, is that of olfactory gradients. In theory, any relatively stable chemical gradients in the atmosphere which could be detected via the birds' olfactory system could provide navigational information. Exposure to these cues during early life could allow individuals to produce a locally learned map of gradients, which could be extrapolated to more distant locations. More wide-ranging flight experience would increase the range of the map. The availability of such information would depend on how able birds are to sense very small gradients in various chemical compounds, and how stable such gradients are.

Evidence for the role of olfactory cues in navigation originates with Papi (Papi et al., 1971; Papi et al., 1972), where birds rendered anosmic (unable to smell) via olfactory nerve section were unable to home from unfamiliar but not familiar locations. This suggested that some form of chemical signal allowed birds to identify location outside of their familiar area, essential to navigation. Further experiments in which young birds were shielded from air currents showed that exposure to these currents was necessary for successful navigation in later life (Wallraff, 1970; Wallraff, 1979). Studies of atmospheric gradients via gas-chromatography samples of a 400km^2 area found relatively stable gradients of volatile hydrocarbons, with differential orientations sufficient to produce a gradient map (Wallraff, 2004). However, it remains contentious whether such gradients would be sufficiently stable, especially over land (Wiltschko and Wiltschko, 2017a). The role of wind exposure may be to give a directional reference to the observed gradients, as young birds shielded from this during a 3-month sensitive period post-fledging cannot learn to navigate successfully (Gagliardo et al., 2001a; Ioalè et al., 2008).

Instead of a strict bicoordinate gradient map which allows exact position to be determined, the olfactory map is thought to provide information about the direction of displacement, based on gradient differences and wind directions (Gagliardo, 2013). However, the mechanism of olfactory processing in the context of navigation remains unclear, as well as the range at which such a map could be used.

Information from the position of the sun

Due to its predictable apparent movement across the sky, the sun may be used for directional information. Although the absolute position of the sun could be used, this can be broken down into its horizontal and vertical components, usually described as its azimuth (relative direction on the local horizon), or its elevation (zenith, the angle between the horizon and the centre of the sun's disc). Either component, or both, could be used as a directional reference providing that necessary time-compensation occurred to account for the change in position over the course of the day. Similarly, variation in the sun's arc across the course of the year would mean that experience of seasonal changes may be necessary. On top of this, geographical variation in the apparent arc of the sun may mean that any learnt associations may be restricted to a relatively local area.

The first evidence for the use of solar cues in bird navigation came from studies of migratory songbirds. Kramer studied Starlings in cages with six windows, restricting vision to the outside world (Kramer, 1950). An observer could sit below the cage and observe the movements of the birds. This setup demonstrated that 'migratory restlessness' corresponded with the direction of migration in free-flying birds, and manipulation of the sun's visible position through the windows with mirrors demonstrated that the bird's orientation corresponded with where they thought the sun was. This provided early evidence that migratory birds use the sun to orient their flight, and lead to the development of Kramer's sun-compass hypothesis.

Hypotheses concerning which particular aspects of the sun's movement provided navigational information continued to be developed following Kramer's sun compass hypothesis. Matthews (Matthews, 1953) developed the sun-arc hypothesis, which proposed that the sun could be used to provide both a locational and directional reference: both map and compass. In this theory, latitude and longitude can be calculated from the sun's position alone, via extrapolation of the full arc of the sun following observation of a small portion of it. Once the arc is known, the highest point indicates geographical south, and the position of local noon can be compared with that of the known position of noon from home. The difference in altitude between these noon positions can be used to calculate latitude, and difference in azimuth gives longitude. However, subsequent experiments by Kramer (Kramer, 1953) and Hoffman (Hoffmann, 1954) suggested that only the azimuth of the sun, not its elevation or path across the sky, was important for orientation.

The ability of pigeons to judge the position of a light source has been tested using conditioning experiments, where the birds were trained to press one key at a particular azimuth, and a different key at any other azimuth. McDonald demonstrated an accuracy of between ±3.4° and ±5.1° across the

birds tested (McDonald, 1971). These show less accuracy than was originally predicted as necessary (Schmidt-Koenig, 1990), but do not necessarily reflect how well the sun compass performs in a field setting.

Due to the variable nature of the sun's position, associations between it and compass directions would have to be learned through exposure. Evidence for such learning can be seen where birds have had restricted access to the sun during an early sensitive period and are subsequently unable to navigate using solar cues. However, only a portion of the sun's movement needs to be observed for time-compensation to be learned (Budzynski et al., 2000). Studies have also shown that birds are able to compensate for the variation in the rate of azimuth change of the sun – much slower at dawn and dusk than around mid-day (Wiltschko et al., 2000). As the only innate mechanism, magnetic cues are likely to be used to calibrate the sun compass during the learning period (Wiltschko and Wiltschko, 2003).

Due to the necessity of time-compensation for the use of solar cues for compass information, manipulation of a bird's internal clock, its circadian rhythm, can be used to test reliance on the sun compass during navigation (Hoffmann, 1960). This manipulation takes place via a process known as 'clock-shift'. Here, experimental birds are kept in either a fast or slow-shifted light-dark sequence, offset with respect to the normal cycle, typically by 6h: they experience night either 6h early or late, which re-sets their circadian rhythm over time to this new cycle. Once clock-shifted, birds miss-judge the position of the sun based on the change in their internal clock, and therefore should demonstrate a predictable deflection from the homewards compass direction (Schmidt-Koenig, 1960a; Schmidt-Koenig, 1960b). Change in sun azimuth during the day averages 15 degrees per hour (although this varies with season, time of day and location), so a 6h shift should produce a 90-degree deflection (Alexander and Keeton, 1974). Full deflection would be expected if the sun compass was being relied upon completely for directional information. However, this is rarely seen in experiments, possibly due to the use of alternative sources of compass information (Wiltschko et al., 1994; Chappell, 1997; Gagliardo, 2005).

Calibration of the sun compass is thought to occur during an early sensitive period, approximately 1-3 months after fledging. However, calibration continues to occur after this, as demonstrated by raising birds under clock-shifted conditions (Wiltschko et al., 1976). Here, birds were raised under a 6h CCW clock-shift. With access to free flight under the sun, they were able to learn to navigate successfully under this modified photoperiod. However, allowing the regular photoperiod to be restored resulted in a flight deflection equivalent to a 6h CW shift. After several flights, however, the birds were able to orient as well as unshifted controls, but did not show a response to further clock-shift treatment,

suggesting that they had down-graded the importance of the sun compass as a navigational cue, and were instead relying on other factors. After a year adjusting in unshifted conditions, the birds learnt to navigate successfully under the normal photoperiod and showed a response to clock-shift, suggesting that the sun compass was now being used once more, following ongoing recalibration.

Landmark features

In contrast to a gradient map which may be extrapolated to unfamiliar areas (outside of direct flight experience), landmark features are only available as navigational cues in the familiar area, where the bird has experienced them, and has been able to form positional or directional associations. The exact nature of which landmark features may be salient in bird navigation will depend on the nature of the birds' visual system, such as its field of view and ability to discern detail. Whatever and wherever they may be, stable landmark features could be associated with a home direction (e.g. that mountain range is East of home), or potentially memorised as a sequence of steps independent of compass directions (e.g. follow the boundary of field and forest then turn towards the church spire).

Visual landmarks had been generally ignored as an important cue in pigeon navigation, following early experiments which failed to find a significant effect of visual impairment on homing performance and orientation (Schmidt-Koenig and Schlichte, 1972; Schmidt-Koenig and Walcott, 1978). Direct investigations into the role of visual landmarks in anosmic birds navigating in the familiar area used frosted lenses to remove object vision but found that birds were still able to orient with reasonable accuracy (Benvenuti and Fiaschi, 1983). However, many still argued that the wealth of temporally stable positional and directional information provided by visual landmarks meant that it was highly unlikely that any navigational system would have evolved to ignore them.

The first direct evidence as to the importance of visual cues in the familiar area came from Guilford and Braithwaite, testing the difference in homing speed and initial orientation between birds who could and could not view the landscape five minutes before release, where both groups could view the sun (Braithwaite and Guilford, 1991a). They found that birds able to view their surroundings had significantly faster mean homing times than those unable to view the landscape until release, although there were no significant effects on trajectory. Later modifications of this test procedure which ensured that the birds had good access to olfactory cues whilst enclosed replicated the findings, suggesting for the first time that visual information was important in familiar area navigation (Burt et al., 1997). Once GPS technology became more widely available for navigational studies, a repeat of

this experiment demonstrated that birds able to view the landscape showed reduced initial circling behaviour than those who could not pre-view the landscape (Biro et al., 2002).

Some subsequent experiments into the importance of visual landmark information took an alternative approach, via arena tasks which allowed the manipulation of available landmark information (Chappell and Guilford, 1997, 1995). The first study used clock-shift to test whether the pigeons relied predominantly on visual landmark or sun compass information in a food-searching task, and found that, even under familiar conditions, the sun-compass was favoured. However, the following study found that pigeons would respond to visual cue shift with a predictable deflection if three-dimensional not 2-dimensional cues were used. This suggested that the nature of visual landmarks would be important in terms of their salience in navigational tasks. The inability of birds to memorise two-dimensional cues and transfer the information between sessions has implications in real navigational tasks, as distant landmarks on the horizon are effectively two-dimensional. The nature of the pigeon visual system must be considered when addressing which features of a landscape might be used in navigation (Holland, 2003).

The way in which landscape information is used in navigation has long been a subject of debate, with two main possibilities: landscape features used as beacons to be piloted between, not requiring the use of a compass, and a 'mosaic map' made up of a memorised layout of landmarks via the compass directions which connect them (Wallraff, 1974). Subsequent sections will address these possible strategies, the information that they require, and the evidence surrounding navigation in the familiar area.

Signal combinations and redundancies

Due to the way in which the non-innate navigational senses are calibrated, there is an inherent redundancy in the information available. As the only innate sense, the magnetic compass is thought to be used to form directional associations with other cues, such as sun position and olfactory cues (Wiltschko and Wiltschko, 2003). Therefore, the magnetic compass and sun compass should, in theory, provide the same information, although each may be more or less reliable under different conditions. For example, evidence suggests that the magnetic compass is used under overcast skies when sun information is unavailable (Keeton, 1969; Keeton and Gobert, 1970). Early experiments releasing pigeons under overcast conditions found that pigeons were still able to orient correctly, prompting the investigation of an alternative day-time compass, now known to be the magnetic compass.

Relationships between sun and magnetic compass use have also been demonstrated via clock-shift experiments. As many clock-shift experiments produce a lower-than-expected degree of deflection, it

was theorised that the magnetic compass may also be used, leading to a compromised direction and reduced deflection. Experiments attaching bar magnets to the pigeon's back to disrupt use of the magnetic compass showed that deflection under clock-shift increased, suggesting that the magnetic compass was contributing to the chosen direction when available (Wiltschko and Wiltschko, 2001; Wiltschko and Wiltschko, 2007; Gagliardo et al., 2009b). Therefore, there appears to be some integration of the sun and magnetic compass in the navigational process, although the extent to which this occurs is unknown. The sun compass appears to be dominant where available, but birds which have been deprived of access to the sun during the sensitive learning period are still able to navigate using the magnetic compass, disrupted by attachment of magnets (Wiltschko et al., 1981).

Although there is theoretically reasonable redundancy between compass cues (signals which provide directional information), map cues (providing positional information) are rarer. In unfamiliar terrain, olfactory cues are thought to be the primary source for positional information. Under Kramer's map and compass theory, both positional and directional information is required for navigation to occur successfully. Therefore, if either map or compass cues are absent, navigation will not be possible. This may explain why anosmic birds are only able to navigate in the familiar area, where an alternative positional cue is available in the form of landscape features.

The availability of learnt visual cues from the landscape in the familiar area provides more alternative sources of information. As discussed, it is debated whether familiar area navigation requires the use of a compass, and it is theoretically possible for navigation to occur on the basis of a memorised sequence of landscape features alone (pilotage). This would imply redundancy with other navigational factors including sun and magnetic compass, as well as olfactory information. Experiments preventing various cue use suggest that different cues can be used interchangeably. For example, birds with object vision impaired via the use of frosted lenses are still able to home fairly successfully, which reduced interest in the role of visual cues for many years (Schmidt-Koenig and Schlichte, 1972).

In familiar area navigation, the sun compass may be used as a reference to learn spatial relationships between landmark features, as demonstrated in hippocampal lesion studies (Bingman and Jones, 1994). This means that they at least partially provide redundant information. However, the sun compass appears to be used in preference to visual cues, as demonstrated by clock-shifted birds which show deflection even when the loft is in sight (Schmidt-Koenig, 1958; Armstrong et al., 2013). In a lab experiment designed to mimic a navigational task, birds were found to preferentially use the sun compass to locate food, even when fixed feature cues were available (Chappell and Guilford, 1995).

The relationship between visual cues and olfactory cues in familiar area navigation has also been investigated. With either sense impaired, birds are able to home successfully. However, impairment of both senses simultaneously decreased homing performance significantly, suggesting that one or other 'map' component is still necessary for navigation in the familiar area (Benvenuti and Fiaschi, 1983a; Gagliardo et al., 2001c).

This relationship can also be investigated in combination with the sun compass, using clock-shift as an indicator of sun compass use. For example, clock-shifted pigeons rendered anosmic do not show the expected level of deflection when released at a familiar site (Bingman and Ioalè, 1989). This suggests that, in the absence of the olfactory map, the sun compass is of reduced importance when visual landscape cues are available. However, increased scatter of the vanishing bearings suggests that the sun is still involved in the use of landmark cues. Clock-shifted birds without olfactory impairment showed greater deflection, suggesting that the map and compass system used in unfamiliar areas is still preferentially used in familiar area navigation.

In summary, the cues available for bird navigation are currently understood to be in the form of a magnetic inclination compass, sun compass, olfactory map, and visual landmarks. When navigating in an unfamiliar area, the olfactory map is necessary for navigation in combination with compass cues. These compass cues appear to be used in combination, but with the sun compass dominating in most situations. In the familiar area, map and compass navigation using the olfactory map and sun compass seems to be preferentially used, but visual landmarks can be used when olfactory map factors are unavailable and may be responsible for some of the reduced clock-shift deflection seen, due to the conflicting information.

Navigation in the Familiar Area

Navigation in the familiar area has the potential to exploit memorised local features, which are not available when navigating unfamiliar locations. Therefore, different cues are available, and are likely to be used in different ways depending on the availability of salient features for memory formation in a local area. Evidence that a 'familiar area map' is used comes from studies of the role of visual landmarks in navigating the familiar area. I will discuss what these studies have found, and what relevance this brings to the study of cue integration.

Studies of familiar area navigation have been recently enhanced by the development of GPS tracking technology, which has allowed the tracks of individual birds to be analysed along their entire length. This technology means that researchers no longer need to rely on vanishing bearings, and can collect sufficient data to avoid averaging across groups of birds. In the context of clock-shift studies, GPS can

be used to see if initial deflections are maintained along the entire flight path, or if corrections are made. If a compass is being used to constantly update trajectory, deflection should be consistent; if updates are being made at particular waypoints in the route, these may be identified; if course corrections are being made, these should also be apparent, and help to determine how different sources of information are being used in combination.

Biro et al. found that results were varied between individual birds: some birds displaying route stereotypy showed no deflection; less experienced birds showed some deflection (although not 100% of expected); some birds showed deflected route recapitulation behaviour, where a stereotyped route was followed in parallel (same flight directions as the original route, but offset to the location of the new release site) (Biro et al., 2007). This highlighted the importance of individual differences in experience, and the possibility that multiple strategies can be used between individuals, and between flights. Similarly, how far along the homing route deflection is maintained is highly variable, with some pigeons showing deflection even when the loft is in visual range (Armstrong et al., 2013). Such variable results would have been hidden in studies where averages were taken across birds.

The use of GPS tracks has also enabled the analysis of individual pigeon routes, and mapping onto landscape features. The discovery that some birds develop stereotyped flight paths (route stereotypy; route recapitulation) in the familiar area which were individually consistent but not maximally efficient, suggested that birds were learning individual routes instead of displaying flexible navigation on subsequent releases. Birds displaced from their chosen routes would return to their normal flight path, even if this was not the most direct route home (Biro et al., 2004; Meade et al., 2005), suggesting that the birds may be navigating via a learned series of landmarks, supporting the pilotage strategy. However, replication of these results has been variable at different locations (Biro et al., 2007; Wiltschko et al., 2007), suggesting that the salience of local landmark features may be important when choosing a navigational strategy. For example, an analysis of landscape complexity found that there was an optimal 'edge density' at which route learning occurred, where visual information was neither too sparse nor too overwhelming (Mann et al., 2014). Above this optimum level, such as in some urban or forested areas, a decline in route fidelity was seen. One study identified that pigeons tend to follow highways in familiar areas, which correspond roughly to the home direction (Lipp et al., 2004), where the degree of following increased with area familiarity, supporting the role of learning. However, it is unclear whether this strategy represents a memorised route or learned stabilisation of a compass course. Later studies have further investigated which landscape features are associated with track features, via 'waypoints' where track variability is lowest (Mann et al., 2011), further highlighting the importance of particular types of landmarks.

Variation in the local types of landscape features and evidence that landscape type is important means that site-specific differences in the use of landscape cues should be expected. The influence of site-specific differences has been found in clock-shift studies, which aim to determine how different landscape features affect the choice of navigational strategy. Mora et al. found that birds navigating from a familiar area with discrete visual landmarks (wind turbines) showed reduced deviation under clock-shift compared to those navigating from a familiar area without distinct landscape features (Mora et al., 2012). Other experiments have also highlighted the role of site-specific differences in the amount of deviation under clock-shift (Bonadona et al., 2000; Wiltschko and Wiltschko, 2007; Filannino et al., 2014), suggesting that multiple aspects of landscape complexity and the availability of salient landscape features are important in the learning of a familiar area map.

Recent studies of familiar area navigation have highlighted the importance of individual and site-specific differences. The availability of particular landscape features and individual homing experiences mean that a variety of homing strategies appear to be used, from apparent map and compass navigation, to following landmark sequences. This suggests that different types of navigational cues can be used to different extents, depending on circumstances. Although many factors contribute to the choice of navigational strategy, understanding more about which cues are important in each may reveal how the information is processed and combined. Put together, the use of salient visual landmarks in combination with clock-shift procedures could be used to investigate the integration of different cues in familiar area navigation. The methods by which this could occur will be discussed in the following section.

Psychological Approaches to the Study of Cue Integration

When navigating, a bird must use the available information to find and take up a particular heading. Due to redundant or complementary information provided by various cues, a navigating bird has access to multiple sources of information by which this directional estimate can be made, but how should these sources of information be combined? Little has been done to attempt to understand the complexities of cue integration within avian navigation, but this is not the case in human psychology, where cue integration has been studied for many years. In this section I will consider the models developed in the field of human psychology to describe optimal cue integration, the methods by which these models can be tested, and how such methods could be applied to the problem of pigeon navigation.

Maximum Likelihood Estimation

In order to interact with the world, an animal's sensory system must be able to encode and integrate available sensory information. However, information and encoding processes are noisy; they have inherent variance associated with them. As each signal available has an associated variance, they should be combined in such a way that the overall variance in minimised, and therefore the reliability of the estimate is maximised. This can be done via a maximum likelihood estimate (Ernst and Banks, 2002). Under this framework, an optimal estimate can be produced by combining the estimates from each signal, weighted by the variance associated with each. As variance is inversely proportional to reliability (Knill, 2006), a signal with high variance should be given a reduced weighting in the combined estimate, in order to maximise reliability. Under a maximum likelihood estimate framework, an optimal estimate is the one which has the lowest variance. This can be considered as a weighted sum model, where the overall estimate is produced by summing the estimates for each signal, weighted by its relative variance (Ernst and Banks, 2002).

Two important properties emerge from a system using maximum likelihood estimation to integrate multiple cues: dynamic weighting, and robustness. These properties must be understood when making predictions about and designing experiments to test cue integration.

Dynamic weighting describes a process whereby weights can change dynamically as the reliability of the available signals vary (Landy et al., 1995). Without dynamic change, the system would only be accurate under a very narrow range of conditions and would therefore not be able to produce an optimal estimate at all times. Dynamic weighting has been observed in populations of sensory neurons, where neurons apply different weights to their sensory input dependent on cue reliability (Angelaki et al., 2009). This could theoretically occur on a cellular level by the setting of sensory thresholds which mean that low-variance signals produce greater cellular stimulation (Ganguli and Simoncelli, 2014).

As well as dynamic weighting, system robustness is also an important consideration. In a robust system, small changes to the underlying distribution of signal data will not produce large changes in the estimate. The influence of discrepant cues should decrease as the amount of discrepancy increases, so that estimates are not biased towards abnormal data points. When discrepancies are extreme, "robust procedures" can occur, such as down-grading or discounting the inconsistent stimuli entirely (Landy et al., 1995). This is important when considering experimental design, as any experiments which test the response of a system facing highly discrepant signals may not see any signal integration due to robust procedures, despite integration occurring under normal conditions.

However, it may be possible to record a switch between optimal integration of signals, and robust behaviour, where one signal is chosen in favour of the other.

Experimental approaches to investigate cue integration

Two main experimental methods can be defined when investigating cue integration: single-cue and two-cue (or cue-conflict) experiments. Both approaches have their advantages and drawbacks, especially when experimental sensory manipulation is limited.

In single cue experiments, a sensory estimation task is presented where only one cue is made available at a time. This allows probability distributions of each estimate to be created, over the course of multiple trials, which can be compared between signals to see which have greater variances, and therefore predict what the relative weightings should be when cues are combined. Subsequent experiments with two cues available in combination can show how the distributions change with multiple sources of information available, and test whether the variance is reduced, as would be predicted under a MLE framework. The primary drawback of single-cue experiments is limiting the observer to the use of a single cue, which may not always be experimentally feasible.

In contrast, cue-conflict experiments can be used to directly investigate the relative weighting of cues as signal reliability changes, by experimentally manipulating the cues to introduce a conflict. Starting with two cues in complete agreement, gradually increasing discrepancy between the cues (introducing conflict) should produce a dynamic re-weighting of the system. In a robust system, it may be possible to identify the level of discrepancy at which a particular cue is discounted in the estimation process. Cue-conflict experiments allow dynamic weighting and robustness of a system to be investigated. However, they must be designed carefully to study the area in which conflicting cues are still integrated, before any robust procedures occur.

Approaches in birds

The vast majority of work on cue integration has taken place using human subjects, in highly controlled conditions where available sensory information can be fully manipulated. Therefore, there are inherent difficulties in transferring these techniques to field studies, where control is greatly limited. However, it may be possible to apply such techniques to pigeons, in order to investigate the amount of uncertainty associated with each cue, which cues are combined, the relative weighting of cues and if dynamic weighting occurs. I will discuss how single-cue and two-cue experiments may be used to study cue-integration in birds, what such experiments would predict, and what difficulties may arise.

One of the greatest unknowns in navigational cue is the amount of uncertainty associated with each cue. One psychophysical approach to analysing uncertainty is to calculate discrimination thresholds. This is the difference in the value of a stimulus necessary for an observer to discriminate between two stimuli, set at a particular success rate, usually 75% of the time. Uncertainty is proportional to the standard deviation of the probability distribution and can therefore be related to variance and weighting in an MLE framework. If the uncertainty associated with different cues, e.g. sun compass vs magnetic compass or visual landmarks, could be assessed, then it may be possible to predict how they should be weighted and compare this to observed values to see how well signal integration fits to optimal integration theory.

Single-cue experiments in birds have thus far been used to assess whether or not a cue is being used in navigation, not to what extent the information is integrated. Unlike in laboratory experiments where the cues available can be controlled, field experiments must rely on sensory manipulation to prevent access to certain cues. Many techniques exist to prevent cue use and therefore limit perception to a single cue. However, this is complicated by the necessary use of combined cues in map and compass navigation, which can distort navigational strategies chosen when some cue use is prevented. For example, anosmic birds show reduced use of the sun compass when navigating in the familiar area (Bingman and Ioalè, 1989), as the map and compass system cannot be used without access to the map, downgrading use of both cues when one is made unavailable. Similarly, it can be difficult to untangle stress effects of sensory manipulation on navigation, with direct effects of sensory manipulation. It may be possible to conduct some arena experiments in which cue availability can be more easily controlled without sensory manipulation.

The focus of past cue-conflict experiments in birds has not been to quantitatively test the degree of cue integration or assess the relative weights of available cues. Instead, they have been used qualitatively to address whether or not certain cues have any input on the navigational system, e.g. input from the magnetic compass (Wiltschko and Wiltschko, 2001; Wiltschko and Wiltschko, 2007; Gagliardo et al., 2009b), or from visual landmarks in familiar area navigation (Holland et al., 2000; Gagliardo, 2005; Wiltschko et al., 2005). Most of these experiments use the standard 6h clock-shift to produce approximately 90 degrees of predicted deflection, although some 4h shifts have been used (Munro and Wiltschko, 1993; Wiltschko et al., 2000; Padget et al., 2018). These are all very large conflicts, which may represent a significant shift in cue weighting, as a result of highly discrepant stimuli. Robust behaviour may also have occurred, with one or other signal being removed from the estimation process. In contrast to these experiments, small amounts of clock-shift increased incrementally could be used to assess cue weights in pigeons and identify if there is a cut-off point

where the conflict is too large for integration to occur. The primary challenges of this approach are gaining sufficiently accurate tracks to detect small deflections resulting from small amounts of clock-shift, and accounting for the large individual variation seen in previous clock-shift studies.

Overall, theory and techniques from human psychology on the integration of sensory cues could be applied to studies of cue use in pigeon navigation, to assess how different map and compass cues are integrated. Using a maximum likelihood estimation framework of cue integration, several predictions can be made: a) homing performance should be better when multiple versus individual cues are available; b) clock-shifted birds should show a reduced percentage deflection with increasing clock-shift amount, as the increasingly discrepant signal has its weighting reduced; c) increasing cue variance should dynamically reduce its weighting in a combined estimate, e.g. increasing the noise of visual signals with increasingly distorting lenses, or increasing inhibition of olfaction. Experiments could be designed to address these specific predictions.

Lateralisation of Cue Use

One area which may be promising in the investigations of cue perception and integration is lateralisation – perception and processing of different cues occurring in different brain hemispheres. Comparative studies of pigeon brains and behaviours have identified many differences between mammals such as the laboratory rat (Bingman et al., 2005), other birds such as chicks (Tommasi et al., 2003; Wilzeck et al., 2009), and between pigeons with and without homing experience (Mehlhorn et al., 2010), suggesting the significance of homing behaviour on the evolution and development of brain lateralisation. Experiments concerning the role of lateralisation in homing behaviour generally involve either surgical ablation of brain structures, or covering of one or other eye, either in arena or open field settings. I will explore what these experiments have shown in relation to navigational cues, and what further information could be gained.

Lesion experiments

One class of experiments focusses on introducing lesions into the brain, to test the importance of that brain region in a particular behaviour. Either bilateral or hemispheric ablation of structures is used to assess the overall importance, or the potential for lateralisation. In the study of navigation, tests usually occur on the avian hippocampal formation, thought to be functionally homologous to the mammalian hippocampus (D. Székely, 1999), where encoding of spatial information and memory formation occurs (Morris et al., 1982).

Early field experiments studying the orientation of pigeons following surgical ablation of the hippocampal formation found that, relative to controls, test birds showed greater deflection under clock-shift when navigating in the familiar area (Gagliardo et al., 1999). Control birds did not show the predicted deflection suggesting the use of a pilotage-like strategy, whereas the routes learned by the hippocampal formation-impaired birds relied on sun compass information, suggesting that the hippocampal formation is necessary for the learning of routes by landmark associations alone. Following studies looking at the role of the left and right HF in this context found that birds using only the right hippocampal formation were worse at orienting from unfamiliar areas (Gagliardo et al., 2001b), but there was no evidence of lateralisation in the familiar area, with both left and right hippocampal formation-ablated birds able to home from familiar sites, but requiring the sun compass (Gagliardo et al., 2002). Therefore, the hippocampal formation as a whole seems to be necessary to allow navigation without the use of a sun compass, and to allow course correction following clock-shift; clock-shifted birds with an impaired hippocampal formation were less able to reorient even when presented with a very distinct landmark feature of a coastline (Gagliardo et al., 2009a). This suggests that the hippocampal formation is required to form a coarse landscape representation necessary to make course corrections.

As well as open field releases, surgically manipulated birds have also been tested in an arena setting, where cues can be more tightly controlled and manipulated, and there is no risk of losing birds which are unable to home successfully, and therefore cannot be included in the results as the efficacy of the surgery carried out cannot be confirmed. A square arena is a popular tool, where birds are trained to find food relative to the arena shape (geometric cues) and a particular 'landmark' (feature cues). Experiments in chicks have shown that hippocampal formation ablated birds are only able to learn using feature cues, suggesting that the hippocampal formation is required for the formation of memories from geometric cues, and that feature cues may be processed outside of the hippocampal formation (Tommasi et al., 2003). Similar results have been found in pigeons, where only feature cues could be used in hippocampal formation ablated birds (Vargas et al., 2004).

Arena experiments have also been used to set feature and geometric cues in conflict. Under these conditions, both right and left hippocampal formation-ablated birds were able to use both kinds of information in parallel and singly to locate the goal, but left hippocampal formation-ablated birds relied more on feature cues when the two cues were set in conflict (Nardi and Bingman, 2007). This suggests an asymmetry in the processing of geometric and feature cues in the hippocampal formation, with the left hippocampal formation being most crucial in the representation of geometric information. An investigation of the role of compass cues in an arena setting found that, when feature

cues and sun compass information were conflicting, left hippocampal formation-ablated pigeons did not use sun compass information, relying on feature cues. Right hippocampal formation-ablated birds could use the sun compass, but still showed a preference for feature cues (Gagliardo et al., 2005).

Put together, experiments studying the role of the hippocampal formation in navigational memory formation suggest that the hippocampal formation is required to encode geometric relationships in long-term memory, with the left hippocampal formation being particularly important. Likewise, learning routes without an intact hippocampal formation required the sun compass, and birds did not show any ability to navigate based on a sequence of landmark features alone. The role of lateralisation, although visible in morphological analysis of brain structures (Mehlhorn et al., 2010), remains unclear in a behavioural context.

Visual occlusion experiments

In contrast to surgical approaches, visual occlusion experiments work on the basis that birds have a strongly lateralised visual system, both in terms of the retina (Hart et al., 2000), and the near complete decussation of the optic nerves to the contralateral optic tecta (Cowan et al., 1961; Letzner et al., 2014). This means that covering one eye effectively restricts visual processing to the contralateral brain hemisphere and can therefore be used to limit where information is collected, and where it is processed.

Like with the surgical experiments, field and arena approaches have been used, with eye patches applied with water-soluble glue to the feathers around the eye. Field experiments showed early issues, due to the tendency for birds to show a systematic bias in orientation towards the uncovered eye (Diekamp et al., 2002; Prior et al., 2004), likely in order to centre the visual field. However, this makes it difficult to interpret any possible lateralisation, and has led to contrasting results. For example, Prior et al. found that birds using the right hemisphere (i.e. with the left eye uncovered) showed poorer initial orientation (Prior et al., 2004). However, GPS tracking experiments looking at route fidelity in the familiar area found a right-hemisphere advantage, with birds able to more closely follow their learned route when the left eye vs the right eye was uncovered (although all test birds developed novel routes) (Martinho et al., 2015; Pollonara et al., 2017).

Difficulties in the interpretation of field experiments, even with the advantage of GPS tracking technology, means that arena studies are often more informative. Birds using the left hemisphere in food-searching tasks showed greater confusion when conflicts between geometric and feature cues were introduced, in a set-up designed to mimic stages of navigation in the field (Prior et al., 2002). When landmarks were moved to a new position, birds with the right eye uncovered directed more

search time towards the new location as predicted by the feature cues, suggesting that this eye/hemisphere has a greater role in feature processing, whereas the left eye/right hemisphere may be more involved in the use of geometric information. Separate processing of these cue types is also suggested by Wilzeck et al., who found that more integrated cue use occurred when binocular vision was available, compared to either monocular treatment (Wilzeck et al., 2009). Since piloting in a field setting involves the memorisation of geometric relationships between landmark features, lateralisation may have a role in the navigational strategy used.

Applications in the study of cue integration

These experiments suggest that there may be a difference in how landmark and compass cues are perceived or processed in homing pigeons, with lateralisation of the visual system. If so, it should be possible to manipulate which cues birds are able to use, via occlusion of one or other eye, the success of which could be tested via clock-shift procedures (or feature shift in an arena setting) to put compass and landmark information at odds, and see whether or not the birds respond with the predicted deflection. Such experiments have been attempted with surgically manipulated birds, but not yet in term of lateralisation of the visual system. Given the difficulties of field experiments using visually occluded pigeons, arenas designed to test the relative use of feature and compass cues in each eye may be informative as to how information is processed and memorised in pigeon navigation.

Conclusion

The map and compass model for navigation in the unfamiliar area is an enduring paradigm in bird navigation theory, and decades of research into map and compass cues has led to a general understanding of the sun compass, and introduced the roles of geomagnetic, olfactory and visual landmark cues. Interest in familiar area navigation has been more recent, but developments in high resolution tracking technology are beginning to reveal which cues are important, and how factors such as landscape complexity and the presence of discrete landmarks can affect how easily a familiar area map can be learned. Studies into the roles of different brain regions and lateralisation into cue use, processing and memory formation are beginning to provide more details on a neurological level, which are informing how navigational behaviours are interpreted.

Unanswered questions

Despite nearly 70 years of research into avian navigation, there are still many unanswered questions concerning cue use and cue integration. How much cue use relies on experience and local conditions remains unclear, as is the role of individual learning experiences in navigating the familiar area. Some

attempts have been made to uncover putative cue hierarchies, but little has been done to investigate the way in which cues are integrated in the decision-making process, or how the relationship between cues changes if cue reliability is altered.

Recent evidence suggests that individual birds use different navigational strategies in the familiar area and can change strategies between homing flights (Schiffner et al., 2018). Little is currently known about how or why these differences occur, in terms of cue availability, reliability and integration. It may be that local variation in cue availability and individual differences in learning capacity and memory are responsible for these differences, but how cue uncertainty varies is unknown.

Studies looking into the lateralisation of cue use in terms of perception and processing have found many asymmetries in cue perception and processing, which may be exploited experimentally to manipulate which cues are accessible to the birds, and how navigational strategies and ability to learn are altered accordingly. Very little is still known about how learning occurs, and which cues and cue features are important in the learning process.

Approach of this thesis

The aim of this thesis is to address how compass cues, well understood in true navigation, are integrated into the familiar area map of homing pigeons. Cue conflict experiments in the form of clock-shift are ideal for studying the relationship between cues and integrating approaches from human psychology will allow me to model cue integration at a level not yet attempted. Using small values of clock-shift should introduce a small enough conflict to analyse the relative weighting of different cues combined in the navigation process, with the aid of high-resolution tracking technology to detect small changes in angular deflection along the course of a homing route. I will be able to take advantage of cue use lateralisation to manipulate how accessible map and compass cues are, to test which cues are involved in route learning.

Methods Review: Development of Techniques in the Study of Homing

The field of avian navigation, particularly the use of homing pigeons as a model species, has seen great changes over its lifetime of the last 70 years, both in the understanding of the mechanisms involved and how this has shaped experimental design, and the technologies available to allow tracking and analysis of pigeon navigation. Methods of data collection have changed dramatically, but data analysis methods are sometimes slow to catch up with the changing data types and quantity. To give context to the methods and analyses I have used, this section will briefly consider the historical methods used to study pigeon homing, how they have developed since early experiments in the 1950s, and how modern approaches can be used to answer questions which would have been impossible to study in previous decades. I will begin with data collection, methods used to manipulate cue access, finishing with common methods of data analysis.

Practical approaches to data collection

The earliest approaches to studying homing behaviour in a field setting relied on two key pieces of information: vanishing bearings (the absolute bearing of the bird at the point at which it disappeared from view following release) and homing time (how long it takes a bird to reach home following a release). Any activities within the homing route were a scientific mystery. Although both factors remain important and are often still recorded, technologies have improved to allow investigation of the homing route, more accurate calculation of bearings, and more complex statistical analyses of paths.

Vanishing bearings remain a critical factor in orientation experiments, but traditional approaches suffer for a number of reasons. Firstly, vanishing bearings are traditionally recorded via one or two observers, typically using 10x40 binoculars, and estimating the direction to the nearest 5 degrees. In some cases, the initial flight path would be estimated by eye using a map (Braithwaite and Guilford, 1991). This makes the measurement relatively inaccurate, especially when considering possibly small deflections from the expected home direction. Although they give an estimate of initial direction, they do not provide any information as to the large period of flight between the vanishing point and the home loft, leaving the majority of the homing flight a mystery.

Additionally, analyses tended to average individual bearings to compare between treatment groups, which can obscure individual differences in homing strategies used (Schiffner et al., 2018). This is particularly important as only when analysed individually can strategies such as parallel route following (Biro et al., 2007) be seen. If averaged, individuals which do and do not respond to clock-

shift (i.e. do or do not use the sun compass when navigating a particular route) will produce an overall result which suggests intermediate deflection, even though this is not seen on an individual level. Therefore, such averaging gives a misleading impression of the response to clock-shift conditions.

Attempts to analyse the homing route began with such approaches as following pigeon flocks in an aeroplane (Hitchcock, 1952; Hitchcock, 1955), and using radio telemetry (Schmidt-Koenig and Walcott, 1978). However, both of these techniques are expensive and have a relatively poor accuracy. Early route-recorder or direction recorder technology (Bramanti et al., 1988; Papi et al., 1991; Holland et al., 2000) improved the accuracy of the data, but had issues such as having to assume a constant flight speed of 60km/h, and needing to know the start and end coordinates with enough accuracy to reconstruct the path from the recorded headings (Meade, 2005 for review of these technologies).

However, progress has been made via the use of GPS trackers, increasingly reduced in size whilst increasing in battery and storage capacity, and often associated with the recording of other physical and physiological features. Some early work using GPS devices was used to tackle issues of landmark use during the flight (Biro et al., 2004), route recapitulation (Meade et al., 2005), and the tracking of clock-shifted birds (Meade, 2005; Gagliardo et al., 2009b). More complex devices have been used to analyse the visual attention of pigeons (Kano et al., 2018).

The advantages of recording the tracks of individual pigeons are many: whereas traditional methods relied on vanishing bearings as a proxy for the presumed home direction as chosen by the pigeon, track data allow the identification of course corrections along the route. When mapped on to the physical landscape, tracks can be used to identify if particular landscape features are being followed (Lipp et al., 2004), if the landscape features affect route learning (Guilford and Biro, 2014; Mann et al., 2014), or to identify possible decision points (Schiffner et al., 2013).

Cue removal procedures

Preventing birds from using some of the available cues in order to isolate the information gained from individual cues is a useful technique when studying the signals used in navigation. However, these tests are only valid if the cue removal is complete and does not otherwise impact the behaviour of the bird (e.g. increased stress) in a way which could influence the navigational behaviour seen. Therefore, this section will briefly explore the techniques used to prevent the use of certain cues, and potential issues which arise.

Olfactory cues - there are multiple methods by which anosmia can be induced, with varying degrees of permanence, completion and invasion. Methods include olfactory nerve sectioning (Papi et al.,

1971), inactivation of the olfactory epithelium by Zinc Sulphate (Gagliardo et al., 2001c), nose plugging (occlusion of nostrils or insertion of nasal tubes) (Benvenuti and Fiaschi, 1983b), and nasal anaesthesia. The more invasive procedures may cause significant levels of stress, which can alter homing performance. Not all methods are complete, leading to some uncertainty. The preferred method is generally zinc sulphate treatment, which gives a high level of anosmia in a non-invasive manner (Benvenuti et al., 1992). A control treatment of a saline wash is used.

Visual cues – manipulating vision to remove landscape vision whilst maintaining use of the sun compass is challenging, but experimenters in the past have used frosted lenses for this purpose (Schmidt-Koenig and Schlichte, 1972; Schmidt-Koenig and Keeton, 1977). Pigeons wearing the lenses still respond to clock-shift treatment, suggesting that they retain the ability to read the sun compass. However, it is unknown how the manipulation of vision influences behaviours outside of homing, which may interfere with the navigational process. More recently, visual occlusion has been achieved via the use of eye-patches, temporarily glued to the feathers around the eye using a water-soluble glue (Diekamp et al., 2002). However, occlusion of both eyes using eye patches is challenging as many birds refuse to fly with both eyes covered. Similarly, caution must be taken when covering only one eye, as some evidence suggests that the bird's flight trajectory is skewed in the direction of the uncovered eye.

Geomagnetic cues - the preferred method to prevent use of the magnetic compass is the attachment of a magnet to the back/head of the pigeon, using brass bars as a control. The main issue is that disruption of the magnetic sense may have an impact on non-navigational aspects of behaviour (e.g. a stress response) which can lead to apparently poor navigation. This was tested by Papi et al., who found that the disorientating effects of magnetic treatment were similar to that of disruption of the opioid system (Papi et al., 1992). However, the presence of a strong magnetic field is thought to entirely mask any geomagnetic cues, making this treatment one of the most reliable cue-removal procedures.

Sun compass – The typical way to prevent use of the sun compass is to conduct test releases on overcast days, where the sun is presumed to be fully obscured. That pigeons with their magnetic compass disrupted flown under cloudy skies fail to orientate correctly suggests this is the case (Wiltschko et al., 1981), but it is unclear how fully weather conditions affect use of the sun compass. Aside from this, the sun-compass use must be manipulated via clock-shift procedures designed to change the directional inference that birds make from the position of the sun (Alexander and Keeton, 1974).

Statistical approaches

With changing data collection methods, a change of analysis is also required. Traditionally, analysis centres around the plotting of vanishing bearing on circular plots, and the calculation of mean vectors for experimental groups. A mean vector has two components: a direction, and a length, calculated from multiple points to be averaged. The direction is the circular mean of the averaged points, and the length (measured between 0 and 1) showing how concentrated the points are, with a higher number showing more concentrated data, and a lower number showing increased scatter. Traditional circular statistics can then be used to compare the average orientations of experimental groups (Batschelet et al., 1965).

Directional Analysis

When dealing with GPS tracks rather than single vanishing bearings per bird, one standard approach is to compute the *virtual* vanishing bearings – the bearing of a bird at the point it would have vanished from view using traditional observation methods. This provides a more accurate measure than recording the bearing by eye, and makes modern experiments more comparable with results from past experiments where GPS tracks were not available (but see (Padget et al., 2018) for a discussion of the drawbacks of virtual vanishing bearings in clock-shift experiments).

A complementary approach is to calculate instantaneous deflection (Armstrong et al., 2013; Filannino et al., 2014; Padget et al., 2018). This is calculated as the circular angle between the current bearing of the bird's track, and the beeline home, at set intervals (e.g. one-minute intervals) along the recorded track. The advantage of this method is that it allows deflection to be analysed along the entire track, particularly useful in clock-shift experiments, as it can show whether deflection under clock-shift remains constant over the course of a flight. Track data can also be used, in combination with sun azimuth values at each time/location point along the route, to calculate the expected deflection of clock-shifted birds at each point along the track. Instantaneous deflection can then be expressed as a percentage of predicted deflection (Filannino et al., 2014).

Track Phases

When analysing flight data, it is often useful to distinguish between the initial circling exploratory flight which first occurs after a bird is released, from the navigational flight, and then from the circling which occurs around the loft when landing. This may be done via visual inspection of the tracks to choose a distance-based cut-off point e.g., (Meade et al., 2006) cropped all tracks at a 100m radius around the

release site to cut out non-navigational circling behaviour. However, this is highly subjective, and the length of each phase can vary significantly between birds.

A more nuanced approach was developed termed point of decision analysis (Schiffner and Wiltschko, 2009), using empirical measures to determine when a flight pattern changes from exploratory to homing behaviour. This method uses cumulative velocity and steadiness to identify a switch of flight behaviour. Steadiness is calculated by taking consecutive flight directions and calculating a mean vector over a particular range, e.g. each 15-second period. The length of the mean vector gives an indication of how similar the flight directions are, with a lower length when direction is highly changeable. A steady increase in vector length shows a decrease in turning, suggestive of more consistent directional flight. A cut-off relevant to the particular dataset can be used to determine the point where steadiness represents navigational flight. Point of decision analysis, therefore, can be used to consistently split tracks into distinct phases, which can then be separately characterised.

Characterising homing tracks

Features of a homing track can be characterised in various ways, but features of interest tend to be length, speed profiles, efficiency, tortuosity, and number of stops. Track length can be simply calculated by summing the distances between consecutive points along the entire track, and efficiency is then calculated as the distance flown relative to the shortest possible route; shortest distance/distance flown. The relative straightness of a track may be of interest, as increased tortuosity and circling indicate uncertainty, in comparison with a direct flight path. Tortuosity is usually calculated using turn angles, e.g. average turn angle per meter (Biro et al., 2006). Turn angle between pairs of points (Miller et al., 2011), with this averaged over track sections to give a measure of tortuosity (Mora et al., 2012). Tortuosity can also be expressed in terms of mean vectors lengths, as with the steadiness calculation above (Gagliardo et al., 2011). A speed profile can be used in combination with this, as flight will generally be slower during these uncertain phases. As each GPS fix is time-stamped, distance over time can be calculated to produce speed.

Track Similarity

One area of increasing interest is that of track similarity, either to neighbouring birds, or to repeated tracks from the same bird when considering stereotypic route formation. Early versions of this method involved the calculation of the area between two tracks, as a measure of similarity (Biro et al., 2004; Meade et al., 2005). Following on from this, a method of nearest-neighbour analysis was developed whereby for each point along a track, the distance to the closest point on a neighbouring track is calculated (Biro et al., 2006). This was subsequently developed to produce a nearest neighbour vector,

with a longer vector between a point and its nearest neighbour showing reduced similarity at that point along the tack (Biro et al., 2007), which can be used to compare similarity between the tracks of different birds, or between repeated flights of the same bird to assess route fidelity (Guilford and Biro, 2014; Mann et al., 2014). The nearest neighbour distance can be assigned a negative or positive value, depending on whether the nearest point lies to the left or right of the compared track – such an approach has been taken when studying clock-shift, to see whether shifted birds tend to stay on the shift-predicted side of their previous tracks (Armstrong et al., 2013). Another method of measuring route fidelity is to calculate a mean track, averaged from several other tracks, and then to calculate the variance of the averaged flights around this track (Freeman et al., 2011). This information can be used to assess how route fidelity varies across different track phases.

Conclusion

The rise of small-scale tracking technology over the last few decades has led to significant changes in the kinds of data available in pigeon homing studies. This, in turn, has meant that new methods of analysis have had to be developed to cope with the increase in data, and to analyse parts of the flight which were previously hidden. Current levels of technology allow analysis of all phases of a homing flight, especially important when considering the influence of the sun compass and landscape features, as response to cues may not be consistent over the course of a flight. In this thesis, I will be utilising GPS technology and using a variety of statistical approaches to analyse homing tracks.

Glossary of Terms

Clock-shift	An experimental technique where the internal clock of the experimental subject is reset via an artificial light-dark cycle, with a general rule of one day required per hour of shift.
Cognitive map	A concept of a mental representation of the relative locations of spatial features, learnt through experience. The map can be formed from any sensory modality.
Continuous flight bearing	Calculated as the absolute bearing from the release site to each point along a GPS track.
Deflection/Deviation	Following a clock-shift procedure, birds are expected to miss-identify compass directions on the basis of the sun's azimuth, and therefore show an angular deflection from the home course (a 6h clock-shift gives an expected deflection of approximately 90°, a standard amount used).
Gradient map	A proposed system by which birds could learn to recognise relative positions via a system of overlapping gradients. Some current evidence points towards gradients of olfactory information.
Instantaneous deflection	A calculated measure of flight trajectory from GPS tracks, calculated as the angular difference of the current track at any one point, and the home direction at that point.
Map and Compass	The original theory proposed by Kramer to conceptualise avian navigation as a two-step process. In the first step, a 'map' is used to identify position relative to a goal and calculate the necessary compass direction needed to get there. In the second step, a 'compass' is used to find this direction in reality.
Migratory restlessness	Caged birds will exhibit flapping behaviours during the natural period of migration, in the approximate direction of their migratory flight as it would be at a given point along the migratory route for a particular time in the migratory period.

Mosaic Map	A form of familiar area navigation using landmarks which have learned associated compass directions to produce a route towards the home target (a.k.a. site-specific compass orientation).
Navigation	Setting a course towards a remote goal, a general term covering all areas of navigation. Some authors use in place of true navigation, but this can make the general use of the term confusing.
Pilotage/piloting	A method of navigation via which a memorised series of landmarks within visual-range of each other are followed towards the goal.
Preferred compass direction	A frequently observed bias in compass orientation independent of site, but often population specific. Function/cause unknown but observed in homing pigeons and many migratory bird populations.
Release site bias	A site-specific deflection from the home direction which is consistently seen across independent releases. Such biases are thought to occur due to local inconsistencies in the map component of the navigational system, e.g. olfactory anomalies.
Route Reversal	Young pigeons inexperienced with homing show a route reversal behaviour following passive displacement, where they repeat the out-going journey in reverse in order to find home, rather than approximating a beeline route.
Route Stereotypy	Over the course of many releases from a single site, pigeons show increasingly idiosyncratic routes which are not maximally efficient and will return to these learned routes if displaced from them. Some cite this as evidence for pilotage (a.k.a. route following; route recapitulation).
Time compensated sun compass	Time compensation is necessary to use the sun as a compass, due to the characteristic patterns of movement across the sky over time. Birds learn to associate the sun's azimuth (angle against the horizontal plane, independent of elevation from the horizon) at different times of day with compass directions, via the innate magnetic compass. This is a learnt process which occurs during the first 1-3 months and is enhanced by

	exploratory flights. Experience of all times of day and seasonal changes are necessary for complete time compensation. The learning process is specific to the local area, as the pattern of sun movement varies across the globe.
True Navigation	Navigation from an unfamiliar location, often used as a synonym for map and compass navigation.
Vanishing bearings	The absolute bearing of a bird at the point at which it disappears from view.

Chapter Summaries

Is There Visual Lateralisation of the Sun Compass in Homing Pigeons?

Our understanding of how the sun compass is used in homing is generally good – where available, the azimuth of the sun is used in a learned, time-compensated manner to give a directional reference. However, as a visual cue, it is possible that the processing of this information through the visual system occurs in a lateralised manner, due to the asymmetric nature of the avian brain. This experiment trains pigeons with either their right or left eye occluded, in an arena setting to locate a food reward, with both solar and visual landmark cues available. These are then set in conflict by rotation of the arena, to see whether there is a cue-type preference between the right and left visual hemispheres.

Repeated training of homing pigeons reveals age dependent idiosyncrasy and visual landmark use.

Homing in the "familiar area" requires a level of familiarity with a release site and the surrounding landscape. In order to investigate how familiarity interacts with navigation, repeated releases from the same site have been used, and revealed increases in efficiency, and the generation of idiosyncratic routes which are repeated by individual birds. Additionally, releases from near-by locations from the training location have shown that many birds return to their learned route. This experiment repeats this procedure at a location with distinct visual landmarks in the form of the Menai Strait, as well as using two age groups of pigeons to determine how flight experience and the salience of landscape features influence route-forming and the homing strategy chosen at off-route release sites.

Using small values of clock-shift to investigate how homing pigeons integrate navigational cues when homing in the familiar area.

Our understanding of the sun compass comes largely from clock-shift experiments, where experimenters keep birds under an altered light-dark cycle to shift their circadian rhythm, such that they predictably mis-read the time-compensated solar compass. Previous experiments tend to use a 6h or 90° clock-shift, so that deflection of the flight path could be easily identified via vanishing bearings, but even these experiments found that deflection was less than would be expected from the amount of shift applied, and it has been theorised that this may be due to the integration of other directional cues. However, it is not well understood how different cues may be integrated in this process. Therefore, this experiment uses two small values of clock-shift to investigate how navigation is affected by differing levels of cue conflict, at different locations with differing availability of visual landmark cues.

Investigating the factors which reduce the deflection seen in flights of clock-shifted homing pigeons navigating in a familiar area.

Following on from the previous chapter, this chapter seeks to investigate the factors which may be causing the reduced amount of deflection seen relative to the sun-compass direction in clock-shift releases. Many previous studies have found that deflection is less than 100%, with various factors being suggested as explanations for this observation. This chapter investigates the correct shifting of the circadian rhythm and use of the magnetic compass as possibilities: if the circadian rhythm is not as shifted as predicted from the experimental design, then a lower deflection may be seen, and use of the magnetic compass in conflict with the sun compass may also/alternatively reduce deflection. To investigate the first possibility, accelerometer data recorded from pigeons under clock-shift were used to analyse their sleep patterns as a proxy for circadian rhythm. For the second, birds were released following clock-shift with magnets attached to their heads in order to interfere with use of the magnetic compass – if the magnetic compass is being integrated with the sun compass to reduce deflection, then we would expect to see an increase in deflection with this treatment, in comparison to clock-shift without magnets.

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Chapter 2

Is There Visual Lateralisation of the Sun Compass in Homing Pigeons?

Charlotte Griffiths, Richard Holland and Anna Gagliardo

Published paper

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Abstract: Functional lateralisation in the avian visual system can be easily studied by testing monocularly occluded birds. The sun compass is a critical source of navigational information in birds, but studies of visual asymmetry have focussed on cues in a laboratory rather than a natural setting. We investigate functional lateralisation of sun compass use in the visual system of homing pigeons trained to locate food in an outdoor octagonal arena, with a coloured beacon in each sector and a view of the sun. The arena was rotated to introduce a cue conflict, and the experimental groups, a binocular treatment and two monocular treatments, were tested for their directional choice. We found no significant difference in test orientation between the treatments, with all groups showing evidence of both sun compass and beacon use, suggesting no complete functional lateralisation of sun compass use within the visual system. However, reduced directional consistency of binocular vs. monocular birds may reveal a conflict between the two hemispheres in a cue conflict condition. Birds using the right hemisphere were more likely to choose the intermediate sector between the training sector and the shifted training beacon, suggesting a possible asymmetry in favour of the left eye/right hemisphere (LE/RH) when integrating different cues.

Introduction

A large body of evidence has shown that brain asymmetry is not an exclusive peculiarity of the human brain, as lateralisation of brain function has been found to be common across vertebrates (Rogers, 1980; Vallortigara et al., 1999; Rogers, 2002; Ghirlanda and Vallortigara, 2004; Ocklenburg and Gunturkun, 2012). Due to the lack of the corpus callosum and complete decussation of the optic fibres, the hemispheres of the avian brain are largely independent. For this reason, lateralisation phenomena in birds can be easily investigated by covering one eye so as to largely exclude the involvement of the contralateral hemisphere. Therefore, the birds' visual system became the subject of investigation for the study of brain functional asymmetries in several contexts such as, imprinting, social recognition, visual learning and spatial cognition (Vallortigara and Rogers, 2005; Mehlhorn et al., 2010; Ocklenburg and Gunturkun, 2012; Pecchia et al., 2013; Manns and Ströckens, 2014).

According to the lateralisation pattern delineated by a number of studies on the visual system of birds, the right hemisphere has an advantage in spatial tasks, in global attention and in social recognition, while the left hemisphere takes the control of behaviours requiring the categorization of visual stimuli and the discrimination of visual details of stimuli (Vallortigara and Rogers, 2005; Manns and Ströckens, 2014).

Visual lateralisation in birds engaged in spatial tasks has been investigated in laboratory settings in both hen chicks and pigeons, and in semi-natural and natural settings in homing pigeons (Tommasi et al., 2003; Wilzeck et al., 2009; Martinho et al., 2015; Pollonara et al., 2017). Laboratory studies in

monocularly occluded chicks tested in a cue-conflict task highlighted the different strategies used by the two brain hemispheres. For instance, chicks trained to find food in the centre of a squared arena, near an object constituting a visual beacon, were tested in monocular condition after the beacon was moved to a new position near a wall of the enclosure. The chicks with the right hemisphere in use preferred to rely on the geometrical properties of the arena by consistently searching for food in the centre of the arena, while those with the left hemisphere searched in the new position of the object (Tommasi and Vallortigara, 2001).

In semi-natural and natural settings, natural spatial cues such as the sun compass constitute an important and often dominant source of information for birds challenged to localise a food reward in an outdoor arena, or to orient towards a goal (Kramer, 1953; Füller et al., 1983; Wiltschko and Balda, 1989; Schmidt-Koenig et al., 1991; Bingman and Jones, 1994; Chappell and Guilford, 1995; Gagliardo et al., 1996; Wiltschko and Wiltschko, 1999; Gagliardo, 2005; Gagliardo et al., 2005; Filannino et al., 2014). Information useful for determining an absolute direction using the sun reference is processed through the visual system. In fact, sun compass orientation requires the observation of the sun azimuth in order to compute an angle to take with it on the basis on the information about the time of the day provided by the internal clock. A clear demonstration of the use of the sun compass by birds is the consistent deviation from the goal direction shown by birds subjected to phase-shift; an altered light-dark cycle is used to manipulate the circadian rhythm of test birds, thereby predictably altering time-compensated interpretations of the sun compass direction (Kramer, 1953; Schmidt-Koenig et al., 1991; Wiltschko and Wiltschko, 1999).

Despite the importance of the sun compass mechanism in birds' spatial behaviours (Kramer, 1953; Füller et al., 1983; Wiltschko and Balda, 1989; Schmidt-Koenig et al., 1991; Chappell and Guilford, 1995; Wiltschko and Wiltschko, 1999), lateralisation phenomena in relation to sun compass-mediated spatial tasks have been poorly investigated. Few exceptions are represented by studies conducted with homing pigeons subjected to unilateral hippocampal lesions. One of these studies on sun compass-mediated spatial learning in an outdoor arena suggested that only an intact left hippocampal formation is sufficient to allow sun compass-based learning (Gagliardo et al., 2005). However, in a cue-conflict situation, in which visual beacons and sun compass information provide conflicting spatial information, the left hippocampal lesioned pigeons, in contrast to controls, often relied on feature cues (Gagliardo et al., 2005). Two conclusions emerged from this study: i) only the left hippocampal formation is capable of supporting sun compass-based learning; ii) the integrity of the hierarchy of strategies for spatial learning, that sees in birds a preferential use of the sun compass over visual features, requires an intact right hippocampal formation.

It is important to clarify that, although the hippocampal formation is involved in sun compass-based spatial learning in a confined condition (Bingman and Jones, 1994; Gagliardo et al., 1996; Ioalè et al., 2000), hippocampal lesions do not affect the use of the sun compass during homing. As well as this, hippocampal lesions, and importantly even unilateral lesions regardless of which side of the hippocampus remained intact, disrupt familiar visual landmark-based navigation, inducing clock-shifted pigeons to totally rely on the erroneous sun compass information rather than on the chain of familiar landmarks leading them home (Gagliardo et al., 1999; Gagliardo et al., 2009a).

Early studies relying on vanishing bearings and homing times found that birds using the right eye (therefore the left brain hemisphere) performed better when relying on visuospatial information (Ulrich et al., 1999; Prior et al., 2004). However, more recent studies using GPS tracking of monocularly trained pigeons suggests that the right hemisphere may play a more important role when establishing and re-tracing routes from familiar sites (Martinho et al., 2015; Pollonara et al., 2017). However, some inconsistency in apparent lateralisation of homing performance may be due to the tendency for monocularly occluded birds to fly towards the direction of the open eye, possibly in order to centre the visual field (Diekamp et al., 2002; Pollonara et al., 2017). For this reason, the deviation induced by clock-shift treatments may be difficult to evaluate in monocularly occluded pigeons during homing, in which the tendency to deviate their flight path towards the side of the open eye also occurs.

In order to investigate the possible lateralisation of the sun compass, we tested pigeons in a food localisation task in an octagonal outdoor arena, in which it is known that birds preferentially rely on sun compass directional information to localise the food reward associated with a sector (Wiltschko and Balda, 1989; Bingman and Jones, 1994; Chappell and Guilford, 1995; Gagliardo et al., 1996; Gagliardo et al., 2005). This setting avoids the in-flight issue of flight bias towards the uncovered eye, whilst investigating a sensory system relevant to wider navigational tasks. This apparatus has been previously used to demonstrate that chicks use sun-compass information to find a food reward (Zimmerman et al., 2003), including showing a significant response to clock-shift, and ruling out the use of a magnetic compass (Zimmerman et al., 2009). Birds were trained to find a food reward in one of eight arena compartments, with sun compass information and beacon cues available, under either binocular or monocular conditions. The arena was then rotated 90 degrees anti-clockwise to introduce a directional conflict between the sun compass and beacon information, and the birds were tested in the absence of a food reward. If the sun compass is dominant in one hemisphere, we would expect birds with the corresponding eye occluded to ignore the sun compass in the test phase, relying on the beacon cues in the arena. However, if use of the sun compass occurs in both hemispheres, left and right monocular treatments should show evidence of sun compass use during the test sessions.

Methods

A total of 27 adult homing pigeons *Columba livia* successfully completed this procedure. All birds had flight experience in the surrounding area of the Arnino field site (Pisa, Italy). For the duration of the experiment, birds were housed in a mesh aviary, open to the elements, and not allowed outside flight. For an individual bird, the experimental duration was generally between 10 and 20 days. Birds had unlimited access to water, but food was restricted to 25–30 g per bird per day during days when the pigeon was being trained in the arena. On days where conditions were unsuitable for training, birds were fed in the aviary. At the end of the experiment, birds were returned to their normal living conditions (food, water and grit ad libitum, outside flight access).

Experimental Apparatus

The arena consisted of a large octagon, approximately 2.6 m in diameter, 0.75 m high, with opaque sides to restrict the view of the surrounding landscape, but a mesh top through which the sky and therefore movement of the sun could be clearly seen. The arena was placed in the middle of a field, so no identifiable landmarks could be seen from within the arena. In the middle of each of the eight sides, a 25 × 25 cm square hole gave access to a wooden box, which could contain a food reward. This box had a wooden block in the centre, behind which the food reward could be concealed. Above each box entrance on the arena wall was a coloured beacon, 25 × 25 cm (green, black, barred white, yellow, barred blue, grey, red, and blue). In the centre of the arena was a central supporting pole, with a release box. Birds were placed under this box in the centre of the arena, then released at a distance by the experimenter, via a rope used to lift the box (figure 1).

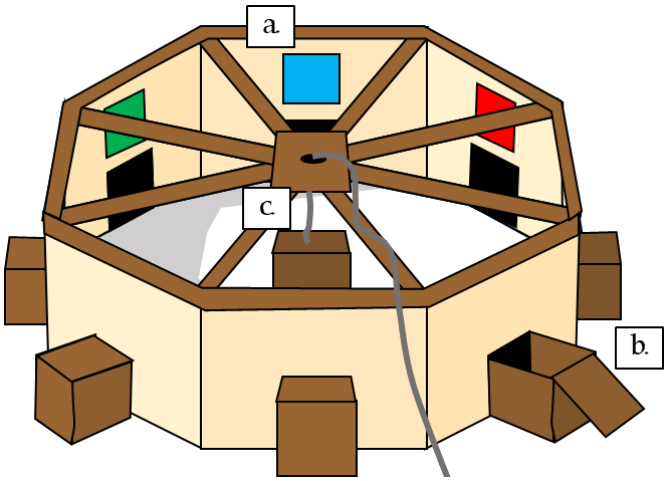

Figure 1: Diagram of the arena set-up. (a) Shows the colour beacons attached to the walls of the arena; (b) shows the reward boxes, which open from above to insert and remove the food rewards; (c) shows the release box—the pigeon is placed under the box, which is then pulled up with the attached rope to release the bird into the arena.

Experimental Groups

Pigeons were randomly assigned to one of three experimental groups: controls, where no eye caps were attached and pigeons maintained binocular vision; left eye/right hemisphere (LE/RH) where birds had an opaque eye cap attached to cover the right eye; and right eye/left hemisphere (RE/LH), where birds had the eye cap covering the left eye, with 9 birds in each experimental group. For the monocular groups, the eye cap was removable using a Velcro attachment. The feathers were trimmed around the assigned eye, and a ring of Velcro attached using a non-toxic glue. The eye cap could then be attached using the corresponding Velcro side when necessary (removed when birds were not in training, e.g., in the aviary at night). This Velcro ring falls off eventually, although some birds learnt to remove the ring, and had to be abandoned from the experiment.

Experimental Procedure

This experiment consisted of two key phases: training and testing. In the training phase, birds would first become familiar with the arena, then be trained to collect a food reward from a randomly assigned box. Training was divided into the following sessions:

1. Pre-training one: food was present in all of the eight boxes (eight pieces of corn in each) and was visible from the centre of the arena. The session ended when the bird had eaten the food in all the boxes, or after thirty minutes. The session was repeated until the bird had learnt to eat all the food.
2. Pre-training two: food was present in all the boxes as before, but this time concealed behind the central blocks, so that the bird would have to enter the box in order to find the food reward. As in pre-training one, the session would continue until all the food had been eaten or after thirty minutes had passed, and the session would be repeated (once per day) until the bird had learnt to eat all the food.
3. Pre-training three: one box was assigned to each bird, which would contain the food reward for the remainder of the experiment. This training session consisted of ten trials in the same day. In each trial, food was hidden in the assigned box (six pieces of corn per trial), and the trial ended when the pigeon found and ate the food, or after thirty minutes had passed.
4. Training sessions: each training session consisted of ten trials in the same day. In each trial, food was concealed in the assigned sector, and the trial ended when the pigeon entered any box (i.e., training without correction). If the bird entered the rewarded box, it was allowed to eat all of the food before being removed. If the bird entered a different box, it was removed without a food reward. Training sessions continued until the training threshold was met.

The birds were trained up to a criterion of a minimum of 24 correct choices in three consecutive training sessions, discounting the first training session, with at least 8 correct choices made in the final session. Sessions taken to reach the criterion ranged from the minimum possible of four, to ten. All training was conducted on days when the sun was unobscured by cloud, and between 6 a.m. and 12 a.m., and 4 p.m. and 9 p.m., when the arena was at its coolest.

After training had been passed, birds could be tested. In the test session, the arena was rotated 90 degrees anti-clockwise, so that there was a conflict between the coloured beacons and the sun compass direction identifying the training sector. The training session consisted of five trials, in which no food reward was given, each trial ending when the bird entered the first box. Between test trials, the bird was removed from the arena, with a break of 5–10 minutes between test trials. No other training was performed during this time, and the pigeon was returned to the arena for the next test trial following the break.

Data Analysis

For the training sessions, the number of sessions taken by the three groups of pigeons to reach the criterion and the average time in minutes taken to reach the chosen box was compared with the Kruskal–Wallis test. Finally, a linear mixed model was produced to assess possible differences in the number of errors made by the three groups, using the R package lme4 (Bates et al., 2015). The number of errors per session was modelled with session and treatment as fixed effects, and individual as a random effect, allowing for by-individual random slopes for the effect of session.

The five choices made in each test session were compared with the last five choices of the final training session, in order to determine how choices differed between the conditions. For each bird, the assigned training direction was set to 0°, to make each bird comparable. Therefore, when the arena was rotated −90° during the test session, the direction given by the beacon cue was −90°, vs. the 0° of the sun compass direction.

For each bird, the mean vectors for the last five training trials and the five test trials were calculated. These were then used to calculate the mean vector for each experimental group. The paired Hotelling's test was used to compare the mean vector length and direction between the test and training trials for each group.

For the test session, a Rayleigh test of Uniformity was used to test for randomness in the individual median distributions of the three groups of pigeons, considering as expected direction either the sun compass training direction (360°), or the direction of the training beacon (270°). The distributions of the mean vector lengths of the three groups were compared using the Kruskal–Wallis test in order to

compare the level of consistency in directional choices during the test session. The Watson–Williams test was applied to the individual median directions in order to compare the orientation of the three groups of birds. As the test was conducted in absence of a food reward, the Watson–William test was also applied to the first and second directional choices, in order to evaluate a possible effect of learning during the following choices. By using the Mann–Whitney U test, a direct comparison between the two monocular groups was also performed by considering the rate of choices for the sector identified by the training direction, the sector identified by the training beacon and the sector located in between.

Ethical Statement

The pigeons were bred and manipulated in accordance with the 57 EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The experiment was approved by the Animal Welfare Committee (OPBA) of the University of Pisa and by the Italian Ministry of Health (permit number 227/2019). All birds used for the procedure were captive-bred and had experience of experimental conditions from previous release experiments. Birds had free access to water for the duration of their use in the experiment and were removed from the experiment if they were unable to use the apparatus. For the monocular birds, feather trimming and gluing of the Velcro rings took place using cloth restraints to prevent movement which might cause harm. A non-toxic glue was used, which allows the Velcro ring to fall off over time. If loose enough, the Velcro was removed at the end of the experiment. Eye-caps were only worn during the experimental procedure and removed during rest periods and overnight to minimise distress. As the experiment was conducted during the summer in an open field, shade was provided for the birds waiting to be tested, and the arena was periodically cooled with water to prevent the floor from reaching an uncomfortable temperature. Birds had a set ration of food for each day, and any which did not reach this ration through use of the arena were fed the remainder at the end of the day. Trials were spaced out so that each bird had a significant break between trials in the arena, as other birds took their turn. Birds were used for an average of one week and were returned to standard living conditions afterwards.

Results

Learning

A total of 27 homing pigeons completed training to the test criteria, with nine birds tested for each experimental group. The number of training sessions required to meet the training criterion ranged from the minimum of four sessions, to 10 (median number of session to criterion: C 4; LE/RH 5, RE/LH 5, see Figure 2 for individual training times). There was no significant difference in the number of

sessions required to complete training between the three experimental groups (Kruskal–Wallis: H2 = 0.522, P = 0.770).

To compare the rates of task learning, the number of errors per session was modelled, with session and treatment as fixed effects and individual as a random effect in a linear mixed model. Analysis of the model found that a linear relationship between the number of errors and the session number, that it fit a normal distribution (visual interpretation of histograms of the response variable) and that residuals were not significantly heteroscedastic (Breusch-Pagan test for heteroskedasticity, p = 0.052). However, significant positive autocorrelation was present (Durbin-Watson statistic of 1.0891), so the errors were taken every other session (1, 3, 5, 7 and 9) to account for this (Durbin-Watson statistic of 1.8057). Comparing the full model and a model without treatment as a fixed effect in a likelihood ratio test revealed no significant effect of treatment on error rate (Chi-square test: $\chi^2_2 = 2.654$, P = 0.2665). Session, however, was a highly significant predictor of error number (Chi-square test: $\chi^2_1 = 33.462$, P = 7.266×10^{-9}). Therefore, the rate of task learning does not appear to differ between experimental groups. Time taken to make a choice (minutes) was also similar between the experimental groups during training (Kruskal–Wallis Test: H2 = 3.854, P = 0.1456; median times in minutes : 1.0, 1.2, 2.4 for C, LE/RH and RE/LH, respectively), and during the test sessions (Kruskal–Wallis Test: H2 = 1.5776, P = 0.4544; median time in minutes 1.0, 1.4, 1.2 for C, LE/RH and RE/LH, respectively).

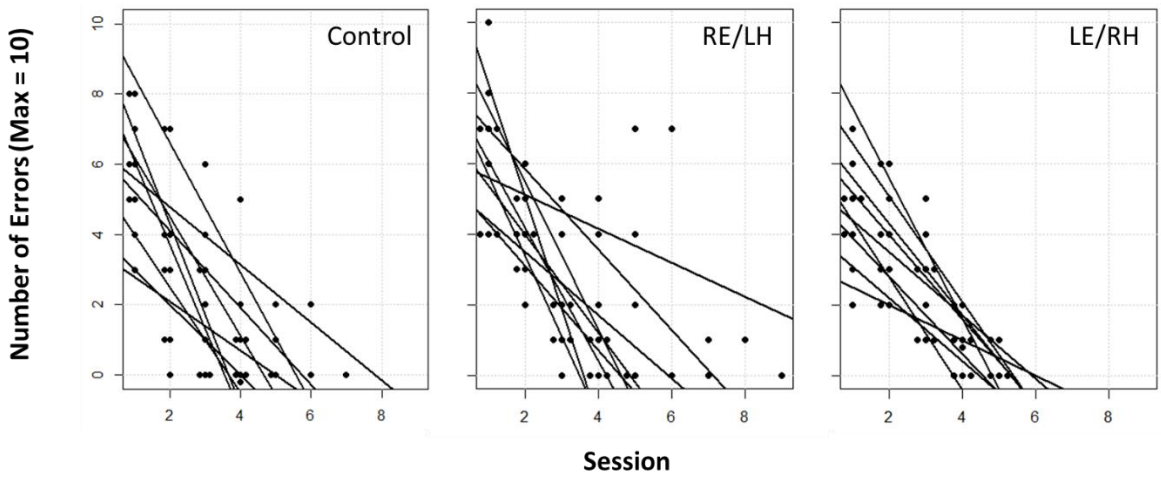

Figure 2: Rates of task learning across the three experimental groups, measured as the number of errors per session (n = 10 trials per session) against session number. Each point represents an individual bird's performance in that session, and each line signifies the regression slope for each individual bird.

Directional Choices

To compare between training and test sessions, the last five training trials and five test trials were used to calculate pairs of individual mean vectors for each bird (Figure 3), which could then be compared between experimental groups.

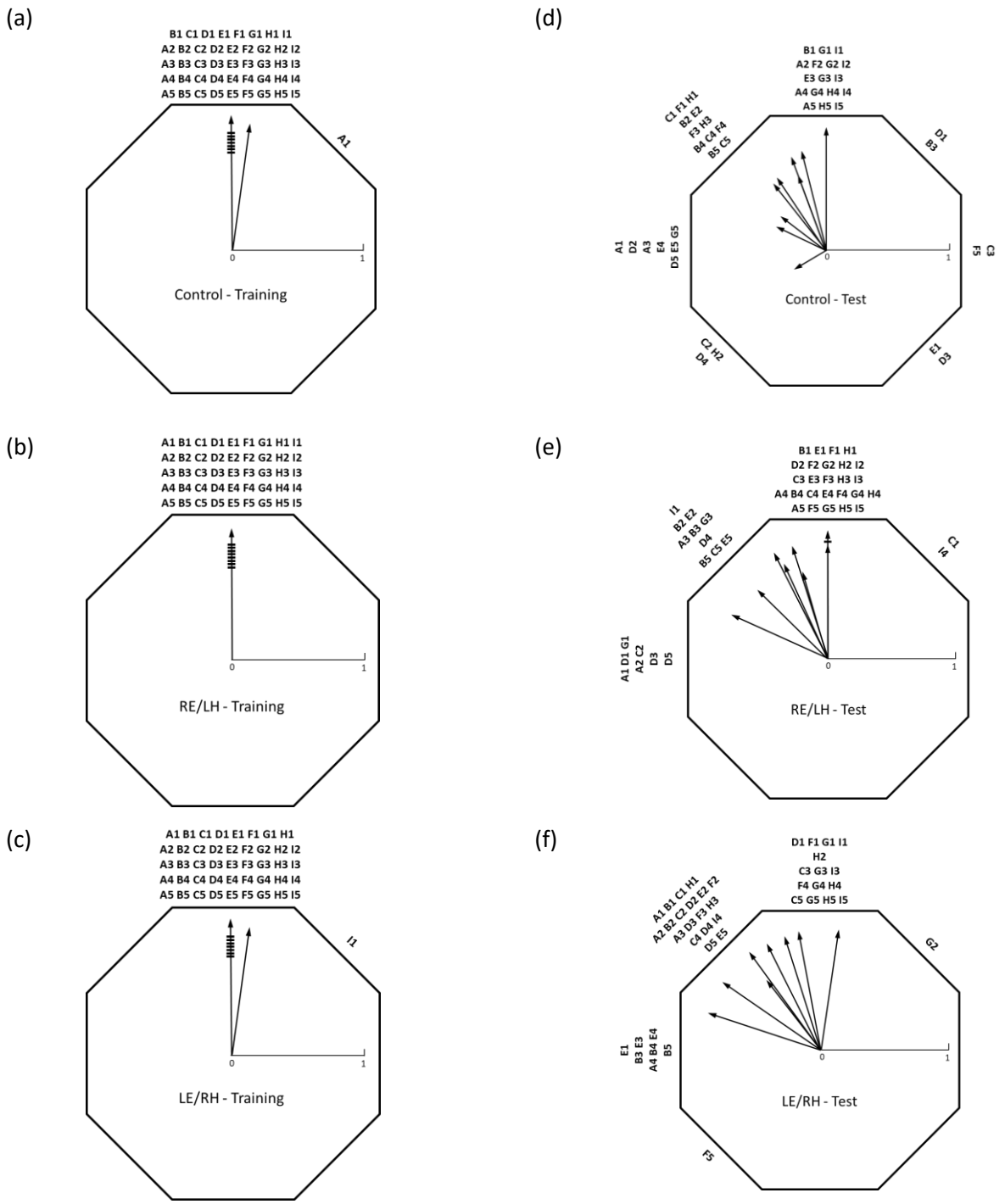

Figure 3: Directional choices of birds in the final training session (a-c) and the test session (d-f). Each of the nine birds per treatment are labelled A-I, with trial number (1-5) included for each choice. The internal arrows show the individual mean vector for the directional choices of each bird, the length of which can be read from the scale bar. Lines cutting an arrow show additional birds which share the same mean vector. Each bird's training direction has been normalised to the top of the octagon, with the test beacon direction to the left.

The Hotelling's test for paired data revealed a significant difference between the training and test orientation in all three experimental groups (paired sample Hotelling's test: C $F_{1,16}$ = 16.03412, P = 0.00102; RE/LH $F_{1,16}$ = 4.833955, P = 0.04269; LE/RH $F_{1,16}$ = 8.61383, P = 0.00971), showing that the conflict of cues produced a change in orientation in the three groups of birds (see also Table 1).

Table 1: Second order mean vectors for training and test trials. In both cases the rewarded training sector was set to 360°; r second order mean vector length; α second order mean vector direction. Other explanation in the text.

Group	**Training**		**Test**	
	r	α	r	A
C	0.999	001°	0.848	322°
LE/RH	0.999	001°	0.901	324°
RE/LH	1.000	360°	0.935	339°

When the Rayleigh test of Uniformity was applied to the individual median orientation considering the training direction (360°) as the expected direction, all three treatments displayed an orientation significantly different from random (Rayleigh test of Uniformity, Control mean resultant length = 0.6720 P = 0.0014; RE/LH mean resultant length = 0.8705, P = 8.0380×10^{-6}; LE/RH mean resultant length = 0.7307, P = 4.859×10^{-4}). When the beacon direction (270°) was considered as the expected direction only, controls and LE/RH distributions were significantly different from random (Rayleigh test of Uniformity, Control mean resultant length = 0.5179, P = 0.0129; RE/LH mean resultant length = 0.3419, P = 0.0748; LE/RH mean resultant length = 0.5271, P = 0.0116), suggesting a less consistent attention of the right eye/left hemisphere system for the beacon information.

However, no statistical differences in orientation among the three groups during the cue-conflict test emerged (Watson–Williams Test: $F_{2,24}$ = 0.89307, P = 0.4226). Consistently, no difference among groups emerged in the first (Watson–Williams Test: $F_{2,24}$ = 0.127, P = 0.881), or in the second trial (Watson–Williams Test: $F_{2,23}$ = 0.274, P = 0.762) of the test session.

The Kruskal–Wallis test applied to the individual mean vector length (r) distributions of the three groups highlighted a significantly different level of consistency in the individual directional choices in the three groups (Kruskal–Wallis Test: H2 = 7.9109, P = 0.02). In particular, the control group exhibited smaller individual mean vector lengths (median r: C 0.69, LE/RH 0.93, RE/LH 0.88; see Figure 4) than both monocular groups (Tukey HSD: q = 4.868, P = 0.0058 (C vs. RH); q = 4.332, P = 0.014 (C vs. LH)), while no difference between the two monocular groups emerged (Tukey HSD: q = 0.536, P = 0.9).

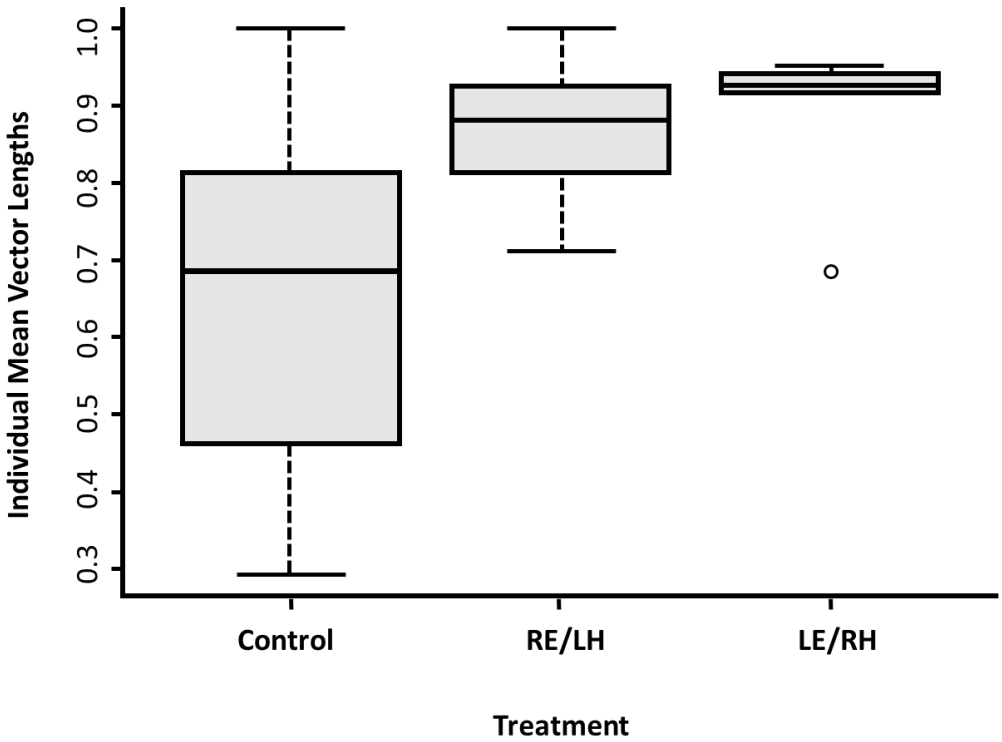

Figure 4: Box and whisker plot of the mean vector lengths for the directional choices of each individual bird across the five test trials, grouped by experimental treatment (see Figure 3d–f for individual mean vector visualisation).

The direct comparison between the two monocular groups on the rate of choices displayed for the three relevant sectors of the arena (training direction sector as defined by the sun compass, training beacon sector and sector in between) showed that rate of choices for the sector in between the two cues set in conflict (training sun compass direction sector and training beacon) was significantly higher for the LE/RH pigeons compared to the RE/LH (Mann–Whitney U test: w = 62.5, P = 0.045; see Figure 5). However, no significant difference was found in the rate of choices for either the training sector identified by the sun compass (Mann–Whitney U test: w = 23.5, P = 0.1355) direction or the sector identified by the training beacon (Mann–Whitney U test: w = 39, P = 0.9197).

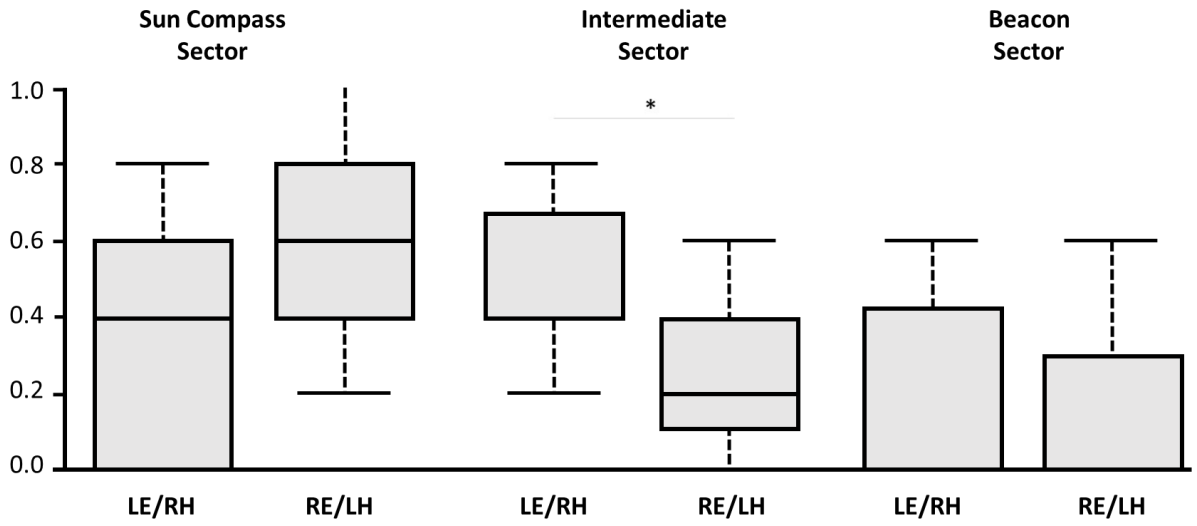

Figure 5: Rate of choices of the two monocular groups for the sectors identified by the sun compass training direction, the training beacon and the sector in between these two (intermediate sector), respectively.

Discussion

Here we present a study designed to determine whether there is a functional asymmetry in the visual system of pigeons in sun compass processing. Our results suggest that there is no clear lateralisation of the sun compass. In fact, both monocular groups displayed no difference in the use of the sun compass vs. intra-maze visual beacons. During the learning phase, the performances of monocularly occluded pigeons challenged to localise a food reward in an outdoor octagonal arena provided with a distinctive beacon in each sector, were similar to those of control pigeons with binocular view. Monocular occlusion did not hinder task learning, with all three experimental groups showing similar error rate curves (Figure 2). This suggests that, even if birds in the different groups were learning using different cues, all were similarly able to learn the task.

Past studies have shown that monocular birds using the left hemisphere performed better in grain-grit discrimination tasks (Güntürkün et al., 2000), as the left hemisphere has an advantage, through the inhibition on the right hemisphere, in responding in an appropriate way in tasks implying a dual choice. Similarly, pigeons with the right eye/left hemisphere visual system perform better in reversal learning tasks, in which birds are requested to inhibit their response to a previously learned stimulus (Diekamp et al., 1999). However, while in both grain-grit discrimination and reversal learning tasks birds have to refrain from pecking inedible items or to an unrewarded stimulus previously associated to a reward, in the present experiment birds have to learn to localise the food reward, on the basis of a beacon distinctive of a specific sector of the arena, or on the basis of a specific sun compass direction, or both. Therefore, the lack of an advantage of the left hemisphere in the learning food localisation in

the present experiment is consistent with a lack of a dual choice implying inhibition by the left hemisphere on the right one.

Fewer monocular birds that started training completed the process, as some learnt to quickly remove the eye-cap, making them unsuitable for the experiment (seven RE/LH birds, six LE/RH and one control bird which failed to learn the task were abandoned). In terms of orientation in training, the remaining birds successfully learnt the task to the required criteria, showing consistent orientation towards the goal location.

During the test session, following a 90° anti-clockwise rotation of the arena, the sun compass information learned during training were set in conflict with the distinctive beacon associated with the presence of food. Group averages of test orientation showed an intermediate direction between the sun compass direction (360°) and the beacon direction (270°) (see Table 1), composed of varying individual strategies, many of which showed switching between the sector identified by the training beacon, the sector identified by the training direction and the sector located between the two (Figure 3). The within-group variation in directional choice and lack of a significant difference in orientation between the test groups suggests that birds in all groups were able to orient using both sun compass and beacon-based strategies, independent of the monocular or binocular conditions. Individual strategies generally showed a high degree of switching, possibly due to the lack of a food reward during the test session, meaning that the birds chose different directions on repeated attempts. However, no significant difference in orientation on the first trial of each test between the groups still supports a lack of treatment-specific effects.

The directional choice distribution of the three groups was significantly different from random when the sun compass training direction was considered as the expected direction. This is consistent with the interpretation that both hemispheres participate in sun compass-mediated spatial learning. Interestingly, the distribution of the choices of the birds processing using the right eye/left hemisphere visual system, and contrarily to both the other two groups, was not different from random if the training beacon direction was considered as the expected direction. This suggests that, although not totally ignoring the learned beacon, they displayed a less consistent reliance on visual feature information.

During the test session, the training compass direction and the colour beacon were set in conflict by rotating the arena 90° anti-clockwise. As the lateral visual field of pigeons, as in all diurnal avian species, is wider than 120° a monocularly occluded bird can view at the same time both the training sector associated to the sun compass training direction and the sector identified by the training beacon. An interesting result emerged from the direct comparison between the rate of choices for

one of the three relevant sectors: the training direction sector, the sector identified by the training beacon, and the sector located between these two. In fact, the birds with the right hemisphere in use displayed a higher preference for the sector located between the training beacon and the training compass direction, compared to the birds with the left hemisphere in use (figure 5). This suggests a possible advantage of the left eye/right hemisphere visual system in integrating information of a different kind, such as the colour beacon and sun compass direction. It is possible that choosing the intermediate sector does not represent higher levels of integration; choice of the intermediate sector could instead be due to an inability to resolve the conflict between the two directions, or as a compromise rather than an integration. It is also possible that, given a range of possibly correct directions indicated by the conflicting cues, the intermediate sector might be chosen randomly as one of three options. However, a random strategy seems unlikely as the birds with the right hemisphere in use display a significant preference for the intermediate sector compared to the birds with the left hemisphere in use.

Prior et al. found that birds using the right-eye/left-hemisphere visual system were more distracted by changing landmark or beacon cues in an indoor arena setting, suggesting that the left hemisphere may be more involved in the processing of such cues (Prior et al., 2002). However, this result might be in line with the view that the right hemisphere has an advantage in integrating different information and therefore producing a more consistent behavioural output.

The involvement of both hemispheres in processing sun compass information revealed by the present work is in line with previous studies on homing pigeons subjected to unilateral hippocampal lesions. It has been observed that bilateral hippocampal lesions disrupt the ability of clock-shifted pigeons released from familiar sites to re-orient homeward by relying on the spatial relationships among familiar landmarks. The consequence of the impaired familiar landmark-based navigation ability is a marked deflection of the clock-shifted pigeons, consistent with the sun compass orientation (Gagliardo et al., 1999; Gagliardo et al., 2009a). Interestingly, the total reliance on the site-specific compass orientation strategy, as a consequence of the impaired pilotage strategy, exhibited by birds with bilateral hippocampal lesions is also shown by pigeons subjected to unilateral hippocampal lesions, with no difference between the side of the lesion (Gagliardo et al., 2002). However, as this study was based on the analysis of vanishing bearings, possible differences between the right and the left hippocampal formation in controlling spatial decisions *en route* are not known.

Ulrich et al. showed that birds using the left eye and therefore the right hemisphere visual system showed a reduced homeward component in overcast, suggesting that they are less able to use alternative landmark (or magnetic) information (Ulrich et al., 1999). Our birds with the left eye/right

hemisphere visual system in use did not show significantly less ability to use the beacon cues, in fact they demonstrate a slight tendency towards the beacons, although we have to make clear that colour beacons in an arena are not the equivalent to familiar landmarks in a large-scale navigation context. In fact, hippocampal lesioned pigeons, that are impaired in familiar visual landmarks-based navigation (Gagliardo et al., 1999; Gagliardo et al., 2009a), displayed a total reliance on colour beacons, and not on a specific compass direction, when challenged to locate a food reward in an outdoor octagonal arena (Gagliardo et al., 1996). It has been consistently shown that hippocampal lesions do not affect the pigeons' ability to orient based on the sun compass in a large-scale navigation task, but nevertheless disrupt the ability to associate the presence of food to a compass direction (Bingman and Jones, 1994; Gagliardo et al., 1996). Similarly, young hippocampal lesioned pigeons kept confined in an aviary open to winds turned out to be unable to learn an odour-based navigational map, most likely being unable to learn the association "sun compass direction-wind borne odours" (Ioalè et al., 2000). Of interest for the present study, the left hippocampal formation seems to be critical for the learning process underlying the olfactory map development (Gagliardo et al., 2001) and advantaged in learning the association "food reward-sun compass direction" in an outdoor octagonal arena void of colour beacons (Gagliardo et al., 2005). By contrast, in the present study, the right eye/left hemisphere visual system did not show any clear advantage in the preference for a sun compass-dependent spatial strategy, suggesting that such functional lateralisation in the use of the sun compass depends on an asymmetry of the left side of the hippocampal formation in sun compass-mediated learning strategies, rather than in the operation of the sun compass mechanism itself, which according to the present data seems to involve the visual system of both sides.

It is important to mention that after the learning phase we did not test the birds after clock-shift in order to verify that they used the sun compass to learn the training direction. However, consistent evidence from different research groups showed that food localisation in outdoor arenas is a sun compass-mediated spatial task, and that sun compass information is even preferred to other stimuli that might be associated with the food reward, such as intra-maze visual beacons (Wiltschko and Balda, 1989; Bingman and Jones, 1994; Chappell and Guilford, 1995; Gagliardo et al., 1996; Budzynski et al., 2002; Gagliardo et al., 2005). One may argue that the pigeons could be using the magnetic compass to locate the food reward, rather than the sun compass. However, a large body of evidence both in field releases and arena experiments has demonstrated that pigeons with access to the magnetic compass and sun compass reliably show deviation under clock-shift, showing that the sun compass is used preferentially to the magnetic compass, even when a cue conflict occurs (Füller et al., 1983; Wiltschko and Balda, 1989; Papi et al., 1991; Bingman and Jones, 1994; Chappell and Guilford, 1995; Gagliardo et al., 1996; Bonadona et al., 2000; Wiltschko and Wiltschko, 2001; Budzynski et al.,

2002; Gagliardo, 2005; Gagliardo et al., 2005; Armstrong et al., 2013). Some studies have shown that the magnetic compass may be involved in re-orientation following clock-shift during flight over unfamiliar areas, after several kilometres of flights either at vanishing to a lesser and variable extent or well beyond the release site area to a greater extent (Wiltschko and Wiltschko, 2007; Gagliardo et al., 2009b; Manns and Güntürkün, 2009). GPS-tracking data showed that a complete re-orientation of the birds occurred after several hours and often after the subjective night possibly due to several factors (Manns and Güntürkün, 2009). This suggests that it is unlikely to have occurred in our arena setting where the test sessions occurred over a narrow time frame. As previous experiments using an outdoor arena have demonstrated that pigeons do use the sun to learn the location of the food reward (Wiltschko and Balda, 1989; Bingman and Jones, 1994; Chappell and Guilford, 1995; Gagliardo et al., 1996; Budzynski et al., 2002; Gagliardo et al., 2005), it is reasonable to assume that the pigeons in this experiment used the sun compass in preference of the magnetic compass, especially as the sun's disc was clearly visible from the centre of the arena at all times.

The asymmetry in the pigeon visual system at neuroanatomical level consists of stronger projection from the right optic tectum to the left nucleus rotundus, which in turn sends fibres to the left entopallium, suggesting an increased passing of information to the left hemisphere (Manns and Ströckens, 2014) in birds viewing with the right eye, compared to birds viewing with the left eye. However, a previous experiment suggested that the tectofugal pathway does not participate in sun compass-mediated spatial learning, as lesions to the ectopallium did not affect the ability of the pigeons to learn to locate a food reward in an octagonal outdoor arena on the basis of sun compass information (Budzynski et al., 2002). By contrast, lesions to the visual wulst affected the birds' performances. Wulst-lesioned pigeons took longer to learn the task, and when subjected to clock-shift treatment displayed inconsistent orientation, suggesting an involvement of the thalamofugal pathway in processing sun compass information (Budzynski et al., 2002).

A previous GPS study suggested that the left eye/right hemisphere visual system has an advantage in developing route fidelity and memorising familiar visual landmarks (Pollonara et al., 2017). Interestingly, birds with navigational experience had an enlarged right optic tectum, which projects to the ipsilateral entopallium, when compared with birds without homing experience (Mehlhorn et al., 2010). This might suggest a critical involvement of the tectofugal pathway in the familiar landmark-based navigation.

The control pigeons showed a higher level of inconsistency in their directional choices in comparison to both monocular groups in the cue-conflict test, as shown by the shorter lengths of the individual mean vectors (Figure 3d and Figure 4). This could be due to increased inter-hemispheric

communication relative to the monocular treatments (Manns and Güntürkün, 2009), suggesting that in a conflict condition the two hemispheres might process different information at the same time, but that the processing of a particular item of information is not largely segregated in one hemisphere. It may otherwise be that binocular birds, having a wider field of vision, are able to collect the discrepant information more easily.

The use of an arena to test cue use allows experimental manipulation to be made which would be likely to influence flight in a non-navigational manner, including the use of eye-patches to monocularly occlude birds. This means that directional behaviours can still be studied, without the complications to flight, whilst providing a proxy for the behaviours we might expect to see in the wild. However, there are limitations to this approach, as the arena represents a learned task, not a natural behaviour, so the directional choices made may not be using the same cues as in a navigational situation, where more factors are present. Additionally, the arena gives limited directional choices (45-degree compartments), so has a low resolution. When considering the integration of cues, it would be necessary to design an arena with a higher resolution of choices (more, smaller compartments), in order to analyse how strongly cues are being weighted.

Overall, these results do not support complete lateralisation of the sun compass use to the left eye/right hemisphere or the right eye/left hemisphere visual system, as both left and right monocularly occluded pigeons displayed a comparable level of preference for the training compass direction in a cue-conflict situation. However, birds processing visual information with the left eye/right hemisphere visual system may be more capable to integrate both directional and feature cues, than the birds with the right eye/left hemisphere in use, as shown by their preferential choice for the intermediate sector.

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Chapter 3

Repeated training of homing pigeons reveals age dependent idiosyncrasy and visual landmark use.

Charlotte Griffiths, Ingo Schiffner, Emily Price, Meghan Charnell-Hughes, Dmitry Kishkinev, Richard A. Holland

Published paper

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Abstract

Recent research into the navigational strategies of homing pigeons (*Columba livia*) in the familiar area has highlighted the phenomenon of route fidelity – birds forming idiosyncratic flight paths to which they are loyal over multiple releases from the same site, and even returning to this path when released from a near-by unfamiliar location. Such results highlight the potential importance of visual landmark cues in the homing process. However, not all birds have been shown to produce idiosyncratic routes or show this route-joining behaviour. Here we use birds with and without flight experience to study the formation of idiosyncratic routes when released repeatedly from a single location, followed by two off-route releases with differing topography to see how flight experience and local landmark features can influence navigational strategy in the familiar area. We found that, over the course of 20 sequential releases, birds with greater flight experience tended to form idiosyncratic routes whereas less experienced birds did not show this tendency. When released from near-by sites (from which the birds had not previously been released), a range of navigational strategies were seen, including flying parallel to the learned route (suggestive of a learned compass direction), a direct flight path towards home (again indicative of compass use), re-joining the learned route, and following the coastline. These latter strategies are suggestive of landmark usage. Analysis using time lag embedding was also used to assess the off-route releases, and the short-term correlation dimension values produced (ranging from 1.5-2.5) were also indicative of strategies using one or two factors (landmarks, compass, or a combination of these two). Individual birds often showed different strategies at different sites, suggesting that the use of different navigational cues is highly flexible and situationally dependent.

Introduction

The study of pigeon homing has for many years been focused on how the birds are able navigate from an unfamiliar location for so-called “true navigation”, usually framed in the paradigm of Kramer’s “map and compass” theory (Kramer, 1953). Here, navigation is broken down into a two-step process, with a “map” providing a relative location from which the appropriate direction can be determined, and the “compass” providing a real-world directional heading. The map stage of navigation theorises a system of relatively stable gradients which vary predictable over a significant distance, such that birds can learn the spatial relationships of these gradient concentrations to produce a mental navigational map (Holland, 2014). A large body of evidence supports a role of olfactory cues in the formation of a map (Wallraff, 2005; Gagliardo, 2013), although alternative roles for olfactory cues have been proposed (Jorge et al., 2009; Jorge et al., 2010; Gagliardo et al., 2011; Gagliardo et al., 2018). Other cues have also been proposed to play a role in the map, such as the magnetic field (Wiltschko and Wiltschko, 2009), infrasound (Hagstrum, 2000; Hagstrum, 2013), and gravity (Blaser et al., 2014). Both magnetic (Keeton, 1971; Wiltschko and Wiltschko, 1972; Wiltschko et al., 1981;

Walker, 1998; Beason, 2005) and solar-cues have been found to provide compass information (Schmidt-Koenig, 1990; Guilford and Taylor, 2014) in terms of magnetic inclination and the time-compensated solar azimuth compass.

When navigating close to the loft in a familiar area, evidence suggests that both the sun compass and magnetic compass still play significant roles. Birds subjected to clock-shift procedures to alter their perception of time of day, and therefore their interpretation of the time-compensated sun compass, show a relatively predictable deflection angle away from the home direction when navigating, and this deflection can be seen even when navigating close to the loft (Wiltschko et al., 1994; Chappell, 1997; Armstrong et al., 2013). Experiments attaching magnets to the heads of navigating pigeons in order to disrupt access to the magnetic compass (by producing a local strong magnetic field) show that deflection under clock-shift increases, suggesting that there is some influence of the magnetic compass on the chosen direction also, and that the cues are being combined (Wiltschko and Wiltschko, 2001; Wiltschko and Wiltschko, 2007; Gagliardo et al., 2009).

When navigating in the familiar area, however, the importance of familiar area cues, particularly visual landscape features, has been highlighted (Braithwaite and Guilford, 1991; Burt et al., 1997), although curiously, not always supported (Schmidt-Koenig and Schlichte, 1972; Schmidt-Koenig and Walcott, 1978). Early studies found that birds deprived of object vision via the use of frosted lenses were able to home from significant distances (up to 130km) (Schmidt-Koenig and Schlichte, 1972), although many birds with frosted lenses were only able to locate the vicinity of the loft (0.5-5km) (Schmidt-Koenig and Walcott, 1978). Surprisingly, birds deprived of object vision and rendered anosmic were still able to orient successfully from a familiar area, again suggesting that object vision is not necessary for homing, and that at least one other cue was necessary to explain homing (Benvenuti and Fiaschi, 1983). However, a subsequent study did not support this and suggested a crucial role for vision if birds were made anosmic (Streng and Wallraff, 1992). Later investigations found that birds able to view the landscape prior to release showed increased homing performance, suggesting that, when available, visual access to the landscape can be important in homing (Braithwaite and Guilford, 1991; Burt et al., 1997).

The introduction of a whole new series of possible cues, unique to the local landscape, means that strategies of navigation in the familiar area may be highly variable dependent on individual location. Studies have highlighted the importance of landscape features such as rivers, roads and hedgerows which make up patterns of straight lines in the environment to determine flight path structure (Lipp et al., 2004), as well as how the density of edges in the landscape influences how well routes are learned (Mann et al., 2014). The importance of landscape features when learning to navigate in the

familiar area is evident and brings in to question how the cues used outside of the familiar area (olfactory, magnetic, solar) are integrated with the visual landmark cues.

Familiarity with an area comes in two forms – general flight experience in the local area, and experience flying from a particular release site. Previous studies have demonstrated that homing efficiency is greater in birds generally familiar with an area in comparison with naïve individuals (Meade et al., 2006), suggesting that familiarity influences the navigational strategy. Studies of birds released repeatedly from the same location show that birds tend to show an increase in route efficiency and fidelity (Biro et al., 2004; Meade et al., 2005; Biro et al., 2006). However, these birds do not produce maximally efficient routes, instead developing individually stereotypical routes, with efficiency reaching a plateau once the route has been established. Furthermore, birds released from sites alongside the learned route have been shown to return to the established route, rather than flying a direct route (Biro et al., 2004; Biro et al., 2006). This suggests that, instead of flying on a direct compass heading towards home, birds use the local landscape cues to inform their routes. One study found that bottlenecks in tracks from repeated releases occurred alongside salient landmark cues, suggesting the learned routes are constrained by particular landmark features (Mann et al., 2011).

However, the Frankfurt group failed to replicate these results (Wiltschko et al., 2007), with pigeons failing to show an increase in efficiency, or produce stereotyped routes. A later analysis found that birds unfamiliar to the area had a similar efficiency to familiar birds, but that birds released multiply from the same site did show a general increase in efficiency (Schiffner et al., 2013). Given the importance of individual landscape features unique to a particular area, it is possible that the ability to learn local routes varies between locations or landscape features (Mann et al., 2014). Therefore, we use a new location to test the hypotheses that birds develop stereotypical routes when navigating repeatedly from a known location, and that they will preferentially return to this learned route when released at a novel site nearby. Additionally, we classify our test groups as “old” and “young” birds, with over ten years or less than one year of experience respectively, to investigate how age and navigational experience affects navigational ability in the familiar area.

According to the predictions of earlier studies, birds forming idiosyncratic routes should show a general increase in efficiency over early releases, but plateau below maximum efficiency (Meade et al., 2005; Flack et al., 2012; Guilford and Biro, 2014). Birds should show an increase in route fidelity, with a bird’s later releases more similar to each other than earlier releases. Once an idiosyncratic route has been formed, it should be relatively distinct compared to the routes of others. Off-route releases may produce a variety of strategies, with an expectation of either a) a direct route home, based on a compass heading; b) a return to the learned route which is then followed home, presumably more

reliant on visual landmark cues (Biro et al., 2004); or c) possible offset of the learned route from the novel release site, similar to the offset routes seen when clock-shifting birds from a learned route (Biro et al., 2007).

Methods

Subjects, Training and Releases

A total of 21 birds in two age categories (ten young birds, only one year old and eleven old birds, nine/ten years old) were trained to fly repeatedly from a single release site at Y Felinheli (within 5km) over the course of two months, from the 19/09/2017 – 14/11/2017, with a total of twenty releases from the site. All of the birds had flight experience in the local area but had not participated in previous homing experiments. The birds were kept indoors usually during the day and did not have independent free access to the outdoors. Old and young birds had a greatly different flight experience prior to the tracked releases from Felinheli (table 1).

During the experimental releases, birds were released individually. The majority of releases took place once per day, apart from pairs 2 and 3, 5 and 6, 10 and 11, and 13 and 14 which took place in the same day (morning and afternoon releases). All 21 birds completed the 20 training flights from Y Felinheli, although a few flights were not successfully recorded. Any incomplete tracks were removed from the analysis. All of the experimental flights were tracked using a GPS recorder (i-gotU USB GPS Travel and Sports Loggers, 15.5g with outer casing removed), with position fixes every second and an accuracy of ±5m. Trackers weighed less than 5% of the birds' bodyweight, and all birds had experience carrying the trackers before the experimental releases. The trackers were attached to the back between the wings using a Velcro strip glued to trimmed feathers. Following the repeated releases from Y Felinheli, two off-route releases were performed, one from just across the Menai Strait on Anglesey, and one further inland (see Figure 1 for a map of the release sites and home loft location). Off-route release 1 was 1.64km from the Y Felinheli release site, and off-route release 2 was 1.16km. Both off-route releases were conducted once on separate days. All birds were housed at the Bangor University Treborth Botanic Gardens loft, under natural daylight. Birds had free access to water and grit, and 25g of food per bird per day.

Table 1: Subject flight experience. Table showing the release locations for the young and old birds demonstrating their range of flight experience. Both the distance and bearing of the release site from the loft is given. Release locations are given by name and coordinates.

Release Location	Bearing from loft (degrees, North=0)	Distance from loft (km)
Young Birds		
Roman camp (53.233818, -4.127970)	58	3.6
Parc Menai (53.206992, -4.185048)	-144	1.4
Llanfairp.g. (53.220457, -4.194661)	-74	1.5
Llandygai roundabout (53.219084, -4.105247)	87	4.5
Old Birds		
Penmon (53.2950670, -4.0611793)	41	11.5
Caernarfon foreshore (53.1393739, -4.2825671)	-140	11.3
Waterloo port road (53.1526017, -4.2649993)	-139	9.4
Plas Menai (53.1698685, -4.2434940)	-138	7.0
Bush road (53.1815830, -4.2025464)	-153	4.4
Beach road (Felinheli 53.1838068, -4.2116039)	-145	4.5
Glan faenol (53.2045917, -4.1968571)	-131	2.1
Treborth sports fields (53.2148962, -4.1763044)	-135	0.3
Cadnant corner (53.2335445, -4.1568771)	30	2.2
Shore at chateau rhianfa (53.2376368, -4.1428572)	41	3.1
Lon ganol (53.2428050, -4.1420308)	36	3.6
Gazelle foreshore (53.2442042, -4.1286935)	44	4.3
Gallows point (53.2547081, -4.1052416)	47	6.2
Beaumaris foreshore (53.2628903, -4.0879517)	48	7.6
Friars bay (53.2764597, -4.0839630)	42	8.9
Traeth lleiniog (53.2907610, -4.0704235)	40	10.7

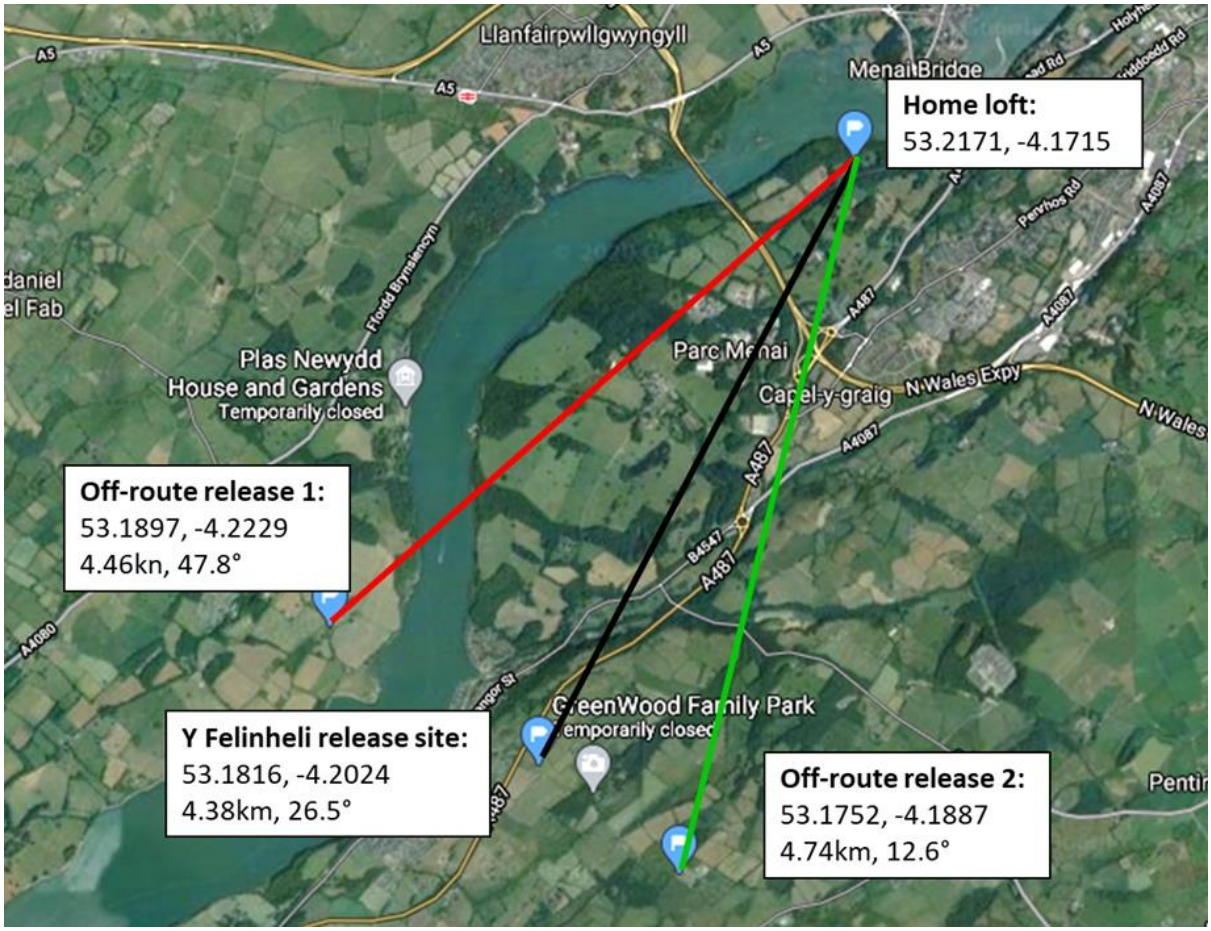

Figure 1: Satellite map of the homing pigeon home and release sites, including the name of each site, the latitude and longitude of each site in decimal degrees and the distance from the release site to the loft in kilometres and the absolute bearing from the release site to the loft in degrees.

Data Analysis

Processing

All tracks were processed and analysed using RStudio. Firstly, a low-pass filter (Butterworth filter, signal package) was applied to the latitude and longitude values to remove noise at the frequency of data collection (i.e. cutting out any higher-frequency signals). Following this, calculations were applied to produce continuous flight bearings (the bearing of each point from the release site) for each point, distance travelled between each point, current track direction, and instantaneous deflection (the difference between the current track direction and the home direction) at each point using the r packages circular and geosphere (Agostinelli and Lund, 2017; Agostinelli and Lund, 2018; Hijmans, 2019). Point of decision analysis was then used to remove early circling behaviour from the tracks (Schiffner and Wiltschko, 2009). The mean vector length of current bearings was calculated across each set of ten consecutive points, and a cut-off for navigational behaviour chosen when three of these consecutive mean vector lengths were all greater than 0.85, implying relatively consistent

direction of flight. This cut-off of 0.85 was chosen after testing multiple values, as providing the best balance between removing circling behaviour without excluding too much of the track. As well as this, a radius of 200m around the home loft was removed from the tracks to discount the circling behaviour around the loft, as this distorts calculations of efficiency. Following this processing, various calculations were performed to analyse the tracks.

Efficiency

Efficiency of a tracks is calculated as the shortest beeline distance divided by the actual distance flown between the start and end points, ranging between zero and one. Efficiency was then compared between young and old birds using an independent t-test, and a linear mixed model (lmer package) was used to analyse the factors influencing change in efficiency, with release number and age as fixed effects, and bird as a random effect (Bates et al., 2015). Data show a linear relationship, are sufficiently normally distributed (visual interpretation of a histogram of the response variable), do not show any autocorrelation (Durbin-Watson Test, D-W statistic = 2.069, p = 0.574), and do not show significant heteroscedasticity (Breusch-Pagan test, BP = 1.963, df = 2, p-value = 0.375). An ANOVA test was used to compare a full and reduced model to identify the significant factors.

Idiosyncrasy

In order to investigate whether birds showed individually unique routes, a mean route was computed for each bird, consisting of the three final successful tracks recorded from Y Felinheli. The mean track was first created as a series of equally-spaced points from the start to the end of the track. For each point along the mean track, the nearest time-independent point on each of the tracks to be averaged was found, and the mean track point moved to the mean of the nearest neighbour points. Once this had been applied to the entire track, points were moved to fill out any gaps and reduce bunching along the mean route by moving any points more than a set distance apart to the half-way point between them.

Once the mean route had been constructed, the nearest-neighbour distances were calculated to three tracks from the same individual (tracks 13, 15 and 16 as these were available for all birds), and to tracks from a different individual (individual chosen at random for each track, tracks 8, 9, 10 and 12). The average nearest neighbour distance for “self” and “other” comparisons was calculated for each bird and compared using a Wilcoxon signed-rank test.

Off-route releases

To investigate the directional choices made when released from the off-route locations, continuous flight bearings were calculated for the mean tracks of each bird (as described above). As the

continuous flight bearings describe the angle from the release site at each point along the track, matching continuous flight bearings from two tracks suggest a copying of the known route. Instantaneous deflection was also calculated for the mean tracks and compared to the instantaneous deflection values from the off-route releases. The mode of each of these measures was calculated to identify the predominant direction of flight over the course of the tracks. These measures, alongside visual inspection of the flight paths, were used to classify the off-route releases into different categories of navigational behaviour.

Time lag embedding

We analyzed data by means of time lag embedding to determine the underlying characteristics of the navigational process used by Homing Pigeons - *Columba livia f. domestica* (Schiffner et al., 2011). Time lag embedding is a method derived from dynamic systems theory, an advanced area of mathematics and physics focused on understanding and describing complex dynamic systems. Time lag embedding, commonly used to characterise dynamic systems (i.e. systems that change over time) (Small, 2005; Nehmzow, 2006), allows the physical/data driven reconstruction of a system in phase space. Observations of a system can be used to construct a multi-dimensional phase space, where each axis in this space represents a parameter of the system (Takens, 1981). The number of parameters (or degrees of freedom) in this space, therefore, is the minimum number of independent variables necessary to describe the system. The proper embedding dimension was determined using a false nearest neighbour approach and the correct time lag was estimated using a non-parametric normalized mutual information algorithm to deal with the sensor noise.

Correlation dimension

In order to estimate the number of navigational factors used by the birds' navigational system we calculated the correlation dimension, a measure of the degrees of freedom of the system. The methods described here have been tested rigorously in mathematical systems where the exact number of degrees of freedom is known and have been applied successfully to tracks of homing pigeons, allowing unprecedented insight into their navigational system (Schiffner et al., 2011; Schiffner and Wiltschko, 2013; Schiffner et al., 2014; Schiffner and Wiltschko, 2014; Schiffner et al., 2016).

The method used to determine the correlation dimension is identical to the original algorithm described by Grassberger and Procaccia (Grassberger and Procaccia, 1983). By estimating the correlation dimension we can determine the nature of the underlying process: deterministic systems have an integer dimension (e.g. 1.0, 2.0, etc.); random systems are dimensionless; chaotic

deterministic systems have a fractal/non integer dimension (e.g. 1.3, 2.1, etc.). Here we specifically estimated the short-term correlation dimension, a lower bound rolling estimate of the actual correlation dimension over a fixed time window calculated over the entirety of the track to analyse the tracks and potential changes in the navigational process throughout the pigeon's journey to its home loft.

Theoretical considerations

When considering an animal's navigational process, the degrees of freedom, as represented by the correlation dimension, indicates the number of independent sensory modalities involved in the navigational process - where the same sensor could pick up several independent cues. Applied to tracks of an animal the correlation dimension thus, allows us to draw conclusions about the navigational strategy used; low correlation dimensions suggest simpler forms of navigation, like navigation based on point-like information, while higher correlation dimensions suggest navigation based on multiple environmental gradients (Schiffner et al., 2011).

The effects of the different releases and types of behaviours on the short-term correlation dimension were tested using the Aligned Rank Transformed ANOVA (ART-ANOVA), a non-parametric approach utilizing GLMM and Global ranking to ensure normal distribution of the data (Wobbrock et al., 2011). For post hoc comparison, we employed least squared means using the Tukey method for multiple comparisons. While this method can ensure that data are drawn from a normal distribution, it still requires testing for homogeneity of variances. The Levene's test, used to test for homogeneity of variances, ensured homogeneity of variances in all tests. In each test we considered the effect of either the release number or the type of behaviour and the distance from home as fixed effects, and the bird's identity as a random effect.

Ethical Statement

All applicable animal welfare guidelines were followed including the ASAB/ABS Guidelines for the treatment of animals in research. The project was reviewed by Bangor University AWERB and received approval for work to be carried out (approval number: CNS2017EJP01).

Results

Efficiency

Efficiency was calculated with the exclusion of early circling and circling around the loft, as described in the methods. There was a general increase in efficiency over the first five releases, with young birds showing a much lower efficiency in the first few releases (Figures 2 and 3). Comparison of the groups

using an independent t test found a significant difference in efficiency between young and old birds during the first 4 releases (t-test: t = 3.5261, df = 55.374, p-value = 0.0008551), but not during the final 4 releases (t-test: t = 1.1744, df = 99.421, p-value = 0.243), (figure 3). The mean efficiency stabilises between 0.8-0.9, showing that the birds are not using maximally efficient routes, even when removing circling behaviour. A linear mixed model found that Release but not Age group were significant predictors of Efficiency (ANOVA: p = 0.001324).

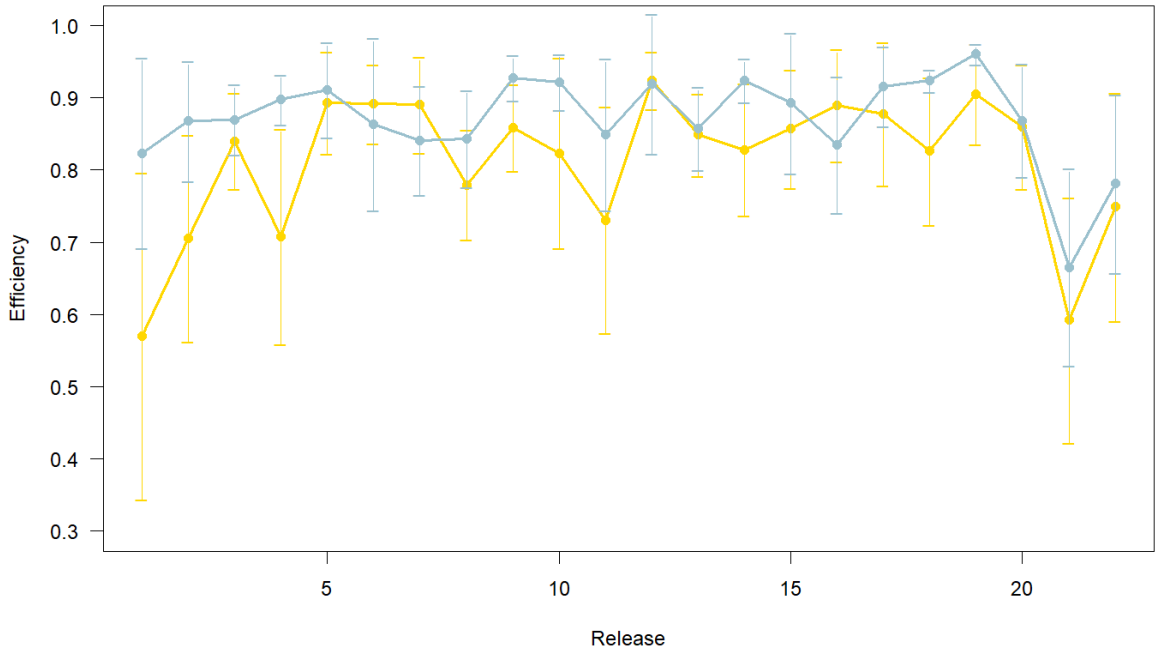

Figure 2: Mean efficiency of each release split into young (gold) and old (light blue) birds, with 95% confidence intervals shown. Releases 1-20 are from Y Felinheli; 21 is the first off-route release from Anglesey; 22 is the second off-route release (Figure 1).

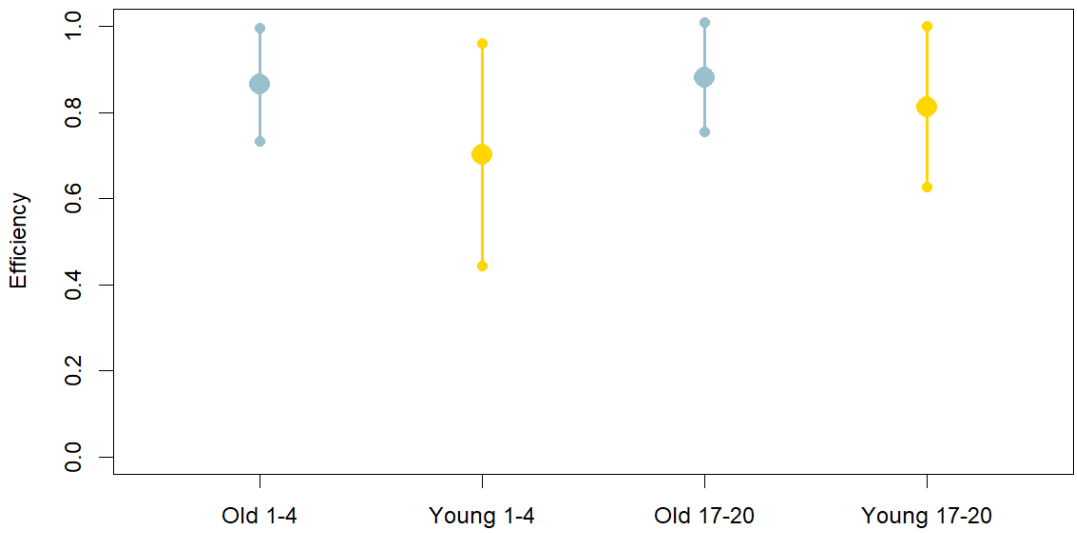

Figure 3: Plot showing the mean and standard deviation of the efficiencies for the first 4 and last 4 releases from Y Felinheli, split by age group (old in blue, young in yellow).

Idiosyncrasy

Mean tracks were calculated from the final three releases from Y Felinheli for each bird. Figure 4 shows these tracks, as well as the mean efficiency averaged across the last three tracks, and the mean area between the tracks is also given in km^2. No significant difference was found in the area between the final tracks between the old and young birds (Wilcoxon signed-rank test: W = 41, P = 0.349). No correlation between mean efficiency and area between tracks was found when a linear regression was applied (ANOVA: $F_{1,19}$ = 0.0125, P = 0.912). Comparison of the calculated mean tracks (Figure 4) to "self" and "other" tracks produced a list of average nearest neighbour distances for each individual. Using a Wilcoxon signed-rank test, we find a significant difference between the self-comparisons and other-comparisons of mean nearest neighbour distance (Wilcoxon signed-rank Test: V = 218, P = 1.918×10^{-3}), with self-distances being lower on average than comparisons to the tracks of other birds (Figure 6).

Figure 4: Mean tracks (black) produced using nearest neighbours to average the final three successful recordings for each bird (orange) from the Y Felinheli release site, young birds (yellow) and old birds (blue). The mean area between the final three tracks has been given for each bird (km^2), as well as the mean efficiency of the final three tracks.

Figure 5 breaks down the above to compare the "self" and "other" distances between young and old birds. There is a significant difference in the "self" distances between young and old birds (Mann-Whitney U Test: U = 28, P = 0.034) with old birds showing lower nearest neighbour distances than young birds. However, there is no significant difference in the "other" distances between young and old birds (Mann-Whitney U Test: U = 61, P = 1). The significant difference between self and other comparisons is maintained when looking at old birds alone (Wilcoxon signed-rank Test (paired): V = 65, $P = 1.953 \times 10^{-3}$), but not young birds (V = 47, P = 0.240).

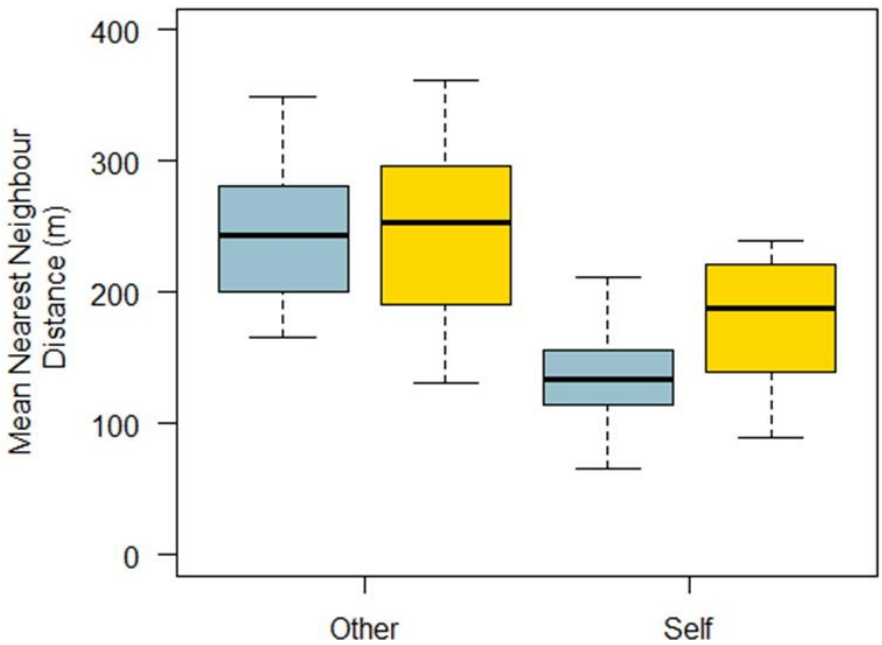

Figure 5: Box and whisker plot comparing the average "self" and "other" nearest neighbour distances for each bird, between young (gold) and old (light blue) individuals. Centre line gives the median of the groups, the box edges the first and third quartiles, and the whiskers the maximum and minimum of the groups.

Short-term Correlation Dimension Analysis

Using time lag embedding to calculate the short-term correlation dimension for each track of repeated releases we observe a highly significant effect of the release number on the correlation dimension (ART ANOVA: $F_{16,2903} = 44.565$, $P < 2 \times 10^{-16}$), which is also confirmed by the post hoc comparisons. Figure 6 suggests that this transition is not instantaneous, but rather gradual. The majority of initial flights have a higher correlation dimension (Green colours: 2-2.5) compared to the later flights (Red colours: 1-1.5). These final values are close to what we would expect if the birds would switch to following landmarks to aid their navigation. Two caveats though, the short-term correlation dimension is generally lower than the actual correlation dimension and there is a lot of variation in this data set. However, because of the high variation and the fact that we are looking at averages it is evident that at least some of the birds do occasionally follow landmarks.

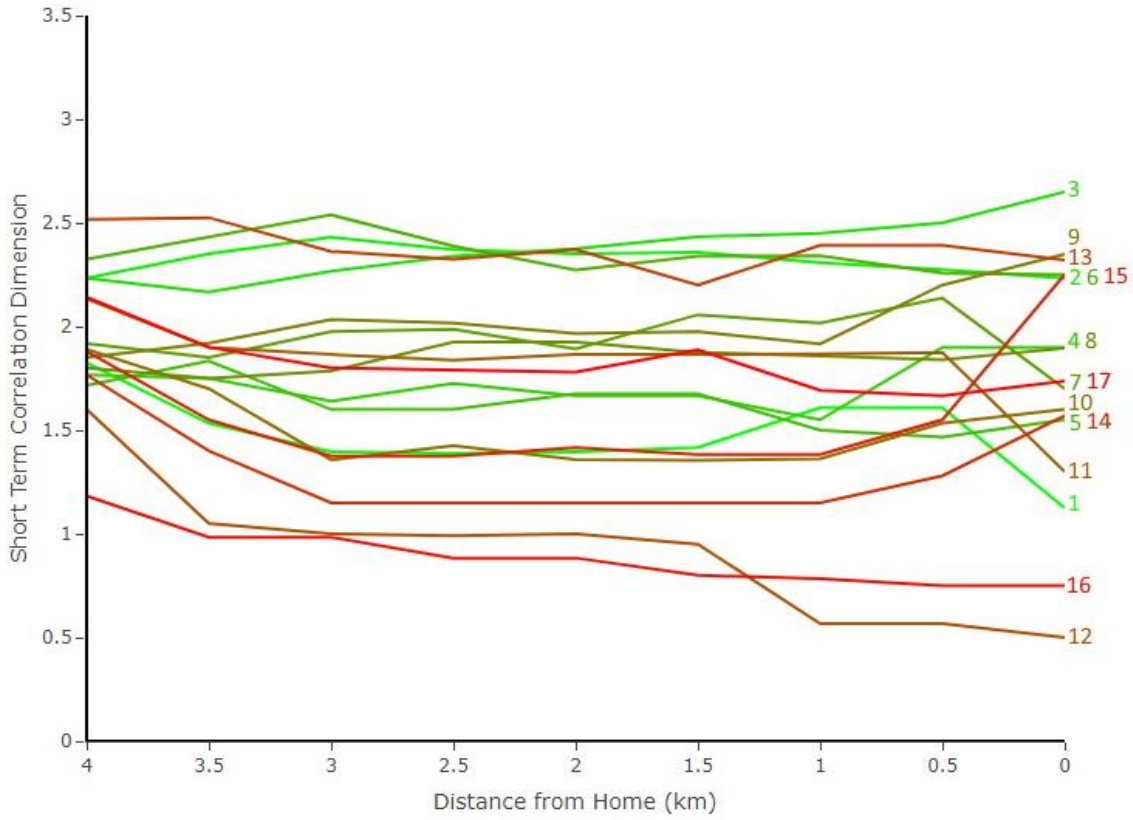

Figure 6: Averages of the short-term correlation dimension for the individual releases, shown as a gradient with earlier releases in green transitioning through brown to later releases in red. Release number has been labelled at the end of each line, in the matching colour.

Off-route Releases

Figure 7 shows the mean track and off-route releases for the young and old birds. From visual analysis of these tracks and use of continuous flight bearings and instantaneous deflection, off-route return strategies have been classified (Table 2). Flight strategies have been broken down into several classes: a coast-following (CF) strategy seen from the first-off route release where the bird follows the Menai Strait coastline of the Anglesey side before crossing the Strait close to home; a direct route (D) where the bird flies a relatively straight course between the release site and home; parallel (P) where the bird flies parallel to the Y Felinheli route, and corrects towards home later on; joining (J) behaviour, where the bird clearly flies to and then along the established learned route; and finally cross (C) where the bird appears to cross but not join the established route.

Continuous flight bearing is a measure of the absolute bearing of a point from the release site. As the absolute bearing of the home loft from the first off-route release site is 47.8° and from the second off-route release 12.6° (Figure 1), a modal continuous flight bearing near these values for each release suggests a relatively direct route. Similarly, an instantaneous deflection of 0° suggests direct

homewards flight, e.g. bird 919 with a continuous flight bearing of 43 and instantaneous deflection of -2 for off-route release 1 and a continuous flight bearing of 9 and instantaneous deflection of 3 for off-route release 2, both suggesting a direct flight path. The homewards bearing from the Y Felinheli release site is 26.5°, therefore modal continuous flight bearings near this on the off-route releases suggest a parallel of the learned route, e.g. birds 158 and 262, with continuous flight bearings of 26 for off-route release 2. A chi-squared contingency table analysis of flight strategy for each location finds no association between age and flight strategy from the first off-route release site (Chi-squared test: $\chi^2_3 = 0.952$, $P = 0.813$), but an association close to significance at the second off-route release site with old birds favouring the parallel strategy (Chi-squared test: $\chi^2_2 = 5.45$, $P = 0.066$).

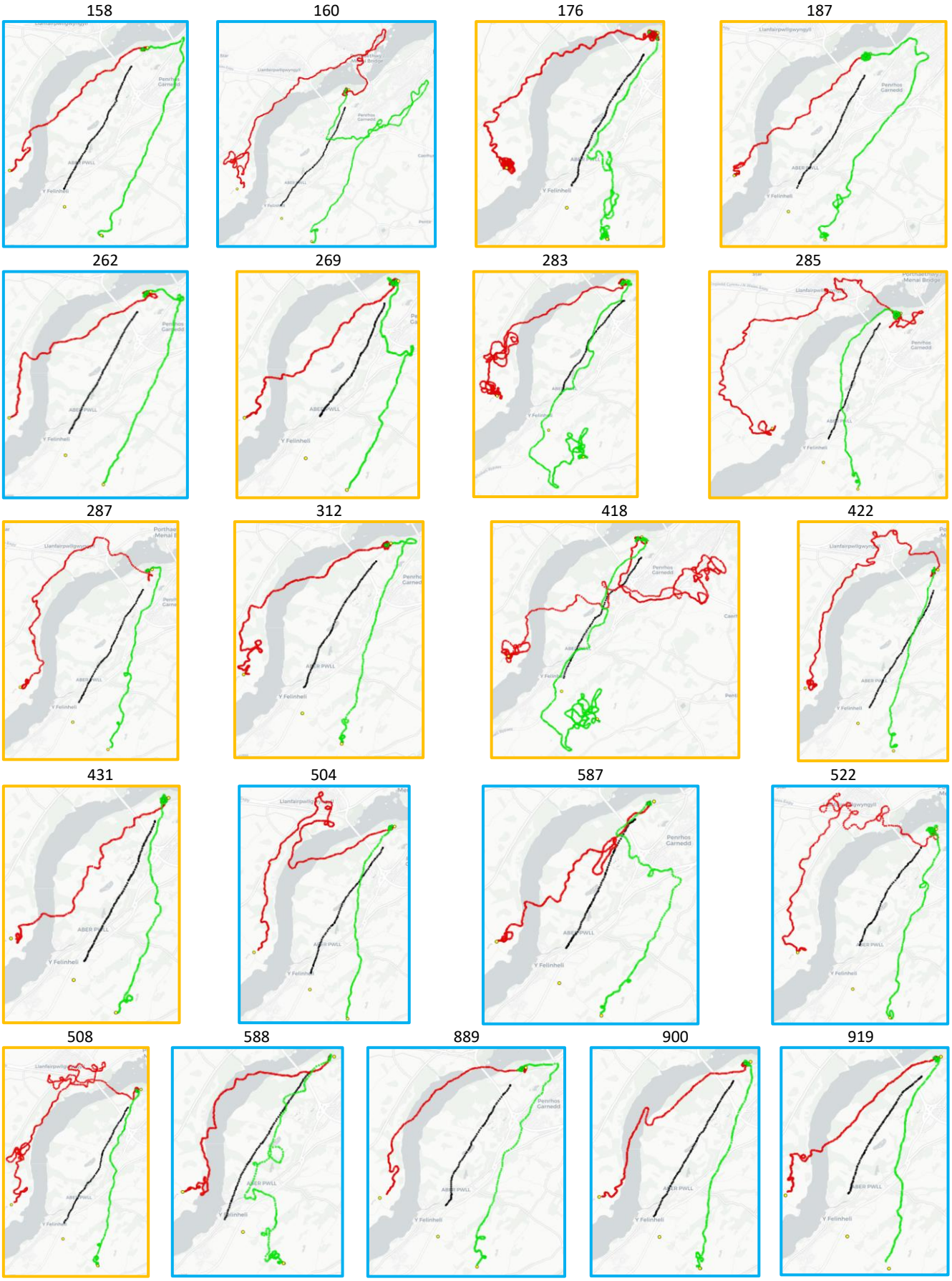

Figure 7: Tracks for each bird showing the calculated mean track from the last three successful recordings from Y Felinheli (black), and the two off-route releases (red and green), young birds are bordered in yellow, and old birds in blue.

Table 2: Individual tracks measurements for off route releases one and two. For each of the 21 birds, mean continuous flight bearings (CFBs, degrees), mean instantaneous deflection (ID, degrees), mean flight efficiency and median short term correlation dimension (STCD) has been given.

	Off-route release 1					Off-route release 2				
Bird	**Strategy 1**	**CFB**	**ID**	**Efficiency**	**STCD**	**Strategy 2**	**CFB**	**ID**	**Efficiency**	**STCD**
					Young Birds					
176	CF	20	3	0.629	2.783	J	4	-6	0.529	1.733
187	D	48	13	0.885	2.633	P	30	63	0.614	1.567
283	CF	8	-64	0.370	2.183	J	3	-6	0.485	2.033
285	CF	21	-30	0.456	1.733	C	-9	-34	0.898	1.580
287	CF	9	-39	0.642	2.400	D	13	8	0.862	1.967
312	D	40	-36	0.641	2.267	D	15	2	0.978	0.550
418	C	67	-45	0.170	2.583	J	7	-10	0.265	1.980
422	P	6	-47	0.590	2.433	D	9	4	0.966	3.300
431	D	45	29	0.809	2.633	D	11	17	0.924	1.325
508	P	19	-69	0.259	1.900	D	10	7	0.937	1.950
					Old Birds					
158	D	41	-17	0.829	2.100	P	26	37	0.783	3.000
160	CF	44	-27	0.324	2.514	P	36	-81	0.300	1.467
262	D	43	-34	0.804	2.300	P	26	27	0.818	1.125
269	D	46	-10	0.848	2.800	P	23	21	0.726	1.125
504	CF	21	-40	0.345	2.067	D	5	-23	0.945	0.200
552	CF	29	-39	0.339	2.550	D	16	3	0.839	0.975
587	J	48	-7	0.535	2.150	P	42	26	0.625	2.500
588	CF	17	-60	0.731	2.250	J	7	-19	0.551	2.225
889	P	-2	-35	0.762	0.620	P	10	-3	0.837	1.220
900	D	23	-37	0.750	0.800	D	7	-7	0.969	0.450
919	D	43	-2	0.805	1.100	D	9	3	0.930	1.450

In terms of mean flight efficiency and short-term correlation dimension of the off-route releases, there is a significant negative correlation (-1.012), with short-term correlation dimension decreasing as efficiency increases (ANOVA: $F_{1,40} = 4.447$, $P = 0.0413$). There was no significant correlation between efficiency of the first and second off-route releases (ANOVA: $F_{1,19} = 2.298$, $P = 0.146$), nor short-term correlation dimension (ANOVA: $F_{1,19} = 1.081$, $P = 0.312$).

Using time lag embedding to calculate the short-term correlation dimension for each track of offs-route releases and comparing the prevalent types of behaviours (direct, parallel and coast-following) reveals a significant difference between direct routes and parallel routes, as well as differences between those two types and the coast following type of behaviour. While there is variation in the correlation dimension estimates indicating that the visual categorisation is not exact, it was still sufficiently accurate to detect significant differences between the different types of routes (ART ANOVA: $F_{2,214} = 44.731$, $P < 2\text{x}10^{-16}$). These differences are also confirmed by the post hoc comparisons, showing again significant differences between the individual types of routes. Figure 8 shows that Coast following has the highest correlation dimension (CF≈2.5). The Direct flights have a slightly lower correlation dimension (DIR≈2.0) and the parallel flights have the lowest correlation dimension (PAR≈1.5).

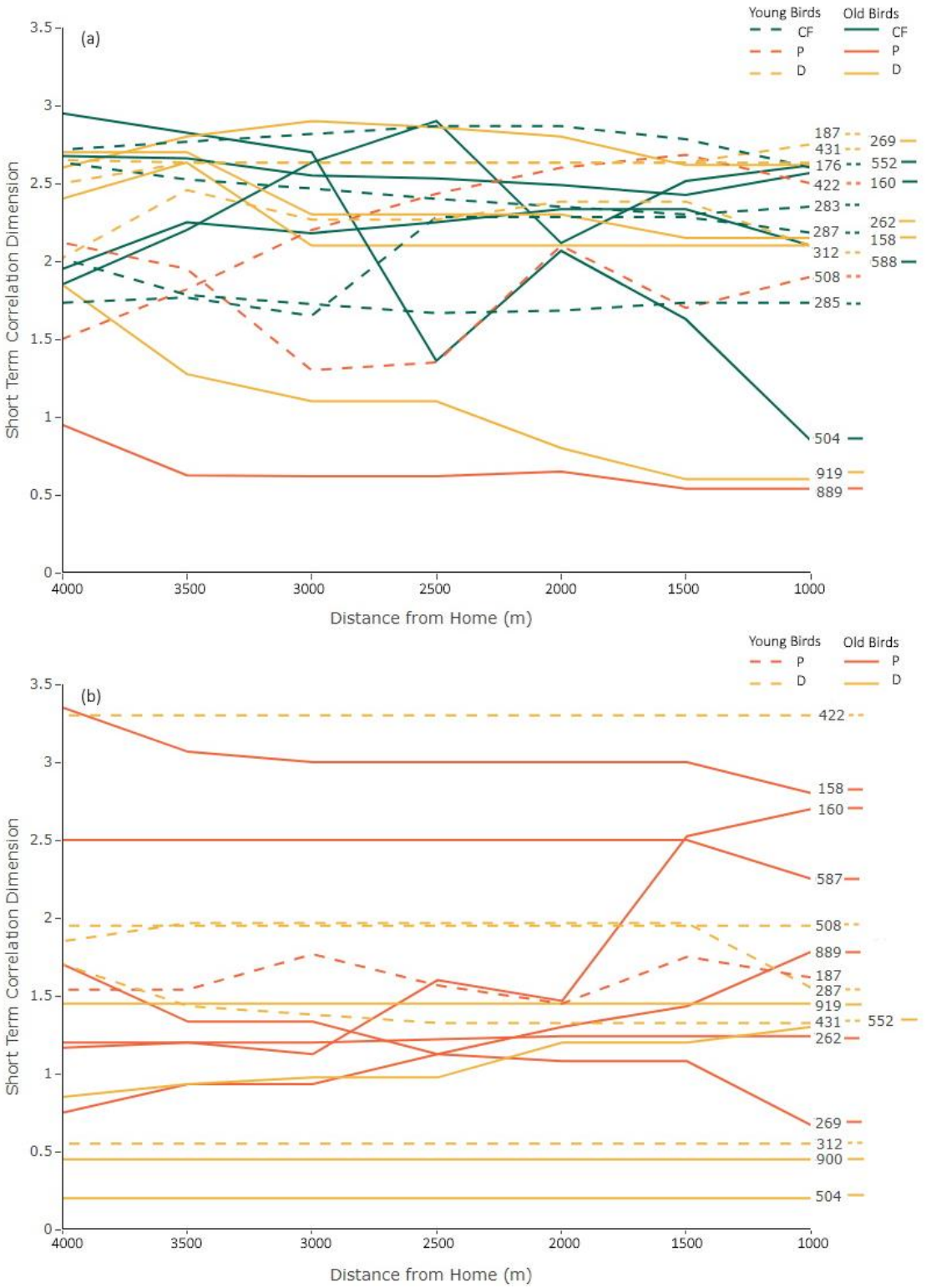

Figure 8: Short-term correlation dimensions at 500m intervals for each bird classified by flight strategy for off-route release 1 (a) and off route release 2 (b). Green lines show the coast-following behaviour (CF), red a parallel route (P) and yellow a direct route (D), with unbroken lines representing old birds and dashed lines representing young birds.

Discussion

Efficiency

Figure 2 shows that average efficiency per flight increases over the first five flights, stabilising between 0.8 and 0.9. This demonstrates an improvement in path efficiency with experience but is still variable and plateaus before reaching maximal efficiency. Given that this measure of efficiency was calculated from tracks where circling behaviour had been removed, the numbers are a true representation of the efficiency of the navigational path. This initial increase in efficiency agrees with previous findings, where birds flew routes significantly longer than the beeline (Biro et al., 2004; Meade et al., 2005). Consistently inefficient routes suggest that the flight path is not based solely on a compass direction, which should produce a beeline home. However, it should also be noted that we would only expect highly efficient routes if birds were suitably motivated to return home. Within the small flight radius of the study this might not be the case.

When comparing young and old individuals (Figure 3) it becomes evident that the inexperienced birds have a much lower efficiency on the first four flights and show a much more significant increase in efficiency than the old birds. After ~5 flights, both young and old birds show similar efficiencies on their learned routes. The earlier success of the old birds suggests that general experience may be important in determining flight efficiency from novel locations. A linear mixed model of route efficiency against flight suggests that both bird age and flight number are significant predictors of efficiency, with a significant interaction between age and flight number. This supports the differing relationship between efficiency increase in young and old birds across the flights.

In contrast to previous work (Biro et al., 2004; Meade et al., 2005; Biro et al., 2006), the Frankfurt group failed to find an increase in efficiency when releasing birds from shorter distances, with only birds being released from 30km showing an increase in efficiency over multiple releases (Schiffner et al., 2013; Schiffner et al., 2018). These birds were all experienced adults with significant homing experience in the region with efficiencies between 0.77 and 0.92 found (Wiltschko et al., 2007), although no significant increase in efficiency was seen over the recorded flights. The range of efficiencies is similar to the range at which the pigeons here stabilise at after the initial learning phase, and agrees with the results shown in Figure 3, where the more experienced birds begin with a much greater efficiency. It is possible that greater general flight experience of the older birds here or the Frankfurt birds means that their flight efficiency is generally high, so no significant increase is seen. In contrast, the inexperienced birds show a significant increase in efficiency over the first few flights. Additionally, the combination of navigational factors unique to the different flight areas between the groups may contribute to which cues are preferentially used (Schiffner et al., 2013).

Idiosyncrasy

Despite the short distance of these flights, birds still show individually distinct paths. Calculation of a mean route based upon the final three successful flights allowed nearest-neighbour distances to be calculated against self and other tracks, giving a measure of similarity to a bird's own tracks and the tracks of other birds. Figure 5 shows that "self" distances were significantly lower than "other" distances, suggesting that individual birds fly routes which are more similar to their own other routes than those flown by other birds. Additionally, we observe an overall reduction of the short-term correlation dimension from the initial to the final releases, suggesting that with increased familiarity birds rely on fewer cues, supporting the formation of idiosyncratic routes based on visual cues (although reliance of visual cues cannot be directly tested without removing object vision, e.g. via the use of frosted lenses). This transition appears to be a rather gradual transition, rather than an abrupt switch between two modes of navigation with individuals switching back and forth between both - which would explain why this behaviour may sometimes to be hard to detect. However, splitting birds into the two age categories (Figure 5) shows that this difference is primarily due to the old birds, with young birds showing higher "self" distances. Only in old birds was the difference in nearest-neighbour distances significant between "self" and "other" tracks, suggesting that the formation of idiosyncratic routes may require more experience.

In previous studies where idiosyncratic routes were identified more experienced birds tended to be used, e.g. all birds older than two years (Biro et al., 2004; Meade et al., 2005; Meade et al., 2006; Biro et al., 2007), or four years (Biro et al., 2006). This suggests that greater flight experience may be an important factor in determining the use of visual cues associated with higher idiosyncrasy. It is possible that the one-year-old birds are more reliant on compass mechanisms to navigate, rather than using visual landmark information, or that younger less experienced birds display more exploratory behaviour. Clock-shift and magnetic treatments would be necessary to determine if compass mechanisms are being used; Biro et al found that clock-shifting birds which had learned a route resulted in an off-set track which paralleled the shape of the learned route, in the clock-shift direction (Biro et al., 2007). This demonstrates that both landmark and compass information is being combined.

Off-route releases

Following the twenty training releases from Y Felinheli, two off-route releases (Figure 1) were conducted to test whether birds would return to their learned route from a short distance away. Our results identify several different navigational strategies, the most obvious being birds flying a relatively direct route from the release site to home. This suggests use of a compass heading to navigate, and is seen from both release sites, across young and old individuals (Table 2). Both of the off-route release

sites are relatively close to the Y Felinheli site, and birds have been trained in the general area, so it is possible that the birds possess a familiar area map from which they can produce the correct headings. Another strategy seen is taking a parallel track to the learned route. This is a relatively common strategy, identifiable as tracks with virtual vanishing bearings which match between the learned and off-route releases. In these cases, birds fly a parallel track to their learned route, usually correcting towards home once they have flown the correct distance. As with the direct route, this strategy suggests that the birds are relying on a compass heading, but this time a learned heading from their repeated releases, which they then copy when released at a nearby site.

A few birds showed the looked-for route joining behaviour, but in contrast with previous studies (Biro et al., 2004; Meade et al., 2005; Meade et al., 2006) this was not a common strategy. Re-joining the learned route suggests that the birds are at least partially relying upon visual cues, specifically the memory of cues associated with the repeated route, as the sites are in close enough proximity to each other that, from an aerial view, several landscape features should be visible from all sites, e.g. the coastline. It is possible that the short distance of the flights meant that joining the learned route was not necessary, with many birds simply taking the direct path. For the first off-route release site, the presence of the Menai Strait as an obstacle may have blocked birds from joining the original route, with uncertainties as to where to cross the body of water.

A final strategy was seen which demonstrated landscape following: when released from the first off-route site on Anglesey, several birds fly along the coast, and correct their route when reaching a particular landmark. For example, four of the old birds fly along the Menai Strait (160, 504, 552, 588), with 160 correcting towards the loft when Menai Bridge is reached, and 504/552 correcting after reaching Llanfairpwllgwyngyll. The same can be seen in many of the young birds. Following of landscape features has been previously documented (Lipp et al., 2004; Mann et al., 2014), and suggests that predominant features may override other navigational cues. However, the following of the coastline may simply be a temporary guide to help maintain the correct heading, as it roughly agrees with necessary heading, meaning that multiple navigational cues are combined, which is supported by the comparatively higher short term correlation dimension observed in birds that are using this strategy.

From the second release site there is far less evidence of following the landscape, with many parallel routes, most predominant in the old birds. The use of parallel routes is reminiscent of (Biro et al., 2007), where clock-shifted birds flew in parallel to the learned route. The authors took this as evidence of combining compass and landmark cues when navigating, which is supported by the variety of strategies demonstrated by these birds. The lack of distracting landscape features from the second

off-route release site may be responsible for the reduced landmark following and predominance of parallel/direct flights.

Most birds did not show a fixed strategy at both of the off-route release sites, suggesting a flexibility of navigational strategy, responding to the particular local conditions of an unfamiliar release site. Many birds which showed a preference for landscape following from the first off-route release site flew either a direct or parallel route from the second site, demonstrating that both compass and visual landmark mechanisms could be used.

The correlation dimension analysis of the off-route releases can allow us to draw some tentative conclusions concerning the number of navigational factors being used across the course of a flight. On the basis that the correlation dimension is a representation of the kind of information being used in navigation, with a lower correlation dimension suggesting the use of point information such as individual landmarks, and a higher correlation dimension suggesting the use of a multi-factorial system, such as a mosaic map, with both map and compass components (Schiffner et al., 2011).

The coast following behaviour had the highest correlation dimension (2.5), suggesting that the birds following the coast were using the coastline as an additional cue instead of relying on it alone, possibly as an addition to compass-based information. The direct flights had a slightly lower correlation dimension (DIR: 2.0) and the parallel flights had the lowest correlation dimension (PAR: 1.5), with such a low correlation dimension suggesting reliance on the lowest number of factors. Given the idiosyncratic nature of the training routes, it is likely that an entrained compass heading may be responsible for the majority of the navigational information, with other factors being used more sparingly during the flight. From figure 6 we can see that the correlation dimension is not stable across the course of a flight, however, suggesting that the number of cues used may vary significantly across the course of a flight. Although this results in a single visually classified strategy, the actual information informing the flight path may be highly variable.

This experiment was designed to test whether there was a difference in idiosyncratic route-learning behaviour and subsequent off-route release strategies between experienced and inexperienced birds. In particular, it compared two extremes of first-year birds with no previous flight experience prior to the season of the experiment, with 9- to 10-year-old birds with a high variety of experience in the local area. This large contrast between the two groups revealed significant differences in initial flight efficiency and idiosyncratic flight paths, but it does not give any indication of at what level of experience these differences start to appear. To further investigate this, it would be necessary to use experimental groups with smaller age/experience intervals between them. Additionally, an experiment designed to separate the effects of age and experience on navigation would give more

information on whether general age-associated processes or specific navigational experience is responsible for differences in efficiency and strategy. Previous work has found that navigational experience is associated with a relative increase in the size of the hippocampal formation (Cnotka et al., 2008; Mehlhorn et al., 2010). However, unlike in mammals, where the hippocampus shrinks in older individuals and is associated with memory issues, age-related memory decline is not associated with a decrease in size of the avian hippocampal formation, with older birds tending to have a larger hippocampal formation than younger birds (Coppola et al., 2016). Despite this, it has been demonstrated that older pigeons do show a decline in spatial memory (Coppola et al., 2015), so it is possible that the hippocampal formation may lose its ability to make new connections. Therefore, an investigation into the navigational abilities of experience pigeons but at entirely unfamiliar locations may reveal more about the relationship between age and experience in navigation.

Conclusion

This study has demonstrated that pigeons can learn idiosyncratic routes when flying repeatedly from a nearby location, but this occurs more readily in birds with greater experience. These idiosyncratic routes were characterised by a low correlation dimension supporting the formation of idiosyncrasies in the routes, that most likely rely primarily or even exclusively on visual cues. We found a general increase in flight efficiency over the first few flights, although this was generally restricted to the young birds, which began with much more inefficient routes. Off-route releases demonstrated a variety of navigational strategies characterised by distinct differences in the short-term correlation dimension and therefore the number of cues involved in the underlying navigational process, although few birds returned to the learned route. Instead, birds used either additional landscape features (in this case the curve of the coastline) or compass directions (either a direct route home or paralleling the learned route) to navigate from the unfamiliar sites. These results suggest that multiple forms of navigational information are important when homing in a familiar area, and that the strategy used is flexible, depending on the particular local conditions.

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Chapter 4

Using small values of clock-shift to investigate how homing pigeons integrate navigational cues when homing in the familiar area.

Abstract

Studies of familiar area navigation in homing pigeons have revealed the importance of several cues, including solar, magnetic, and local landmark features, although there has been conflicting information concerning the relative use of each, and how they may be combined. Here, we used small values of clock-shift, one and three hours, to investigate whether we can observe a change in how much deflection occurs at different levels of cue conflict. Birds were trained repeatedly to fly from two distinct locations with differing availability of large landmark cues, and clock-shift flights took place at these locations. We found that all clock-shift flights showed deflection lower than would be expected from only sun-compass use, and that flights following three hours of clock-shift showed a lower percentage than those with one hour of shift. This effect was more pronounced at the release site with more salient visual cues. This suggests that the sun compass information may be downgraded in the integration process as the amount of conflict increases, and that this may occur more readily when other cues are more readily available to highlight the conflict, in this case visual landmark cues.

Introduction

The ability to navigate over long distances accurately is seen across many different species of birds. Flight means that large distances can be covered quickly and means that birds can have large territories to hunt or forage across. Additionally, birds often need to find the way home from an unfamiliar location, as birds are liable to being blown off-course by weather conditions, or potentially chased out of a familiar area by predators. Therefore, being able to navigate quickly and accurately is beneficial in nature (Wallraff, 2005). Navigational behaviour in birds has been studied for over 70 years, investigating the environmental cues used to inform navigation (Wiltschko and Wiltschko, 2009). Homing pigeons have been a key tool in these studies, with their reliable homing behaviour a useful and manipulable model for studying navigation (Wiltschko and Wiltschko, 2017). Homing pigeons can be trained to return reliably to a home loft, allowing experimenters to release them from known and novel sites and record their flight trajectory, either manually in the past, or using GPS loggers at present. This allows detailed analysis of flight paths, and therefore navigational behaviour, under a variety of conditions (Meade, 2005; Dell'Ariccia et al., 2009; Gagliardo et al., 2011).

The study of homing pigeon navigation has been based upon the map and compass theory (Kramer, 1953), which describes a theoretical process of navigation from an unfamiliar location in a two-step process, the first a "map" stage which identifies a theoretical home direction, and a second "compass" stage which finds that direction to produce a heading. Many different cues have been proposed to provided map or compass information, including olfactory cues (Papi, 1989; Ioalè et al., 2008; Gagliardo, 2013), magnetic cues (Wiltschko and Wiltschko, 1972; Beason, 2005; Beason and Wiltschko,

2015), and solar cues (Keeton and Gobert, 1970; Schmidt-Koenig et al., 1991; Guilford and Taylor, 2014). Inclination of the Earth's magnetic field can be used to provide a magnetic compass, interpreted as either poleward or equatorward, thought to be the only innate navigational sense (Wiltschko and Wiltschko, 2009). During an early sensitive period of development, it is thought that an association is made between the magnetic compass and the position of the sun in the sky (azimuth) to produce the sun compass, which is then used in preference during navigation (Wiltschko et al., 1981; Wiltschko and Wiltschko, 1981). The sun compass is time-compensated, and therefore relies on the circadian rhythm of the bird (Armstrong et al., 2013).

Map and compass navigation describes the process of true navigation – navigating from an unfamiliar location to a known location, but navigation within a known region may provide alternative methods – the use of familiar area cues (Braithwaite and Guilford, 1991; Biro et al., 2002; Holland, 2003). It is possible that birds familiar with a local area may be able to use familiar area landmarks as directional cues, either in combination with compass cues such as magnetic or solar (a "mosaic map"), or solely in a method known as "pilotage", where a series of landmarks are followed in the home direction (Füller et al., 1983; Holland, 2003; Biro et al., 2004). Despite years of research investigating which cues are used in a variety of situations, there is conflicting information about how the cues combine, if certain cues are used in preference to others, and how different situations may influence cue integration.

In order to investigate the use of different cues, cues are set in conflict with each other by a method known as clock-shift; altering the circadian rhythm of the homing pigeons using a shifted light-dark cycle. Typically, this produces a predictable deflection in homing direction, as a result of mis-reading the sun compass (Schmidt-Koenig, 1960; Alexander and Keeton, 1974; Guilford and Taylor, 2014). A 6-hour clock-shift would be expected to produce a 90° deflection from the home direction, based on sun-compass navigation alone. Clock-shifting has traditionally been used to demonstrate the use of the sun-compass during navigation, but over the last twenty years, questions have been raised over the interpretation of clock-shift results.

Although we can make predictions concerning how much deflection should be observed under a certain amount of clock-shift, a full amount of shift is rarely seen (Wiltschko et al., 1994; Chappell, 1997; Gagliardo, 2005). This has been attributed to three possible reasons: a) vanishing-bearing data being averaged across individuals producing a group average which hides individual homing strategies; b) incorrect calculations of expected shift, failing to take into account the effect of time of year and time of day on the rate of change of the sun azimuth; and c) the use of other environmental cues in

combination with sun-compass information to inform the flight direction, which reduces the effect of clock-shift on the deflection seen.

The first of these options, the averaging of vanishing bearing data, is primarily seen in older studies where only vanishing-bearing data have been available for analysis: one data point per bird of the compass direction observed as the bird disappeared from view. The calculation of group mean vanishing bearings can hide individual strategies, where some birds may show greater deflection than others. However, the advent of tracking technology means that much more data are available from individual flights (potentially hundreds-thousands of data points per flight), so individual flight characteristics can be analysed.

The second option, incorrect calculations of expected clock-shift, still remains contentious, as it is unclear whether or not birds consider the hourly/daily variations in azimuth change to inform their sun-compass, or if they use an average rate of change (the 15° per hour assumed in clock-shift calculations). Some evidence suggests that birds are able to compensate for the different rates of change in azimuth across the day (Wiltschko et al., 2000), although this has not been widely repeated.

The final consideration, impact of combining other environmental cues, has been widely studied with conflicting results. In theory, if full deflection under clock-shift is seen when the magnetic compass is no longer available, then the reduction in deflection under clock-shift observed under normal conditions when the magnetic compass is available suggests that the magnetic compass heading is being integrated with the sun compass heading, giving an intermediate direction between the two conflicting cues. Several studies have demonstrated an increase in deflection under clock-shift when the magnetic compass is disrupted using small magnets attached to the head of the bird, suggesting that the magnetic compass would usually be integrated with sun-compass information during navigation (Wiltschko and Wiltschko, 2001; Wiltschko and Wiltschko, 2007; Gagliardo et al., 2009). However, others have failed to show a significant effect of magnetic interference (Ioalè et al., 2006), and the possible integration of landmark cues when homing in a familiar area has been raised (Bonadona et al., 2000; Holland et al., 2000; Gagliardo, 2005; Biro et al., 2007; Mora et al., 2012; Gagliardo et al., 2020). The nature of cue integration in this situation has, however, gone largely unstudied.

This experiment was designed to investigate the nature of cue integration in homing pigeons when navigating in a familiar area, focussing on the impact of varied landmark features on deflection under clock-shift. When navigating in the familiar area, pigeons have multiple redundant directional cues available to them, from which a single directional estimate of the home direction must be produced. Each cue has an associated variance, so may be more or less reliable than others, which may vary

throughout time (e.g. sun compass is less reliable under cloudy conditions). Therefore, it is theoretically optimal to combine multiple cues to account for this variance. This problem of cue integration has been studied in humans in terms of sensorimotor processing, producing models for the theoretically optimum way in which noisy signals should be combined (Ernst and Banks, 2002; Battaglia et al., 2003). This approach will be applied here to study how homing pigeons tackle an analogous integration problem.

The standard practise for clock-shift experiments is to use a 6h shift, with a predicted deflection of 90°. A large clock-shift of this size was traditionally used to make sure that the deflection would be visible when recording vanishing bearings. However, advances in GPS technology mean that much smaller deflections should be detectable, allowing smaller clock-shifts of 1h and 3hrs may be detectible. When multiple cues can be used to inform a behavioural output, in this case the direction of flight towards the home loft, the optimal strategy is to combine the information to produce a maximum likelihood estimate (Ernst and Banks, 2002; Ernst, 2006; Knill, 2006). Each cue will be associated with a certain amount of variance; higher variance is proportional to lower reliability. Combining multiple redundant cues in an optimal fashion, whereby each cue is weighted in proportion to its reliability known as a weighted sum model, produces an overall estimate which has a higher reliability than any of the individual cue estimates (Trommershauser et al., 2011).

In order to investigate whether or not homing pigeons combine available cues in an optimal way, cue-conflict studies can be applied. Here, a small discrepancy is introduced in one of the available cues. Theoretically, when integrated with the other available cues, the discrepancy should skew the overall estimate in the direction of the introduced conflict, but the influence of the un-manipulated cues should mean that the resultant estimate is somewhere between the true value and the estimate given from the manipulated cue. The degree to which the manipulated cue is expected to affect the overall estimate is dependent on its relative reliability compared to the other cues in the integration process. One important prediction of a robust weighted sum model is that dynamic weighting will occur – if the reliability of a cue decreases, then its weighting will be reduced. However, if an extreme conflict is observed, our model of an optimal system should ignore this cue, as such a conflict is likely to represent an incorrect or mis-calibrated signal (Trommershauser et al., 2011).

In the case of clock-shift in homing pigeons, it is likely that studies using a 90° shift produce a conflict far too large to be resolved through cue integration. As sun compass information has generally found to be used in preference to other directional cues when available, large deflections observed under clock-shift may be a result of a break-down in cue integration, where the most historically reliable cue is chosen. In this experiment use of smaller amounts of clock-shift may reveal whether integration of

different cues can be seen. The two release sites differ in the availability of visual landmark cues, therefore it is predicted that birds trained from the site with more reliable landmark cues should show reduced deflection under clock-shift relative to the site lacking in these local cues. As well as this, if increasing conflict down-weights the reliability of the sun-compass, we would expect to see a smaller percentage of predicted deflection under the 3h vs the 1h clock-shift treatment.

Methods

Subjects and Training

Forty-five homing pigeons from the 2020 cohort and twenty-six from the 2019 cohort were housed in the Bangor University pigeon loft at Treborth botanic gardens for the duration of the experiment. Birds were fed 25g of mixed seeds per bird per day, with water and grit ad libitum. All of the birds were trained to be familiar with the local area, with flock releases from various locations around Bangor and on to Anglesey (figure 1). Following flock training, the 2019 birds were trained from a release site on Anglesey, just outside of Llangefni, and the 2020 birds were trained from a release site outside of Caernarfon. All birds received 6 individual training flights from the respective locations, with a weighted release to ensure that the birds would be able to carry the weight of the GPS trackers. A final un-manipulated release at each site with GPS tracking the birds produced a control track for each individual. The birds were tracked using i-gotU USB GPS Travel and Sports Loggers, recording at a frequency of 1Hz. Trackers were attached to the birds via a Velcro strip glued to trimmed feathers between the wings on the back of the bird.

The experimental release sites were chosen for their difference in proximity to salient landmark cues; the Llangefni site has a clear view of the Snowdonia mountain-range which lies behind the target location, and the Menai Strait site sits such that flying directly along the Menai Strait will lead home.

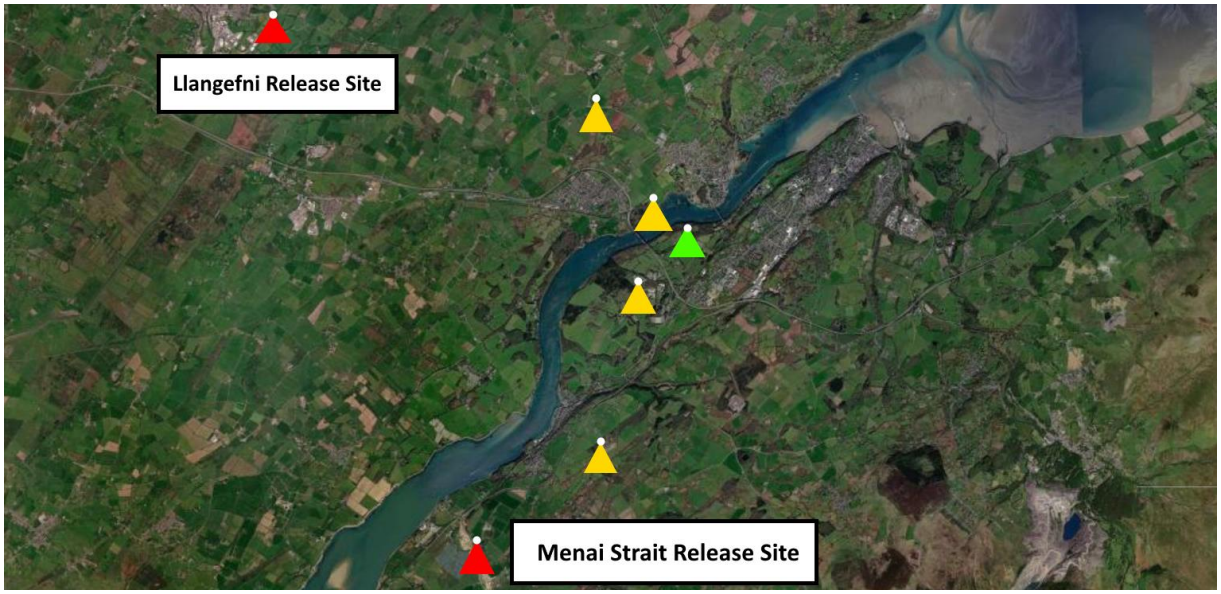

Figure 1: Training and test release sites across Gwynedd and Anglesey (Google Earth). The green triangle marks the pigeon loft at Treborth Botanic gardens (53.217, -4.173), the yellow triangles intermediate flock release sites, and the red pins the experimental release sites (Menai Strait site: 53.164, -4.234; Llangefni: 53.254, -4.295).

Experimental releases

Experimental releases involved clock-shifting the birds either one or three hours. 2019 and 2020 birds were shifted on separate occasions. Within each cohort, the birds were split at random into two groups corresponding to the clock-shift conditions. For clock-shift, birds were housed in a light-proofed shed, with artificial lighting shifted such that artificial sun rise occurred either one or three hours later than actual sunrise. Birds were kept in clock-shift for four days, allowing more than one day per hour of shift. Feeding and cleaning occurred during the artificial daylight hours only. As usual, birds received 25g of food per bird per day, with water and grit ad libitum. Clock-shift releases took place on 02/07/2019, 08/07/2019, 14/09/2020, 27/09/2020 and 01/10/2020. Clock-shift releases took place in the early afternoon under sunny conditions, with low wind (<7m/s). Birds were released singly with the GPS loggers attached and followed with binoculars until they had left the vicinity of the release site, before releasing the next bird to ensure that birds did not fly in pairs. Releases alternated between the two clock-shift conditions, with at least 10 minutes between the release of sequential individuals. Upon their return to the loft, trackers were removed from the birds and the data downloaded.

Data Analysis

GPS tracks were downloaded using @trip PC and exported as GPX files. All data were analysed using RStudio. Tracks were first filtered using a low-pass Butterworth filter, with a cut-off at half the frequency of data collection (1Hz). The filter was applied to the latitude and longitude measurements.

These were then used to calculate a variety of distance and directional values, including the distance from the release site/home loft at each point along the track, distance between consecutive points, cumulative distance flown, turn angle between consecutive points, continuous flight bearings and instantaneous deflection. Continuous flight bearings are calculated as the absolute bearing of a point from the release site, and instantaneous deflection is calculated for each point as the difference between the current track direction and the home direction. Instantaneous deflection can be used as a measure of deflection under clock-shift, as it shows the difference in trajectory at any given point from the intended home direction.

Following the calculation of these measures, the tracks were cut to remove circling behaviour at the release site and around the loft. In order to determine when the bird had left the release site, a radius of 500m was used to draw a circle around the release site, determined from a range of tested distances to be the best balance between removing circling and maintaining as much "navigational" flight as possible, across the different recorded tracks. Once the bird had crossed the circumference of this circle for the final time, it was deemed to have left the release site, and the track was cut to this point. For home circling, a 200m radius around the loft was chosen, and the track cut once the bird had come within this distance of the loft (Griffiths et al., 2021). After these calculations were performed, the tracks were plotted to identify any in which the loggers had failed to properly record, and any such tracks removed. Efficiency was calculated for each track, as the shortest possible distance divided by the total distance flown. Efficiency was compared between treatments for each location using the Kruskal-Wallis test and between locations using the Wilcoxon test.

In order to analyse how response to clock-shift changed over the course of the track at a finer level, instantaneous deflection was found for each bird, at 500m intervals along the track (direct distance along the track from the release site, not the distance travelled by an individual bird). This was then averaged within the experimental groups for each 500m measurement and plotted against distance from the release site. These measures were then taken in relation to the control track (instantaneous deflection of the clock-shift tracks - control instantaneous deflection for each 500m point), and then calculated as percentage of expected instantaneous deflection (based on individually calculated values for each flight, using the time and date of release and the rate of sun movement). A linear mixed model (R package lme4) was produced for this data (instantaneous deflection at 500m intervals), to see which factors influence the instantaneous deflections. A variety of models were produced for the whole dataset and the individual locations to see the estimated effects of distance and the predicted sun-compass direction on instantaneous deflection.

In order to compare the observed deflection of each bird to the value we would expect if sun compass information alone was used, the change in sun azimuth between the actual azimuth and the azimuth at the clock-shift time was calculated for the time of release of each individual. For each 500m instantaneous deflection calculated for the clock-shift tracks, the mean control instantaneous deflection for that distance was removed to find the deflection relative to controls. From this, the percentage of sun-compass direction is calculated using the calculated azimuth change. Linear mixed models were also produced for percentage of sun-compass direction.

The linear mixed models were analysed for their normality, homoscedasticity and for the presence of autocorrelation. Both instantaneous deflection and percentage sun compass direction models were normally distributed. However, both suffered from heteroscedasticity and positive autocorrelation. Both of these problems were solved by taking a random sample from the data, of around 50% of the data points, reducing autocorrelation to within the 1.8-2.1 using the Durbin-Watson test. This sampling process was repeated 10000 times, and the effect estimates and p-values averaged from these repeated results. Effect estimates were found by calculating the mean and standard deviation, as the distribution of results from the repeated trials were symmetrical. However, p-value averages were calculated using the median, as distributions were skewed.

Results

Following initial processing of the data, 120 useable tracks were available for analysis, split into control and clock-shift tracks for each cohort of birds (table 1, figure 2). The 2020 cohort was larger than the 2019 group, so more tracks were available from the Menai Strait release site. Clock-shift releases also have fewer successful tracks, as releases could not be repeated if the logger failed to record.

Table 1: Number of tracks analysed for each of the homing pigeon cohorts, for each experimental treatment.

Bird cohort	Control tracks	+1h clock-shift	+3h clock-shift	Total tracks
2019	35	15	14	64
2020	44	18	18	80

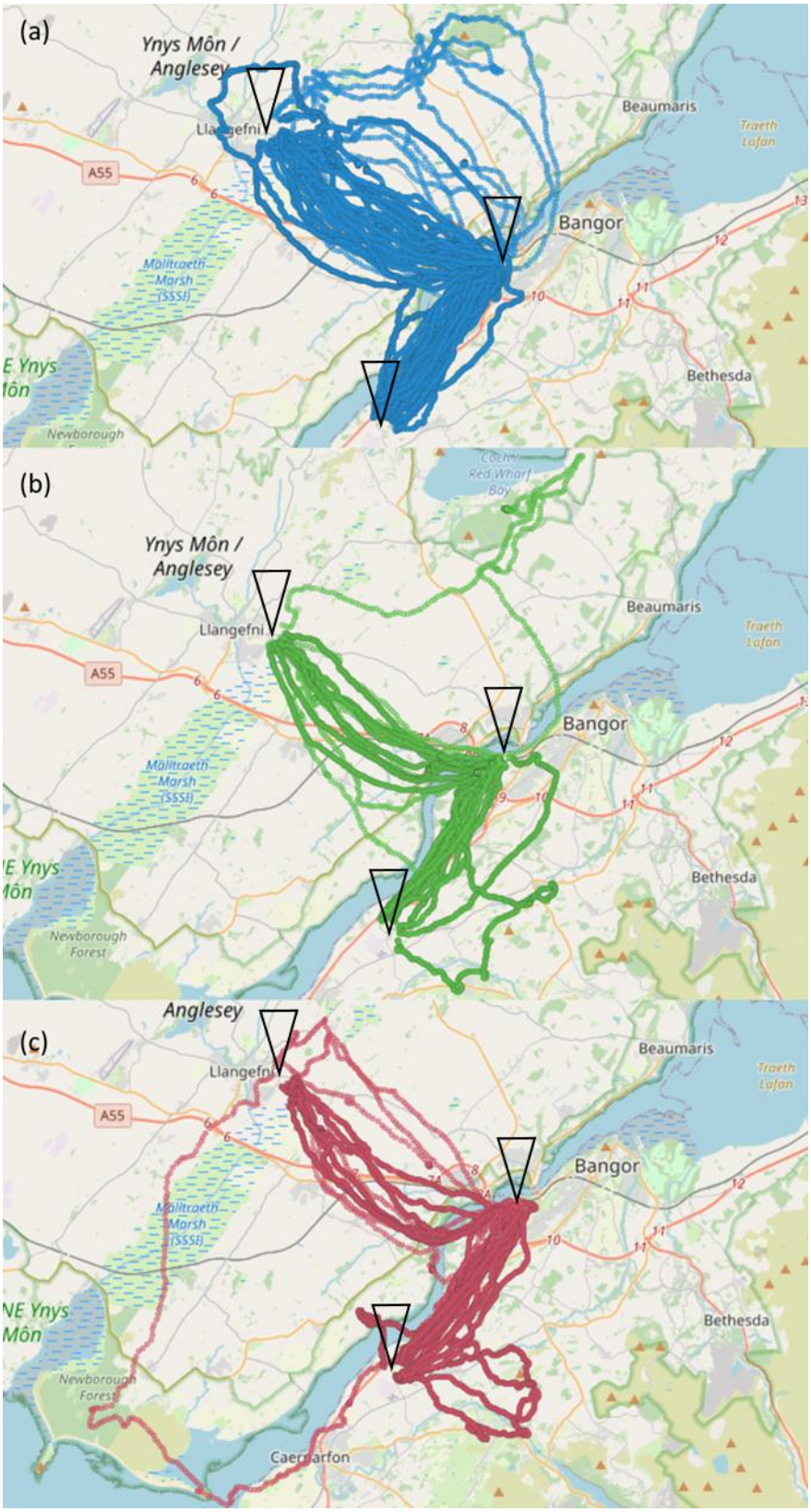

Figure 2: All pigeon flight track recordings used in analysis. (a) blue routes are control flights, (b) green tracks the +1h clock-shift releases, and (c) red the +3h clock-shift releases. For each map, the top left triangle is the Llangefni release site, bottom left is the Menai Strait release site, and the right triangle is the home loft at Treborth. Points are translucent so areas of higher point density appear to be more saturated.

Following track processing to remove non-navigational circling behaviour, flight efficiency was calculated for each individual track. Efficiencies were significantly different between release sites (Wilcoxon rank-sum test: W = 1062, p = 1.80×10^{-8}), but there was no significant effect of treatment on flight efficiency at either the Menai Strait site (Kruskal-Wallis test: $\chi^2_2 = 2.52$, p = 0.283) or the Llangefni site (Kruskal-Wallis test: $\chi^2_2 = 21.8$, p = 0.403) (figure 3).

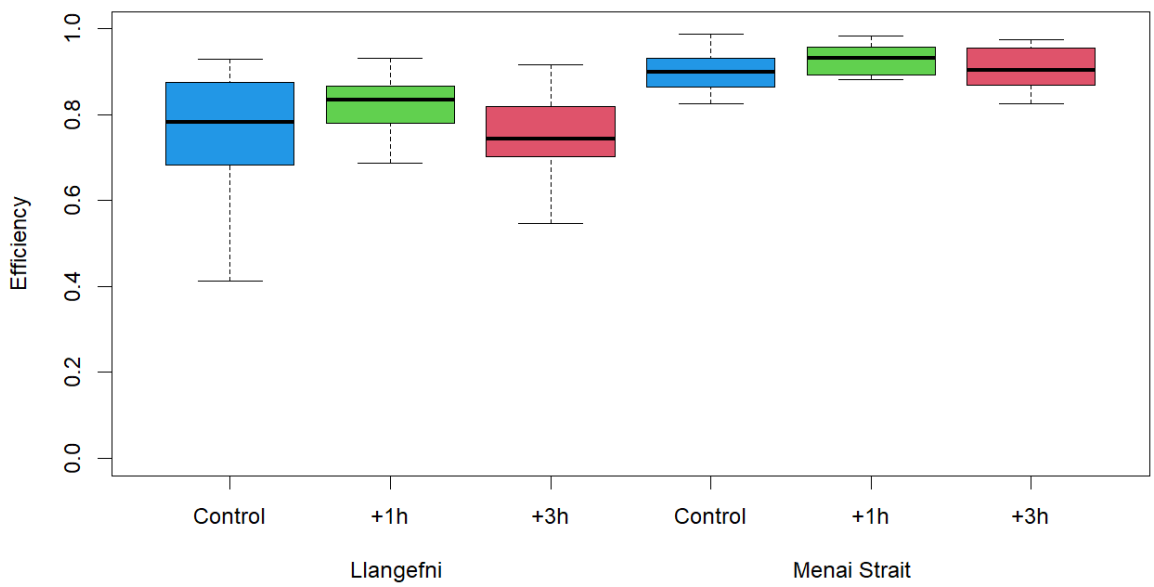

Figure 3: Box plot of efficiency calculated for each of the tracks, grouped by treatment and release location. Efficiency is calculated as the shortest distance between the start and end of the track divided by the total distance travelled, and is measured between 0 and 1, with 1 being a maximally efficient route. Sample sizes for each group shown in table 1. Centre line shows the median, box edges the upper and lower quartiles, and the whiskers the range.

In order to establish the effect of clock-shift over the course of the track, instantaneous deflection for each bird every 500m was plotted against distance from the release site (figure 4). As the control tracks do not lie along an instantaneous deflection of 0, the average control instantaneous deflection at each 500m point was subtracted from the clock-shift instantaneous deflections for that distance for each individual bird, then scaled as a percentage of the of sun-compass direction as calculated using sun azimuth data (figure 5). Full deflection of 100% would be expected if only sun compass information was being used. However, accounting for integration with other cues giving non-manipulated directional information, we would actually expect the percentage to be lower than 100.

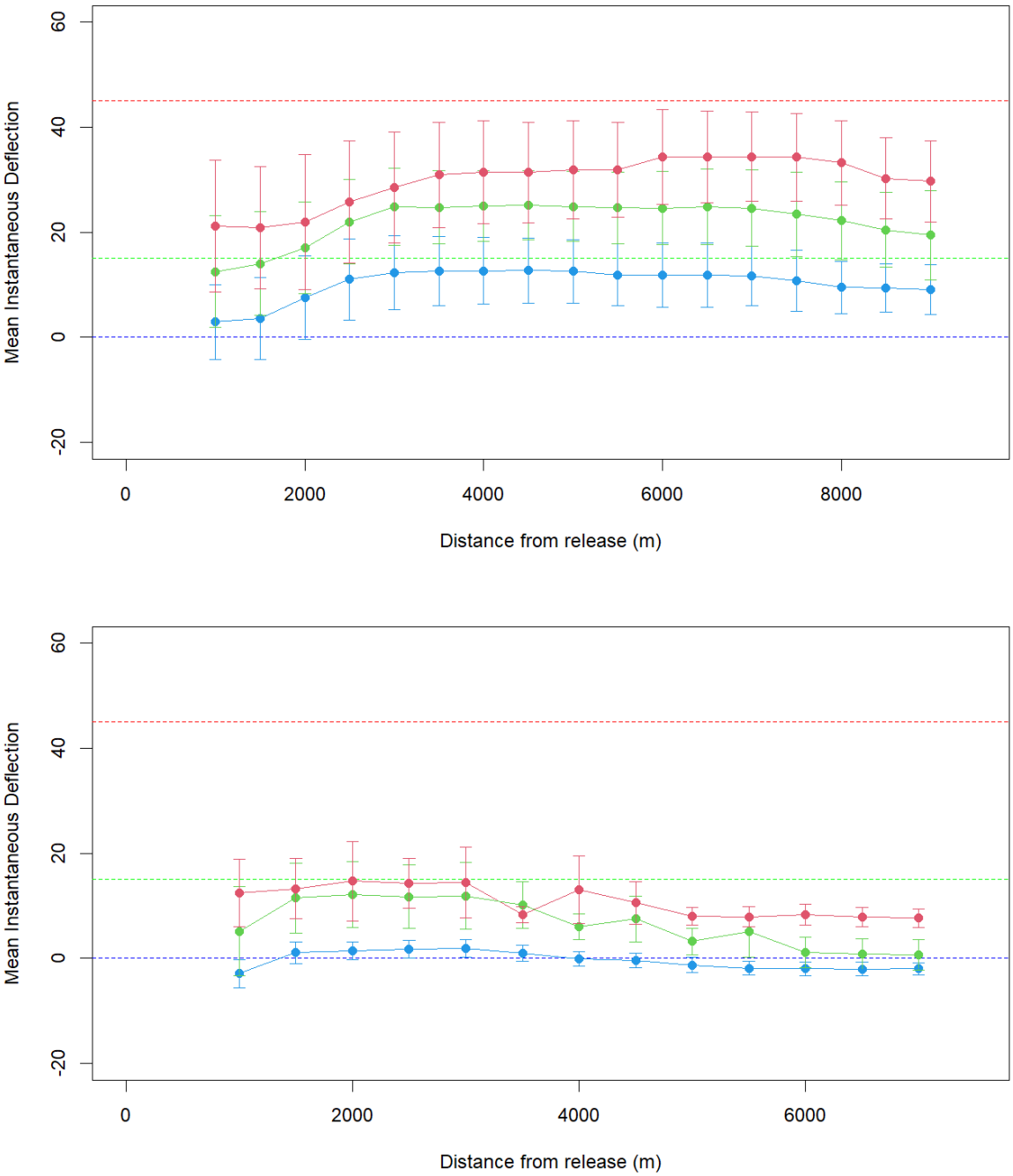

Figure 4: plots of mean instantaneous deflection calculated at 500m intervals for each flight, for the birds flown from Llangefni (top) and the Menai Strait site (bottom). Blue shows the control flights, green the +1h clock-shift, and red the +3h clock-shift. Coloured lines at 0, 15 and 45 degrees show the expected deflections for each of these treatments assuming only sun-compass information is used. Negative numbers show deflection to the left of the home direction, positive numbers to the right. Error bars show the standard error for each point.

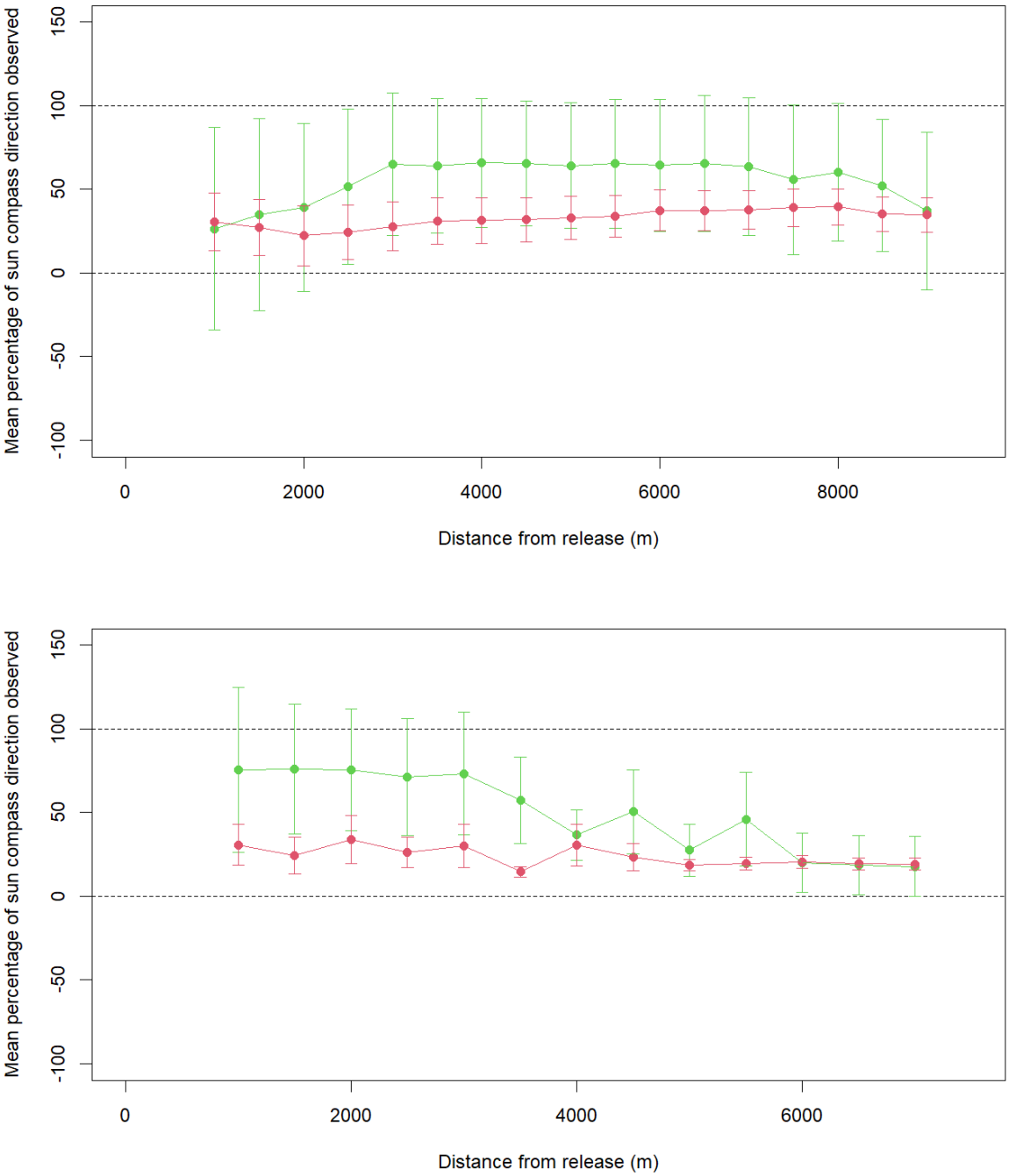

Figure 5: Percentage of sun-compass direction plotted over distance from release site for the clock-shift releases at the Llangefni (top) and Menai Strait (bottom) release sites. The mean percentage of sun-compass direction is calculated by first calculating the difference between the clock-shift track instantaneous deflection and the mean control instantaneous deflection (from figure 4) at each 500m interval, then finding what percentage this is of the sun compass direction as calculated from the change in azimuth. Green points are the +1h clock-shift treatment, and red points the +3h clock-shift. Error bars show the standard error for each mean.

Linear mixed models were produced to analyse how factors including distance from the release site and amount of clock shift affect instantaneous deflection. Models were produced for each location (Menai Strait and Llangefni), with distance in km and predicted sun compass direction as continuous fixed effects, track as a random effect (intercept only) and instantaneous deflection as the response variable. Table 2 shows the analysis of the fixed effects included in this model. There was a significant effect of shift on instantaneous deflection at the Menai Strait site, but a non-significant effect at the Llangefni site. At the Menai Strait site, instantaneous deflection increased by 0.238 degrees per degree of predicted deflection, based on the sun compass direction ($p = 8.70 \times 10^{-4}$). Distance had a significant effect on instantaneous deflection at the Menai Strait site (effect estimate of -1.15 degrees per km, $p = 3.11 \times 10^{-6}$) , but not Llangefni.

Additionally, mean instantaneous deflection per flight was plotted against the calculated sun compass direction, to see the direct relationship between the predicted deflection and actual deflection seen (figure 6) A linear model of this data found a significant effect of predicted deflection at the Menai Strait site (effect estimate of 0.238, $F_{1,78} = 12.15$, $p = 8.09 \times 10^{-4}$), with a weakly non-significant effect at the Llangefni site (effect estimate = 0.283, $F_{1,67} = 3.023$, $p = 0.0867$).

Table 2: Model fixed effects, showing the estimated effect of each variable on instantaneous deflection, and the p-value, as averaged from 10000 iterations of the linear mixed models, run on a random selection of 50% of the total data set. All results given to three significant figures. The average for the estimate is the mean, as data is symmetrically distributed, but median for the p-values, as these have a skewed distribution. Standard deviation is shown for the means, and inter-quartile range for the medians.

	Effect Estimate (mean ± sd)	**p-value (median, IQR)**
Menai Strait		
Predicted sun compass direction (degrees)	0.238 ± 0.0199	8.70×10^{-4}; 1.10×10^{-3}
Distance (km)	-1.15 ± 0.207	3.11×10^{-6}; 2.78×10^{-5}
Llangefni		
Predicted sun compass direction (degrees)	0.285 ± 0.0160	0.0814; 0.0315
Distance (km)	0.456 ± 0.232	0.0773; 0.194

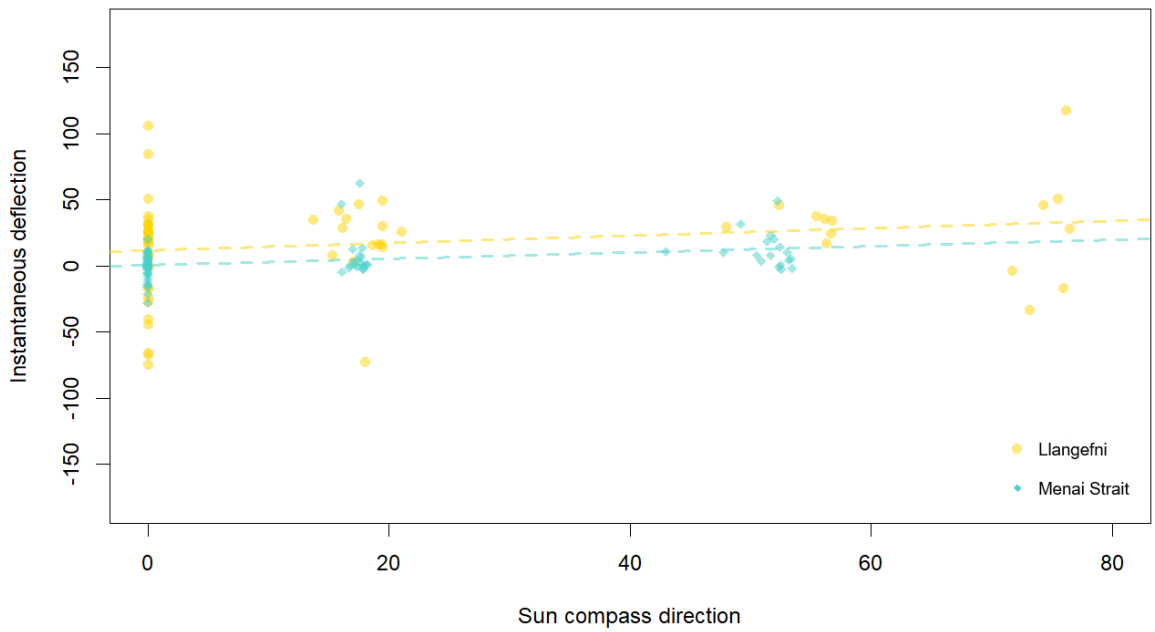

Figure 6: Scatter plot showing the mean instantaneous deflection per flight against the calculated sun compass direction, based on the date and time of release for each treatment. Yellow circles show the birds released from Llangefni, and blue diamonds the Menai Strait site. Dashed lines show linear models for each.

Models were also produced to look for predictors of percentage of sun-compass direction (figure 5), for each location. Table 3 shows the estimates of the fixed effects included in these models. There is a significant effect of predicted sun compass direction on percentage of sun compass direction at the Menai Strait site, with an average of 1.86 decrease in percentage of expected deflection per degree of predicted deflection ($p = 0.0372$). There is also a significant effect of distance, with a 12.9 decrease in the percentage of sun compass direction per km ($p = 1.04 \times 10^{-3}$), and a significant interaction between shift and distance ($p = 0.0147$). At the Llangefni release site, none of the fixed effects had statistically significant effects on percentage of expected deflection.

Table 3: Fixed effects estimates and p-values for the linear mixed models of percentage of sun-compass direction at either the Menai Strait (n = 504, grouped by 36 tracks) or Llangefni (n = 515, grouped by 27 tracks) release sites, shown to three significant figures. Each is calculated as an average from the 10000 iterations of the linear mixed models, each taking a random 50% sample of the data. The average for the estimate is the mean, as data is symmetrically distributed, but median for the p-values, as these have a skewed distribution. Standard deviation is shown for the means, and inter-quartile range for the medians.

	Effect Estimate (mean ± sd)	**p-value (median, IQR)**
Menai Strait		
Sun compass direction (degrees)	-1.86 ± 0.483	0.0327; 0.0497
Distance (km)	-12.9 ± 3.27	1.04×10^{-3}; 4.06×10^{-3}
Interaction	0.266 ± 0.0950	0.0147; 0.0471
Llangefni		
Sun compass direction (degrees)	-0.592 ± 0.206	0.527; 0.197
Distance (km)	1.24 ± 2.19	0.337; 0.538
Interaction	4.28×10^{-3} ± 0.0353	0.356; 0.482

Discussion

This experiment was designed to test the response of birds to small amounts of clock shift at two different familiar release sites with differing availability of salient navigational cues, in order to see whether cue integration changes with the size of conflict between available cues. When using instantaneous deflection values to measure amount of deflection under clock-shift, we do not see instantaneous deflection values which match the predicted deflection, based on the sun compass indicated direction at the time and date of each release for our clock-shift treatments (figure 6). In fact, we see only a 0.238 degree increase in instantaneous deflection per degree of predicted deflection at the Menai Strait site, and 0.285 at the Llangefni site. This is in agreement with many past experiments (although these are usually 6h shifts), where the deviation seen under clock-shift is lower than the predicted deviation based solely on the sun compass (Wiltschko et al., 1994; Chappell, 1997). This supports the theory that the birds are using other cues which are in conflict with the sun compass, producing a heading which is a compromise between opposing directional information. The position of the overall directional estimate between the conflicting cues gives us information about how heavily the cues are weighted in the integration process.

We see a different effect of both distance and predicted sun compass direction between our two release sites. The effect of location is significant as it suggests a potential influence of local landscape features on navigation; the Llangefni release site had no parallel landscape features leading towards the loft, but the clear line of the Snowdonia mountain-range behind the loft to fly towards as a target. In contrast, the direct route home from the Menai Strait release site runs parallel to the Menai Strait (see figure 1), which could act as a prominent visual landmark by which the birds could navigate. The lack of such a proximal landmark at the Llangefni site could mean that the sun compass is more heavily relied upon, giving the larger amounts of deviation under clock shift that we observe. Some previous studies have found possible effects of local landscape features under clock-shift, with differing responses to clock-shift at different locations (Bonadona et al., 2000; Filannino et al., 2014; Gagliardo et al., 2020), as well as the influence of discrete landmarks, with pigeons responding differently to clock-shift at sites chosen to have specific landmark availability (Mora et al., 2012). Our results also suggest that there may be significant effects of location on navigational strategy, suggesting the importance of local landmark features.

In order to account for the variation in the rate of azimuth change throughout the day, we calculated percentage of sun-compass direction (figure 5) and fit a linear mixed model for each of the release sites (table 3). Here we see a significant effect of distance at the Menai Strait site, not Llangefni, with the percentage of sun-compass direction decreasing with distance from the release site by 12.9 degrees per kilometre. The effect is much larger at the Menai Strait site than at the Llangefni site, suggesting that the conflict with the sun compass caused by clock-shift was compensated for more quickly at this location. Previous studies have found varying effects of distance on how birds respond to clock-shift, with evidence of clock-shift within sight of the loft in one instance (Armstrong et al., 2013), although not in another (Graue, 1963, although it should be noted that this experiment used vanishing bearings, with the loft only 0.6-0.8km away, so may not be a reliable measure), and some with both (Holland et al., 2000). Site-specific variation in how deflection changes over distance (Filannino et al., 2014), or a more gradual decrease in deflection over distance (Gagliardo et al., 2009) have also been found. This variation suggests that multiple factors may be involved in how quickly the error in reading the sun compass is compensated for, with the possibility of an influence of familiar landscape cues.

There is a significant effect of the predicted sun-compass direction at the Menai Strait site, with a -1.86 decrease in percentage of expected deflection per degree of predicted sun compass direction. This suggests that the larger conflict produced by the greater amount of shift may be causing the sun-compass to be downgraded in the integration process. In comparison to this, we do not a see a significant effect of predicted sun compass direction at the Llangefni site. Both the 1h and 3h groups

show much less that 100% of the sun-compass direction, but no significant difference between the two, (figure 5). It is possible that here, due to the lack of a proximal landmark cue, that visual cues may be weighted less highly in the integration process, so that the sun compass information has more impact on the overall directional estimate.

There is a significant interaction between distance and predicted sun compass direction at the Menai Strait site, but not at the Llangefni site. A significant interaction term suggests that the response to the altered sun compass direction changes with distance from the release site. From figure 5b we can see that the 1h group shows a decrease in percentage of sun-compass direction as distance from the release site increases, suggesting a decrease in reliance on the sun-compass. However, there is little change in the 3h group as distance increases, likely because the percentage of sun-compass direction starts so low.

When considering the theory of optimal integration, we would predict that greater conflicts between the cues would cause a reduction in the amount of integration seen, as the discrepant cue has its weighting reduced in the integration process (Trommershauser et al., 2011). The results of the percentage of expected deflection at the Menai Strait site support this prediction, suggesting that it is possible homing pigeons may be integrating cues in an optimal manner. However, since many previous clock-shift experiments with much larger shifts (generally 90°) do not find a substantially lower percentage of expected deflection than our three-hour shift, there needs to be questions asked about the factors which are uncontrolled across these experiments. Namely, the one factor impossible to control in field releases are the landscape features. Visual landmarks cannot be manipulated experimentally, nor can they be controlled for between experimental release sites, or between the locations of different research groups. The lack of ability to control this variable, and consequentially its varied nature across release locations means that the weighting of this cue in the integration process may be very different across different locations. This means that it is impossible to make predictions about how cues will be integrated with this unknown, but the framework outlined by optimal integration theory does allow us to explain discrepancies between locations.

In theory, an optimal observer would base the weighting of various cues in the integration process based upon their instantaneous reliability – if one cue has a higher variance at any given point, it has a lower reliability, and therefore will be downgraded (Ernst, 2006; Trommershauser et al., 2011). However, in a robust estimation scenario, a very large conflict between cues should be resolved by rejecting the cue which is historically most likely to be incorrect. Therefore, if a bird has been clock-shifted multiple times in the recent past, it is possible that the sun compass may be deemed less reliable than other cues, and also be downgraded, especially when navigating in the familiar area

where other navigational cues are more abundant. A previous experiment raising pigeons under clock-shifted conditions but with a view of the sun suggested that such dynamic down-grading or up-grading of cues may occur based on experience; birds raised under shifted conditions learned an incorrect time compensation, and once moved to normal conditions showed the effect of shift on subsequent releases. After multiple flights, deflection was no longer present in the flight paths, and clock-shift treatment months later also did not produce deflection, suggesting that the sun was no longer being used in the navigational process (Wiltschko et al., 1976). Therefore, past experiences of birds may be very important to consider when interpreting analysis of clock-shift experiments.

In order to further investigate the possibility of optimal integration, trialling more values of clock-shift (e.g. 0-6hrs at 30-minute intervals) to produce a response curve could see how the integration process copes with increasing amounts of cue conflict, and if there is a point at which the integration process breaks down and a single cue is selected. It would be important in future work to account for a number of variables, especially familiarity with the release site, and previous clock-shift experience. Given the variability of homing flights over natural terrain and the difficulty in controlling cue access during homing, an approach including controlled arena experiments may be beneficial in investigating cue integration in homing pigeons.

Conclusion

This experiment demonstrates that deviation under clock-shift can be detected with clock-shifts as small as 1 and 3 hours, and that even at these small amounts, the amount of deviation seen is still less than 100% of what would be expected if relying solely on the sun compass. Differing responses to clock-shift at our two familiar release sites suggests an effect of the local landscape features on navigation, and the differing rates of reduction in deviation over distance at the two sites supports this. The reduction in the percentage of expected deflection in the 3h shift group vs the 1h shift group at the Menai Strait site may support the theory of optimal integration of navigational cues.

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Chapter 5

Investigating the factors which reduce the deflection seen in flights of clock-shifted homing pigeons navigating in a familiar area.

Abstract

Homing pigeons treated with clock-shift to introduce a predictable error in the time-compensated solar compass often show deflection lower than would be expected based on sun compass navigation alone. Previous studies have highlighted the importance of other navigational factors which may be integrated with the sun compass to reduce deflection, especially the magnetic compass. Here, we test the use of the magnetic compass when birds are subjected to small values of clock-shift, by the attachment of magnets to the head during homing flights. Additionally, to ensure that the full shift was being applied to the birds as expected, accelerometers were used to measure activity during clock-shift treatment, measuring activity levels as a proxy for circadian rhythm. We found that activity data matched the applied shift, suggesting that birds were indeed clock-shifted to the intended degree. There was no significant difference in percentage of expected deflection following clock shift between birds with and without access to the magnetic compass, although percentage of expected deflection was still less than 100%, suggesting integration with a different navigational factor may be responsible.

Introduction

The ability to navigate across a home territory is seen in many species across the animal kingdom and has been extensively studied in birds (Wallraff, 2005). The ability to fly means that birds can cover large distances relatively quickly, often foraging significant distances from their nest or roost. Birds which can bring food back to a nest more quickly can perform more foraging trips, increasing the chances of survival for their offspring. Therefore, natural selection favours those with higher accuracy when navigating.

Homing pigeons (*Columba livia*) are known for their ability to home accurately over significant distances, and decades of research into the mechanisms behind their navigation has uncovered the importance of multiple environmental cues. The paradigm of Kramer's "Map and Compass" theory (Kramer, 1953) describes navigation as a two-step process, whereby a map is used to identify the direction of the target, and a compass is used to find that direction in space. This is still used as the basis of our understanding of navigation today, with a variety of environmental cues able to provide map or compass information.

When navigating from an unfamiliar area, both map and compass cues are required, with olfactory cues thought to be the primary source of map information (Wallraff, 2004; Ioalè et al., 2008; Gagliardo, 2013). Compass cues are available in the form of both the time-compensated sun compass, based on the sun's azimuth (Schmidt-Koenig, 1990; Wiltschko and Wiltschko, 2009), and the magnetic compass

(Keeton, 1971; Wiltschko and Wiltschko, 1972; Beason, 2005). It is thought that the magnetic sense is innate, and that birds learn to use the sun compass via calibration with the magnetic compass (Wiltschko and Wiltschko, 1981); the sun compass is then used in preference when available (i.e. magnetic compass takes over in overcast conditions when the sun compass is unavailable) (Wiltschko et al., 1981).

However, navigation within a familiar area, where a bird has extensive navigational experience, offers additional cues. On top of traditional map and compass navigation there is now the option to integrate familiar cues such as visual landmarks, either using them as a "familiar area map" in conjunction with compass cues (Holland, 2003), or following a series of landmarks home in a method known as pilotage (Füller et al., 1983). However, the evidence supporting use of familiar area cues and how they may be integrated into other methods of navigation has been variable, with some authors finding no significant effects of location (Wiltschko et al., 2005), and others finding site-specific responses suggestive of landmark use (Bonadona et al., 2000; Holland et al., 2000; Mora et al., 2012; Filannino et al., 2014; Gagliardo et al., 2020).

The primary method used to test use of navigational cues is clock-shift: experimental manipulation of the circadian rhythm of a bird using artificial light-dark cycles, to cause a predictable misinterpretation of sun azimuth which results in a directional deflection when navigating (Schmidt-Koenig, 1960; Alexander and Keeton, 1974). If deviation from the correct flight direction towards the predicted clock-shift direction is seen following clock-shift, this suggests that the sun compass is being used for navigation. However, previous studies have found that the deflection seen under clock-shift is often lower than 100%, and a variety of explanations for this observation have been proposed (Wiltschko et al., 1994; Chappell, 1997).

The primary explanation is that information from other navigational cues in conflict with the shifted sun compass are integrated, giving a compromised heading between the conflicting cues. Candidates for alternative cues include the magnetic compass, as well as visual landmark cues when navigating in a familiar area. It is possible to test for use of the magnetic compass by attachment of a small magnet to the head of a pigeon during homing, such that the local magnetic field disrupts use of the Earth's magnetic field. If a "full" deflection is seen with the magnet attached compared to an intermediate heading without, this would suggest that it is the magnetic compass information which is being integrated to produce the intermediate heading between the clock-shifted direction indicated by the sun compass, and unmanipulated navigational cues. Many studies have combined clock-shift and magnets to investigate this, but with variable results, possibly due to different levels of training and local landscape features at different locations (Wiltschko and Wiltschko, 2001; Gagliardo, 2005; Ioalè

et al., 2006; Gagliardo et al., 2009). Additionally, finding a significant effect of magnets does not rule out the concurrent use of landmarks, as multiple different cues may be integrated simultaneously, and this effect cannot be easily seen if they are providing the same directional information (if there is no conflict between the cues).

However, it is also possible that birds may not have had their circadian rhythm fully shifted. Although many dozens of studies have utilised clock-shift procedures, it is unclear whether or not birds are showing the complete change in circadian rhythm that the experimenter has intended. If we observe a direction of flight which is less than 100% of the clock-shift predicted direction, we cannot identify between an incomplete shift in circadian rhythm, and a complete shift which is being integrated with other navigational cues (or a combination of both). Any of these options could produce identical GPS tracks. Therefore, it is essential to demonstrate that birds have received the desired change in circadian rhythm before making any other conclusions about the direction of flight following clock-shift. If we assume that our unmanipulated navigational cues (magnetic compass and landmark cues) are unbiased, then we need to know the actual amount of bias we are introducing with the clock-shift manipulation in order to calculate how the different cues are weighted in the integration process.

In order to test whether our clock-shift procedure is shifting the circadian rhythm by the expected degree, we must first understand what factors are naturally used to entrain it, and the physiological changes which occur as a result. The clock-shift method itself is simple: birds are contained in light-proof rooms with an artificial light-dark cycle applied for several days, offset by the desired amount of time. A 6h shift is standard practice, as this is traditionally the amount of deflection that can be easily identified using vanishing bearing data. However, since the advent of GPS tracking technology, it is possible to identify smaller amounts of clock-shift. The general rule for clock-shifting is to allow one day under the shifted light conditions per hour of clock-shift; however, in practice the clock-shift process may often run longer, until weather conditions are appropriate for the experimental flights. This experiment aims to test how much of a shift in circadian rhythm is produced by this clock-shift procedure.

Circadian rhythm describes the internal clock which controls the daily processes of the body. Although first studied in humans, circadian rhythms are now recognised across many animal species, including birds. The circadian rhythm occurs as a result of hormone cascades which have an endogenous rhythm, but this rhythm is entrained using external sources of information called zeitgebers. The most obvious zeitgebers are photoperiod and temperature, but daily variations in food access can also act to entrain circadian rhythms (Rashotte et al., 1995; Hau and Gwinner, 1996; Rashotte and Stephan, 1996). Social cues may also affect the entrainment of the circadian rhythm in some species, with

rhythms of those in a social group becoming aligned when other external cues are ambiguous (Davidson and Menaker, 2003). When clock-shifting pigeons, it is important to consider the external influences on circadian entrainment independent of the altered light-dark conditions which could be influencing the rate of shift. Therefore, feeding regime was also modified during the clock-shift procedure, with birds fed one or three hours later than usual for the respective clock-shift treatments.

Although the production and maintenance of circadian rhythms in birds is not fully understood, much research has been done into light-sensitive chemical fluctuations, and endogenous rhythms. One key component of this research is melatonin, also known to play a role in the circadian rhythms of humans and other mammals, and potentially many other vertebrates (Cassone, 1990). Melatonin is produced in the pineal gland via O-methylation of N-acetylserotonin, catalysed by the enzyme hydroxyindole-O-methyltransferase (HIOMT), and secreted into the bloodstream (Ralph, 1981). This production is light-sensitive, with increased production in the dark, and decreased production in the light, producing daily fluctuations in melatonin levels which reflect changes in day length over the course of a year, giving both circadian and circannual information (Reiter, 1993). Although pineal melatonin production is light-sensitive, a rhythm of production from a light-dark cycle can be maintained once shifted to constant darkness (Murakami et al., 1995).

Overall, the pineal can maintain an endogenous daily rhythm of melatonin production, stabilised by light-sensitivity which entrains the rhythm to changing daily conditions. The free-running rhythm of temperature and locomotor activity which persists under constant darkness can be rendered arrhythmic in many bird species by ablation of the pineal gland, indicating its role as a circadian pacemaker (Underwood et al., 2001). The fact that reduced melatonin levels allow faster entrainment to altered conditions (i.e. drifting from the set standard conditions) suggests that pineal melatonin rhythms normally play a significant role in maintaining bodily circadian rhythms (Hau and Gwinner, 1995). Additionally, the ease of re-synchronisation afforded by reduced melatonin fluctuation occurs under non-photic zeitgebers such as feeding times, suggesting that the effect is not light-specific, and more likely is due to a masking of the endogenous rhythm (Abraham et al., 2000).

Measuring the circadian rhythm can be done in a variety of ways, although many are invasive and can, therefore, influence behaviour. Measuring blood melatonin levels is a good indicator of the internal rhythm, as well as internal temperature fluctuations. For diurnal mammals and birds, body temperature is lower at night than during the day, and a free-running circadian rhythm of temperature has been recorded in birds with no access to external temporal cues (Refinetti and Menaker, 1992; Refinetti, 2010). Although there are associations between activity level and body temperature, the changes in body temperature are not reliant on levels of activity. Locomotor activity itself can be used

as an indicator of circadian rhythms. This is usually done via some kind of motion detector, such as infrared (Murakami et al., 2001), or a microswitch in the floor of a cage (Oshima et al., 1987; Yamada et al., 1988; Oshima et al., 1989). Measuring activity levels is the least invasive of these methods.

Previously, we used small values of clock-shift at two different release sites with differing landscapes to investigate whether differing levels of cue conflict would influence how much integration occurs between different navigational cues; in this case, the sun compass, and familiar landmark cues. We found that, at higher levels of clock-shift, a lower percentage of expected deflection occurred, suggesting that as the amount of conflict between cues increases, reliance on the conflicting cue is decreased. However, without controlling for other navigational factors, we cannot say that it is the familiar landmark cues which are being used for navigation instead. Similarly, we must account for the possibility that we do not see a complete deflection under clock-shift because the clock-shift procedure does not sufficiently alter the birds' circadian rhythm.

Therefore, in this experiment we again used two small values of clock-shift (1 hour and 3 hours, giving predicted deflection values of approximately 15 and 45 degrees), and tracked the birds for the duration of their time in clock-shift with accelerometers, to monitor their activity levels as a proxy for circadian rhythm. Following this clock-shift, birds were released from a familiar location, with magnets attached to the top of their heads in order to prevent use of the magnetic compass, leaving only the sun compass (the perception of which is altered through clock-shift) and visual landmark cues as directional cues. If clock-shifted birds released with magnetic attachments show a larger percentage of the sun-compass indicated direction than those without, this would suggest that the magnetic compass is being integrated with the sun-compass to produce the overall directional estimate. However, if the percentage of sun-compass direction seen in the experimental release with magnets is not greater than without, this would suggest that other navigational factors are being integrated instead.

Methods

Subjects and training

Fifty-three homing pigeons from two age groups (2019 birds and 2020 birds) from the Bangor University pigeon loft (Treborth botanic gardens) were used for this experiment. These birds were kept indoors and fed 25g of mixed seed per bird per day, with access to water and grit *ad libitum*. All the birds have had local flight experience, two years for the 2019 group, and 1 year for the 2020 group before the field season in which testing took place. All training flight locations are shown in figure 1, and all training flights were conducted as flock-releases.

Following flock training, all birds had a minimum of six individual releases from the test site prior to the experimental release. For these training flights, birds were driven to the release site, left to acclimatise to the new location for ten minutes, before being released individually with five-minute intervals between the release of each bird. No more than one training flight was conducted per day. For the 2020 cohort, all these birds had received individual training flights at the test location the previous year, so a single refresher training flight was conducted before testing, although other training flights had been conducted at other locations. The 2019 birds had not been flown individually from the test site before however, so all six training releases were conducted directly prior to the test procedures. All the birds had experienced clock-shift the previous year but had no clock-shifts in the current season.

Experimental procedure

The birds were split into two groups for clock-shifting to account for space in the clock-shift loft. Prior to going into clock-shift, all birds had the feathers on their back between their wings trimmed, and a strip of Velcro attached using non-toxic glue, to which GPS trackers/accelerometers could be attached. Birds were randomly assigned to either control, 1-hour or 3-hour shift groups, and had accelerometers (Bangor Bio-loggers) attached to the Velcro. These accelerometers were set to record x, y, and z movements 54 times per second for twenty seconds, every ten minutes.

Following attachment of the accelerometer, the control birds were returned to their normal loft under natural daylight conditions, the one-hour group put into a light-proof room with artificial lighting set one hour later than natural timings, and the three-hour group into an adjacent light-proof room, with lighting set three hours later than the natural daylight cycle. Birds were kept under these conditions for at least six days, with food and water changes daily.

Following clock-shift, birds were collected into boxes and their accelerometers removed. A small patch of Velcro was attached to the top of each bird's head and used to attach a small disc magnet (8mm x 1.5mm Neodymium Disc Magnets (N42)). At the release site, i-gotU USB GPS Travel and Sports Loggers, were attached to the Velcro on the back of each bird, recording at a frequency of 1Hz. The birds were released individually from the test site at five-minute intervals, alternating between the three experimental groups. Once they had returned to the loft, the GPS trackers and magnets were removed, and taken for analysis. Experimental flights took place 02/09/2021 and 15/09/2021.

Data Analysis

Accelerometer data

Accelerometer data were downloaded via Arduino and saved in a plain-text format before being imported into RStudio. Many of the accelerometers failed to record for the duration of the time in clock-shift, so any which recorded for less than a day were discounted from the analysis, as they would not provide any information on sleep cycles. For the 54 reading in each second, the standard deviation was calculated for the x, y, and z values. The mean of these three standard deviations was then calculated as a measure of activity levels per second. The data were then further condensed to average the activity levels for each 20 seconds, effectively giving a single activity reading every ten minutes for each bird.

Following this, wake and sleep times were determined for each night under clock-shift. A cut-off activity level was determined for each bird based on their minimum activity level (minimum activity level varied between recordings, threshold was generally set at 10, adjusted up or down if birds had particularly low or high minimum), and sleep time defined as the first time three consecutive activity points fell below this threshold. Similarly, wake time was determined as the first-time activity levels were consistently above this threshold. Finally, the difference in minutes between the sleep time and dusk or the wake time and dawn was calculated, so that if a bird awoke before dawn, a negative value was produced.

For each bird, the sleep and wake times for each day under clock-shift were averaged such that a single wake and sleep time was produced for each bird. These were then grouped by treatment and plotted as a box and whisker plot, and the difference between the group medians analysed using a Kruskal-Wallis rank sum test.

GPS tracks

GPS tracks were handled identically to the previous chapter. Efficiency of each track was calculated as the beeline distance between the start and end of the track divided by the total distance travelled, plotted as a box and whisker plot per treatment group, and compared using a Kruskal-Wallis rank sum test.

Also as previously described (chapter 3), instantaneous deflection was calculated at 500m intervals from the release site for each track, as well as the percentage of sun compass direction seen, based on the exact release times and dates for each individual, in order to account for variations in sun azimuth change over time. These values were grouped by treatment and plotted, as well as being used to produce linear mixed models, to account for the effects of various variables on amount of

instantaneous deflection and percentage of the sun compass direction found. Linear mixed models were assessed for normality, homoscedasticity, and the presence of autocorrelation. The full dataset showed evidence of positive autocorrelation (Durbin-Watson Statistic = 1.04), so the data was sub-sampled, with a random 50% of the dataset being used for the linear model. This was repeated 10000 times, and the effect estimates and p-values averaged from this data set. The mean was used for the effect estimate average as the data were symmetrically distributed, but median was used for p-values, as these tended to be skewed.

Results

From the 53 birds fitted with accelerometers in the loft, 34 produced sufficient data to be included in the analysis. Figure 1 shows a boxplot of the relative wake and sleep times of the three treatment groups. Statistical comparison of the three treatments at dusk found a significant difference in the medians (Kruskal-Wallis rank sum test; chi-squared = 19.1, df = 2, $p < 7.12 \times 10^{-5}$), with medians of 31, 67 and 179 minutes for controls, +1h and +3h clock-shift respectively. Similarly for the wake times at dawn, a significant difference was also found (Kruskal-Wallis rank sum test; chi-squared = 28.9, df = 2, $p < 5.32 \times 10^{-7}$), with medians of -41, 40 and 97 minutes. Sleep times matched well with the light out time under clock-shift, suggesting that the birds cease activity as a result of darkness. However, the birds tended to wake up before the lights came on, so this may be a better estimate of the actual amount of circadian rhythm change achieved. The median +1h shift wake time was 66% of the predicted time if waking up at artificial dawn is our measure of shift, with 54% for the +3h group. If we account for the control group also waking before dawn, however, then the +1h group show 135% of the expected shift, and the +3h group 97%.

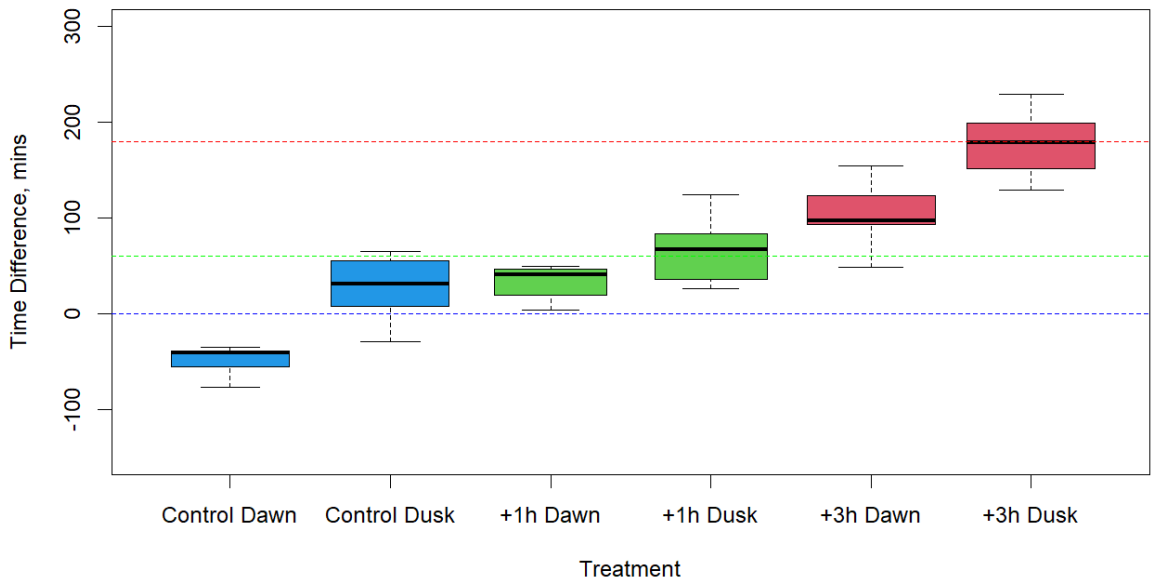

Figure 1: Box and whisker plot showing the time difference in minutes between lights off and sleeping or lights on and waking for pigeons under three clock-shift treatments: natural daylight (Control), 1 hour of clock-shift (+1h) and 3 hours of clock-shift (+3h), split across dawn and dusk. A negative time value shows that birds went to sleep/woke up before lights off/on. The blue, green, and red dashed lines show the expected time differences for the three treatment groups if they were to match their sleeping pattern with the light schedule. The centre line for each box gives the median, the top and bottoms of the boxes the upper and lower quartiles, and the whiskers range. From left to right, n = 11, 11, 10, 10, 13, 13.

Of the 53 birds released for this experiment, 47 tracks were successfully recovered. The GPS tracks for each bird are plotted in figure 2, following processing to remove circling at the release site and around home. Given that the birds were shifted either +1h or +3h, we would expect to see a deflection inland, away from the Menai Straight if the birds were using the sun compass to navigate.

Efficiency was calculated for each track and plotted across the treatment groups (figure 3). No significant difference in efficiency was found between the treatment groups (Kruskal-Wallis Rank Sum, chi-squared = 2.57, df = 2, p = 0.277).

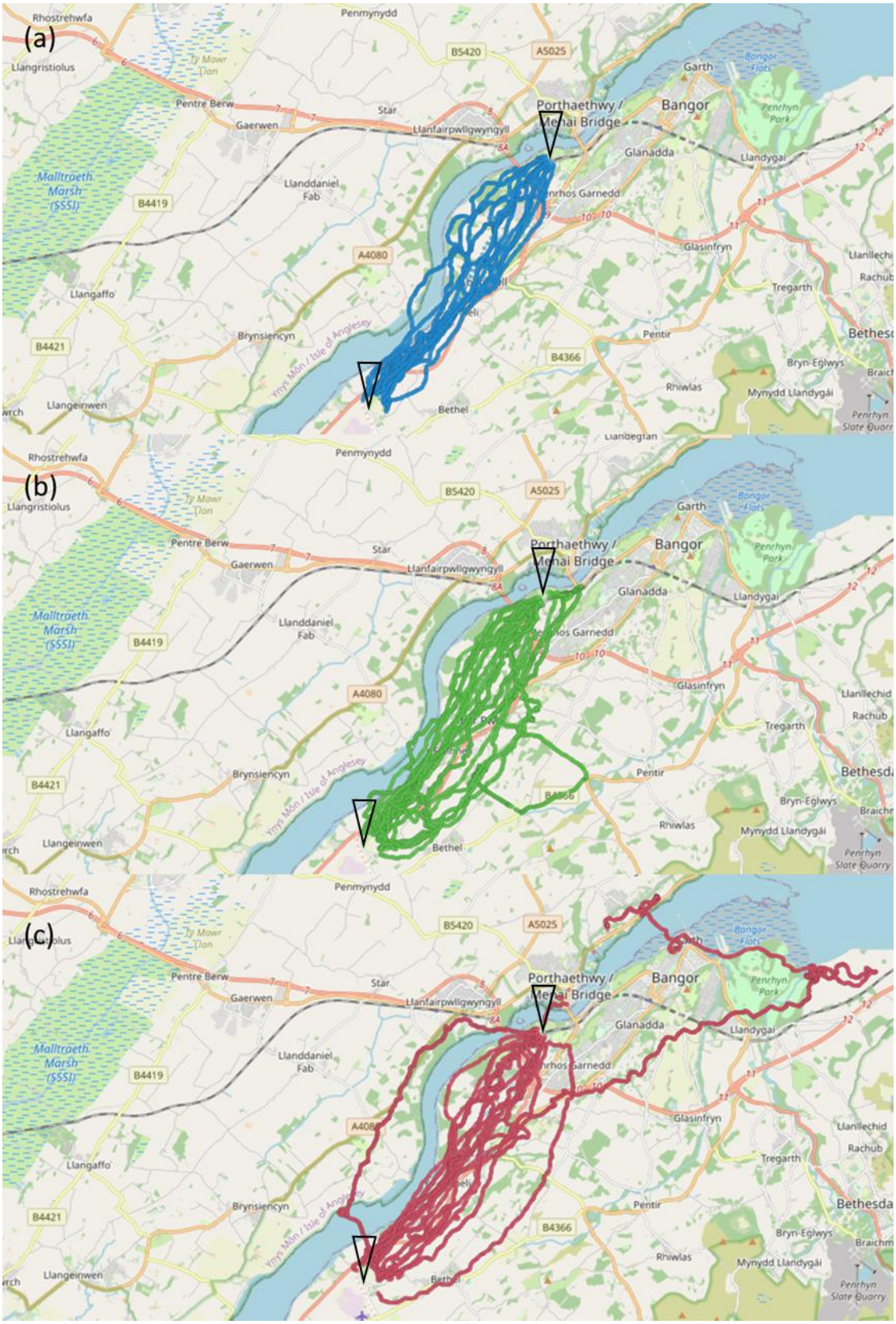

Figure 2: Maps showing all the GPS tracks from the experimental pigeon flights, shown in mapview (RStudio). (a) Blue tracks show the magnet-only birds, (b) green the magnets with 1 hour of clock-shift, and (c) red the magnets with three hours of clock-shift. Triangles indicate the release site (bottom left) and home loft (top right).

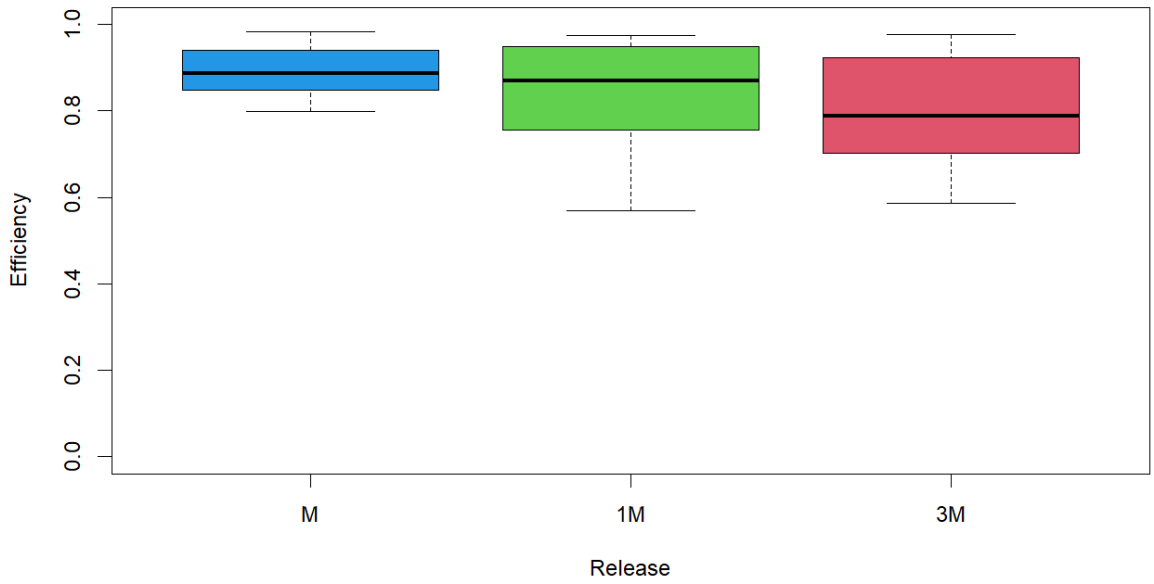

Figure 3: Box and whisker plot of the efficiency of pigeon tracks, calculated as the beeline distance (straight line between the start and end of each track) divided by the actual total distance travelled for each track, shown across the three treatment groups: magnets only (M, n=14); one-hour clock-shift with magnets (1M, n=17); and three-hour clock-shift with magnets (3M, n=16). The centre line for each box gives the median, the upper and lower edges the 1st and 3rd quartiles, and the whiskers give the range.

In order to investigate how the birds responded to clock-shift over time, instantaneous deflection was calculated for 500m intervals from the release site and averaged per treatment group, then plotted over distance from the release site (figure 4). A linear mixed model of these data with predicted sun-compass direction and distance (km) as fixed effects and bird as a random effect found no significant effect of predicted sun-compass direction on instantaneous deflection (effect size = -0.0389, $p = 0.694$) (table 1). There was a significant effect of distance ($p = 2.60 \times 10^{-4}$), with instantaneous deflection decreasing 2.58 degrees per kilometre.

In order to analyse how much deflection we see the percentage of the sun compass direction was calculated for each bird based on the date and time of release and normalised against the control group (figure 5). Percentage of expected deflection peaks at the beginning of the track for both 1-hour and 3-hour clock-shift groups, with the 1-hour groups showing a much greater initial percentage of sun compass direction. Comparing percentage of sun compass direction data between clock-shift with magnets and the clock-shift only data from the same release site in the previous year, we find no significant effect of the presence of magnets on the percentage of sun compass direction ($p = 0.257$, table 2). However, there is still a significant effect of distance, with the percentage of sun compass deflection decreasing by 8.37% per km from the release site ($p = 1.95 \times 10^{-5}$).

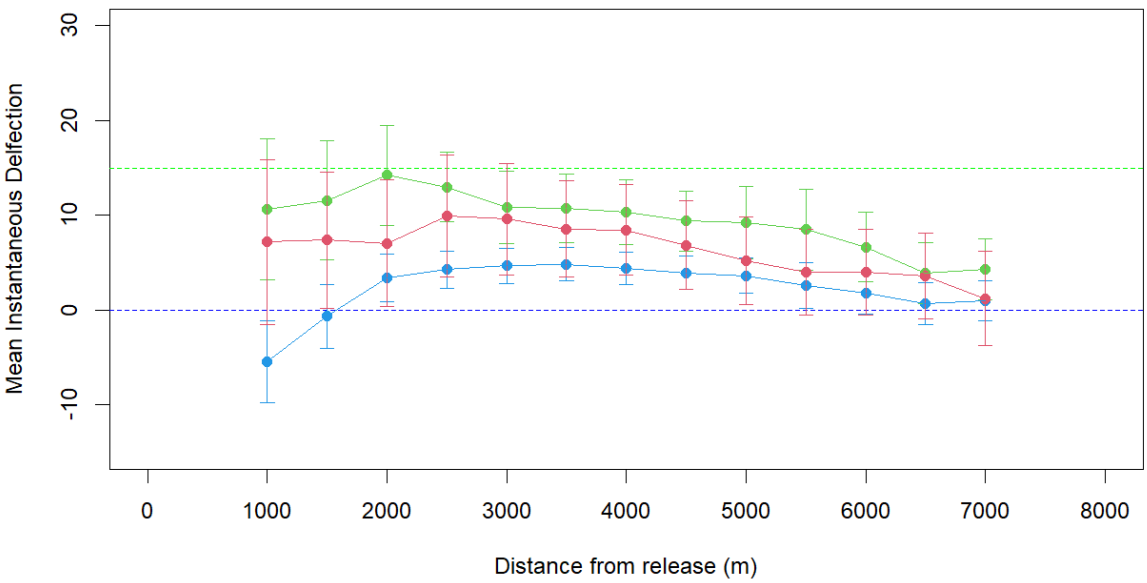

Figure 4: Mean instantaneous deflection averaged across all birds in each treatment group plotted over 500m intervals of distance from the release site. Magnet-only birds are shown in blue, magnets with 1 hour of clock-shift in green, and magnets with three hours of clock-shift in red. Error bars represent standard error for each point. The dashed coloured lines represent the expected amounts of instantaneous deflection for each level of shift (0 degrees for no shift, 15 degrees for 1 hour, and 45 degrees for 3 hours), if we were to see navigation based entirely on the sun compass.

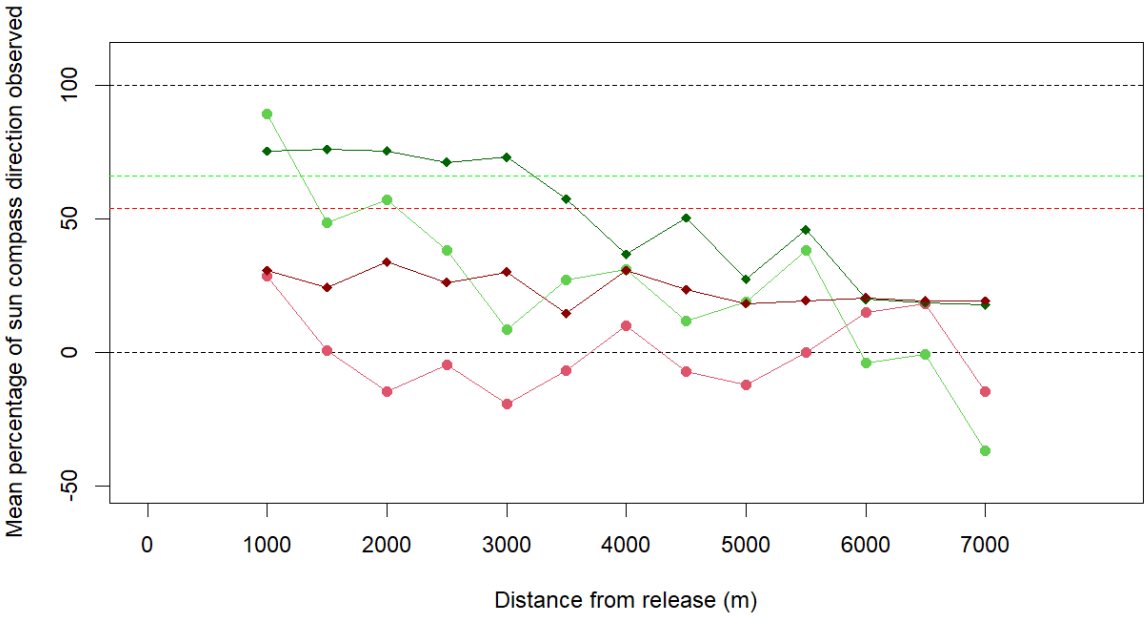

Figure 5: Mean percentage of expected deflection plotted over distance from the release site. The 1-hour shift group is shown in green, and the 3-hour group in red, with circles for the treatments including magnets, and diamonds with darker colours for the treatments without. Black dashed lines mark 0 and 100%, with red and green dashed lines marking the circadian rhythm change predicted from the accelerometer data.

Table 1: Fixed effects estimates and p-values for the linear mixed model for instantaneous deflection, shown to three significant figures. Each is calculated as an average from the 10000 iterations of the linear mixed models, each taking a random 50% sample of the data. The average for the estimate is the mean, as data is symmetrically distributed, but median for the p-values, as these have a skewed distribution. Standard deviation is shown for the means, and inter-quartile range for the medians. Fixed effects are sun compass direction and distance.

	Effect Estimate (mean ± sd)	**p-value (median, IQR)**
Sun compass direction (degrees)	-0.0389 ± 0.0420	0.694; 0.319
Distance (km)	-2.58 ± 0.566	2.61×10^{-4}; 1.65×10^{-3}

Rather than using shift in hours as a factor to predict instantaneous deflection or percentage of sun compass direction in the linear models, the predicted sun compass direction based on the time and date of release has been used. Figure 6 shows a scatterplot of these values for each flight, plotted against the mean instantaneous deflection across the flight, for both 2020 (without magnet) and 2021 (with magnet) experimental groups. This plot shows greater spread in the group without magnets attached.

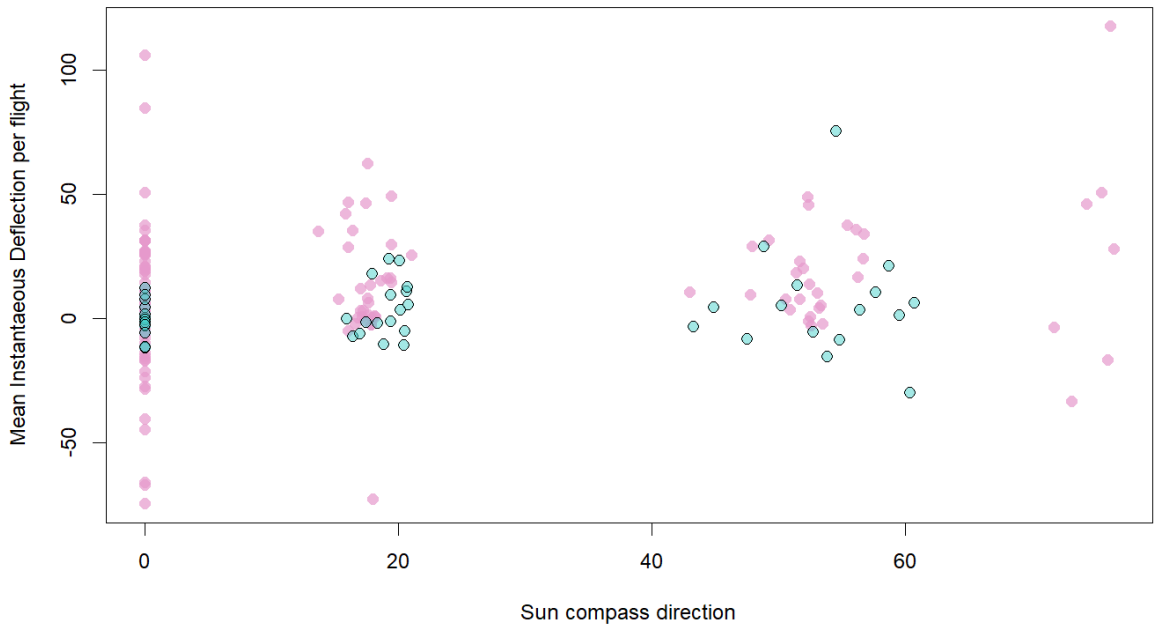

Figure 6: Scatterplot showing the mean instantaneous deflection per flight, plotted against the sun compass direction, for the clock-shift flights from the Menai Strait release site. Pink circles show the 2020 flights without magnets, and blue circles show the 2021 flights with magnets.

Table 2: Fixed effects estimates and p-values for the linear mixed model for percentage of the sun compass direction, shown to three significant figures. Each is calculated as an average from the 10000 iterations of the linear mixed models, each taking a random 50% sample of the data. The average for the estimate is the mean, as data is symmetrically distributed, but median for the p-values, as these have a skewed distribution. Standard deviation is shown for the means, and inter-quartile range for the medians. Fixed effects are the sun compass direction, distance, and the presence of magnets.

	Effect Estimate (mean ± sd)	**p-value (median, IQR)**
Sun compass direction (degrees)	-0.807 ± 0.157	0.110; 0.0779
Distance (km)	-8.37 ± 1.65	1.95×10^{-5}; 1.77×10^{-4}
Magnet (presence)	-19.7 ± 5.63	0.257; 0.183

Discussion

Previous reviews of clock-shift experiments found increased scatter and lower deflection than would be expected based on sun compass information alone at familiar release sites (Wiltschko et al., 1994; Chappell, 1997), and subsequent experiments sought to answer what factors were causing these observations. Although the majority of clock-shift experiments use a 6h shift, our smaller shifts of one and three hours also showed a lower-than-expected deflection than would be expected based solely on the sun-compass.

This experiment was designed to account for possible factors which would prevent “full deflection” being seen when clock-shifting pigeons (i.e. a direction of flight matching the shifted sun-compass direction), in terms of the true amount of change in circadian rhythm seen, and the use of the magnetic compass. From the accelerometer data in figure 1 we can see that the birds under clock-shift appear to cease activity when the lights go out at the artificial dusk, but the control birds remain active for some time after natural sunset. This is likely because, under natural conditions, the light fades slowly after sunset, so darkness is not immediate. Similarly, the control birds wake up significantly before dawn, indicating that the increase in light levels before sunrise might be the actual trigger for activity. Both of the experimental groups also wake up before artificial dawn, suggesting that either (a) they were able to detect light through the clock-shift loft and were disturbed by it or (b) they were waking up at their subjective dawn, meaning that wake time is a better indicator of circadian rhythm. It is also possible that a combination of these factors could be occurring. Although it is possible that external light may be an issue, the wake times for the 1h and 3h groups do not match up with natural dawn. Additionally, the clock-shift loft receives no direct sunlight at dawn, so only

ambient light would be an issue. Visual inspection of the loft would suggest that this ambient light should not be detectible, although the intensity at which pigeons could detect it may be different.

Therefore, it is possible from these results that the pigeons may not have been fully clock-shifted based on our standard procedure. Based upon this and the percentages of shift suggested by the median wake times for the clock-shift groups of 66% for the 1h group and 54% for the 3h group (figure 1), dashed lines representing these figures have been added to figure 5. The lines of percentage of sun compass direction for the 3h shift group still fall well below the red dashed line marking the shift indicated by our activity measures, suggesting that these birds are still not relying solely on information from the sun compass. The 1h shift group starts above the green dashed line but falls below it by the standard vanishing distance of 2km. This indicates that these birds might be using the sun compass to a greater extent, but that it is downgraded in the integration process as the bird travels. However, we must also consider that the use of accelerometers to approximate circadian rhythm may not have been a good measure, as we are using activity as a proxy for the internal rhythm. In order to confirm how useful accelerometery is, recording internal body temperature alongside activity as a comparison could be used to verify or calibrate our measure.

Although the effect of clock-shift is almost always lower than expected at familiar release site, at truly unfamiliar release sites, Wallraff et al found that deflection under clock-shift met expected levels (Wallraff et al., 1999), supporting the notion that it is familiarity with the local landscape at familiar release sites which is necessary to provide a conflict with the sun compass, and reduce deflection.

Studies investigating the effect of magnets on clock shifts from unfamiliar release sites have found variable results, with some authors finding significant effects of the presence of magnets to increase the amount of initial deviation seen (Wiltschko and Wiltschko, 2001), and others finding no difference from clock-shifted birds without magnetic treatment (Ioalè et al., 2006). Results of these initial experiments using only vanishing bearings show high variation due to the nature of only getting a single bearing for each individual flight, whereas later experiments also utilising GPS found more consistent results supporting the finding that, at unfamiliar sites, the magnetic compass plays a significant role in correcting deviation following clock-shift (Gagliardo et al., 2009).

Our study repeated the clock-shift experiment from the site which showed the lowest percentage of the sun compass direction in the previous season, adding magnets to the heads of the pigeons. This would be the site where we would be most likely to detect a change in the percentage of the sun compass direction with the addition of magnets, as a lower overall shift is seen. When comparing the clock-shift releases with magnets to those without magnets the previous year, our linear mixed model finds no significant effect of the presence of magnets on the percentage of the sun compass direction

seen. This suggests that, at least in this case, the use of the magnetic compass is not what is being integrated with the sun compass to prevent full deflection under clock-shift. We still found a much lower than expected percentage of the sun compass direction overall, although this increased with distance from the release site, with distance being a significant predictor of both mean instantaneous deflection and percentage of the sun compass direction.

Percentage of the sun compass direction is an important measure, in order to account for the exact change in azimuth, as the rate of change of sun azimuth is not consistent across the day, moving much faster around noon than at dawn and dusk. Wiltschko et al found that birds were able to show compensation which matched the expected azimuth change at different times of day (Wiltschko et al., 2000), suggesting that this is an important factor to take into consideration. However, many experiments still compare the overall deviations seen to the average expected shift, rather than individually finding the percentage of the sun compass direction, which may cause over or underestimates depending on the time of day that experimental flights took place.

In the familiar area, some experiments have shown a lower-than-expected initial deviation when compared to birds released at the same sites but unfamiliar to them (Gagliardo, 2005), although this has not been the case in other tests (Wiltschko et al., 2005). This suggests potential site-specific differences in navigational cue use, either as a result of different local conditions, training procedures between research groups, or a combination of factors. However, even within familiar areas within a local area, site-specific differences have been found which suggest that the presence of particularly salient landscape features may be of significance in homing (Bonadona et al., 2000). Birds in the present experiment were all familiar with the release site and showed a generally low percentage of the sun compass direction, especially when approaching home. This is in contrast with experiments which have shown an effect of clock-shift even within sight of the loft (Armstrong et al., 2013), although others have found variable strategies among individuals (Filannino et al., 2014), further suggesting potential site-specific differences in response to clock-shift, and use of other cues.

Previous experiments in the familiar area have found that birds rendered anosmic show an even greater reduction in deviation under clock-shift (Bingman and Ioalè, 1989; Gagliardo, 2005; Gagliardo et al., 2020), theorised to be the birds switching to a pilotage-based strategy using familiar landmarks when access to the olfactory map is removed, meaning that a map and compass mechanism of navigation is abandoned, thus the effect of clock-shift on the sun-compass is removed. These experiments support the role of landscape features in familiar area navigation.

It is possible that the birds in this experiment were not relying on compass cues as a result of high familiarity with the release site. Birds which had been released there in both years had a total of twelve

un-manipulated individual releases, whereas birds released there for the first time in the second year had six. This number of releases, combined with occasional releases from surrounding locations to prevent directional entrainment should mean that the birds have learnt relatively efficient routes home (Guilford & Biro, 2014), but evidence from previous studies suggests that they should not be ignoring compass cues based on this level of experience alone (Filannino et al., 2014). The distance to the loft from the release site is towards the smaller end of clock-shift experiments, at around 7.5km. It is possible that the shorter distance means that using compass cues is less important for the birds, but the strong effect of distance from the release site that we see on both instantaneous deflection and percentage of expected deflection (starting high and decreasing over time) suggests that the initial distance is sufficient for at least some compass use.

This experiment used each bird for only one manipulated release each year (one control and one clock-shift track per bird), so we cannot easily assess if there are any idiosyncratic responses to the treatments. It is possible that there is variation among individuals on their reliance on different navigational cues, so repeated testing could be used to assess this, although it would be important to avoid too frequently clock-shifting birds, as they may start to ignore the cue entirely. Previous experiments have indicated that there may be individual differences in homing strategy (Griffiths et al., 2021; Finannino et al., 2014, Freeman et al., 2011), so such differences should be taken into account when discussing homing as a whole.

Conclusion

The results of this experiment, in combination with the experiment from the previous year, strongly suggest that visual landmark cues are primarily used for navigation in the familiar area, at least in our experimental cohort. The results of measuring activity levels under clock-shift suggest that our birds did have their circadian rhythms fully shifted, and thus should be reading the sun compass as predicted by the experimental treatment. We found no significant effect of magnets on the deflection under clock-shift, suggesting that reduction in the percentage of expected deflection occurs as a result of integration with landmark features, rather than (or as well as) the magnetic compass.

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Chapter 6

General Discussion

This thesis set out to uncover which cues are used by homing pigeons during familiar area navigation, and how these cues are integrated, via a combination of field and arena experiments, utilising modern tracking techniques and associated analytical methods. The study of familiar area navigation, initially suffered from the difficulties of analysing homing flights with limited tracking technology (Michener and Walcott, 1967; Bramanti et al., 1988). However, advances in the last two decades mean that information is now accessible across the entire homing route, allowing more information about the impact of different navigational factors to be assessed (Meade, 2005; Dell'Ariccia et al., 2009; Gagliardo et al., 2009; Schiffner et al., 2016).

The experiments in this thesis were designed to test cue integration in the form of cue conflict experiments, introducing a known bias into one navigational cue, in order to see the impact of this on the homing flight. Understanding of cue integration theory from human psychology has been employed in the design and analysis of these experiments to address the question of how different sensory cues are integrated in the homing process.

The use of modern tracking technology in previous studies has highlighted the importance of individual variation in homing strategy which would not have been previously identifiable and has uncovered the importance of experience and learning (Wiltschko et al., 2007; Dell'Ariccia et al., 2009; Flack et al., 2012; Filannino et al., 2014). This thesis investigates how experience with different cues and locations may influence cue choice and relates this to the context of learning cue reliabilities.

Although each individual chapter has a discussion section, this general discussion will look at each experiment through the lens of cue integration and draw overall conclusions from the experiments. I will discuss the additional questions which have been raised as a result of these experiments, and how they may be addressed to further our understanding of cue integration.

Arena experiments

The first experimental chapter of this thesis was designed to investigate whether there was a hemispherical difference in how cues were processed, based upon known lateralisation of the avian visual system (Gagliardo et al., 2005). In particular, whether there would be a preference for the sun compass or visual "landmark" cues. Our results found no difference between the left and right hemisphere treatments, with both groups showing an intermediate response between the sun compass and landmark cues in the test trial, suggesting that integration is able to occur in either monocular group. However, we found reduced consistency of integration in the binocular group compared to either of the monocular groups, suggesting possible differences in the integration process when both eyes/hemispheres were in use (Griffiths et al., 2020).

In this experiment, a 90-degree bias was introduced via the rotation of the training arena, such that the visual cues were 90 degrees clockwise of the sun-compass information. In terms of cue integration, choosing the intermediate compartment suggests that the birds have learnt to use both landmark and solar cues to find the food reward during the training process, as integration, i.e. choosing the intermediate direction during the test trial, requires both cues to be used. This would suggest that there is no general preference between the cues, as both were available at all times during training.

The resolution of this experiment only allowed a single intermediate choice between the direction indicated by the landmark cues and the sun compass, and such it is impossible to accurately gauge the degree of weighting between the two cues in the integration process. If one cue is being weighted more heavily in the integration process, then the directional choice following integration should be closer to the direction of that cue. However, a higher-resolution set-up would be necessary in order to test this hypothesis, and to see whether there is any preferential weighting of cues in each hemisphere, or in monocular vs. binocular birds. One possible approach would be a modified arena set-up with a greater range of directional choices (figure 1). This would give a much greater resolution of directional choice, whilst maintaining the controlled arena environment. However, it would be necessary to demonstrate that the homing pigeons are able to discriminate between these smaller values and learn the task effectively.

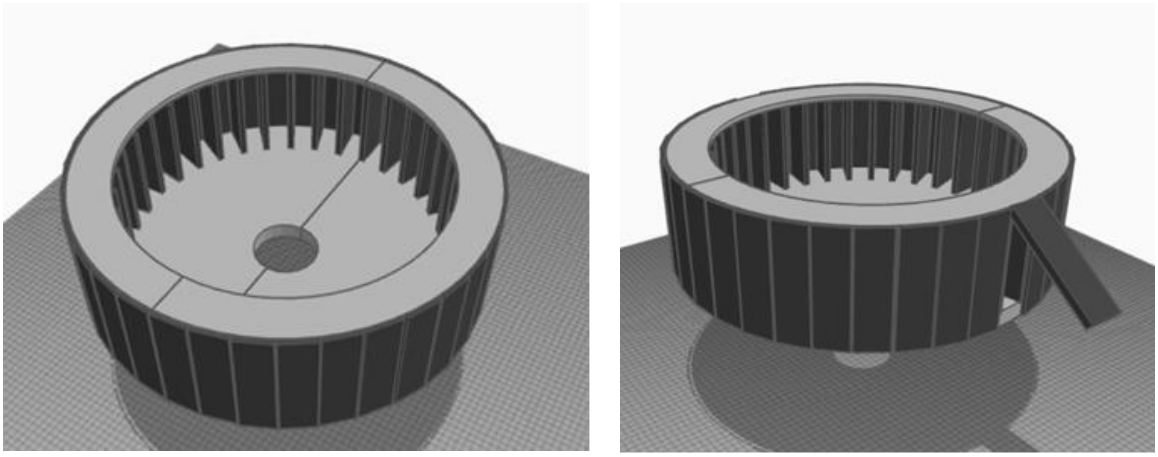

Figure 1: A potential arena design for testing cue integration theory at a higher resolution. This design has 36 10-degree compartments rather than the 8 45-degree compartments used in the lateralisation experiment in chapter 1.

Testing the theory of maximum likelihood estimation

Independent of investigating lateralisation of cue use, this apparatus has the potential to address other aspects of cue integration theory, testing certain predictions that can be made concerning maximum likelihood estimation. For example, the predictions that a) estimate accuracy should increase with the number of cues available; b) the weighting of a cue is proportional to its reliability; the more reliable a cue, the stronger the weighting when integrated; c) creating small discrepancies in cue value should change the overall estimate based on the weight of a cue.

The first prediction can be tested by training birds to find the food reward in the arena with single or multiple cues available to them. Birds using only a single cue should have a less reliable estimate of the correct direction than the birds with several cues available. Birds can be trained to find food with multiple cues available to them (sun compass, magnetic compass, visual cues), and tested under single-cue conditions, or birds could be trained with only single cues available to them, to see how well they are able to learn the task with different cue availabilities.

For the second prediction, we can test whether the weighting of a cue is decreased as its reliability decreases (Ernst and Banks, 2002). For this we must be able to artificially decrease the reliability of a cue, i.e. increase the variance of estimate given by that cue by adding noise to a signal. For the sun compass, this could be achieved by using a frosted covering which scatters light by differing degrees (Schmidt-Koenig and Keeton, 1977; Schmidt-Koenig and Walcott, 1978). For artificial visual cues, cue resolution can be artificially adjusted, and for the magnetic compass, it is possible that electromagnetic interference could be used to introduce noise (Schwarze et al., 2016). For this experiment, birds would be trained under normal cue conditions, and tested under differing cue reliabilities to see if weighting changes dynamically with cue reliability. Such an experiment would only be possible in the controlled environment of an arena setting in order to precisely change cue reliability.

The final prediction is important as it should allow the weighting of a cue to be identified. In this scenario, all cues should be kept consistent, whilst the value of one cue is gradually changed to introduce a conflict. The resulting directional estimate under the cue conflict conditions should allow the weight of that cue to be identified. The primary difficulty here is introducing conflict in a single cue – this could be done relatively easily by moving visual cues within the arena but is more difficult when separating solar and magnetic cues. Clock-shift could potentially be used to introduce a conflict with sun-compass information, but only if we can be sure that the circadian rhythm is being altered by the desired amount.

Figure 2 illustrates the theory behind cue weighting in optimal integration (Ernst and Banks, 2002). The variance of the combined signal is calculated as shown in equation 1, where a and b are the two signal estimates, and c is the combined estimate. The combined signal mean is calculated such that the individual means are weighted based on signal variance; equal variances have a 50-50 weighting, one variance three times as great as the other would be weighted 25-75 (equation 2). Although we may not know the signal variances (inversely proportional to reliability), a cue conflict experiment can theoretically provide us with the mean combined signal estimate, from which the weight of each cue can be calculated (Ernst, 2006).

Equation 1:

$$\sigma_c^2 = \frac{\sigma_a^2 \times \sigma_b^2}{\sigma_a^2 + \sigma_b^2}$$

Equation 2:

$$\mu_c = \frac{\mu_a}{1 + \sigma_a^2/\sigma_b^2} + \frac{\mu_b \times \sigma_a^2/\sigma_b^2}{1 + \sigma_a^2/\sigma_b^2}$$

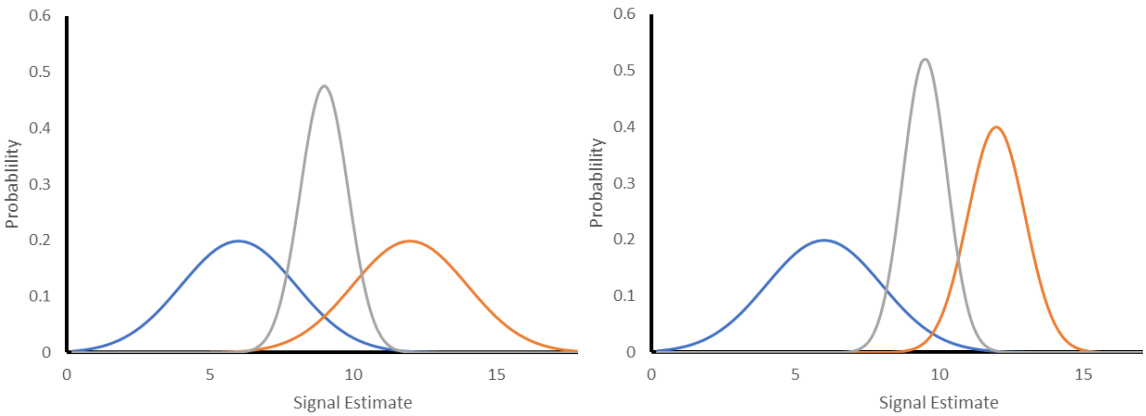

Figure 2: Probability graphs showing two signal estimates (blue and orange) and their optimally integrated combination (grey). Left shows a scenario where both signals have the same reliability (equal variances) and are therefore weighted equally in the integration process. Right shows the second signal (orange) having half the standard deviation of the first and is therefore weighted more highly in the integration process. This results in the combined estimate being closer to the orange signal than the blue.

Small values of clock-shift in a non-arena setting, as in chapters 3 and 4, were shown to be detectible in homing tracks, although deviation under clock-shift was significantly less than 100%. In this case, the intermediate directions chosen were identifiable as percentages of the sun compass direction. Results showed that, as the amount of clock-shift increased, percentage of the sun compass direction

decreased, i.e. at greater amounts of clock-shift (3h rather than 1h), the overall direction of flight was less close to the direction given by the sun compass. From maximum likelihood estimation theory, this could be explained by the downgrading of the sun-compass in the integration process with an increase in conflict size, as lower reliability is detected.

The decision-making process in a maximum-likelihood integration model uses instantaneous reliabilities to inform the weighting of cues. However, in the homing process, we are not seeing the result of a single decision, but potentially hundreds of adjustments to the flight course, each of which may be a result of cue integration. Over the course of a flight, it is possible that the output of cue integration changes based on the changing cues approaching the home loft. For example, if local visual cues are more reliable closer to the loft than further away, then we might expect to see a difference in the influence of clock-shift closer to the loft. The linear mixed models from chapter 4 did find a significant effect of distance on the percentage of sun-compass direction, with the percentage decreasing as distance from the release site increased. This effect was more prominent in the one-hour group than the three-hour group, where there was a much higher percentage of sun-compass direction seen initially.

Robust estimation

As well as weighting cues based upon their instantaneous reliability, we can predict that a maximum likelihood estimation system might show robust behaviour when exposed to a very large cue conflict (Ernst and Banks, 2002). Here, the nervous system may discount a highly discrepant information source, and weight the overall estimate based on the discrepancy between cues as well as their variances. Both field and arena experiments could be used to test incrementally larger values of clock-shift, to see whether we can identify a level of discrepancy where one cue or another is entirely discounted, indicative of robust behaviour. Ideally, intervals as small as 30-minutes (to give an average discrepancy of 7.5 degrees) would give good resolution, but it would need to be demonstrated that such a difference could be detected with GPS on homing flights or trained in the arena. Additionally, this approach would need to be cautious to avoid repeated clock-shifts, as previous experiments have found a reduction in response to clock-shift after multiple rounds (Wiltschko et al., 1976). In these cases, it may be that the sun-compass is found to be an increasingly unreliable cue, so is overall ignored in future cue integration. In an experiment designed to test responses to differing values of clock-shift, we would want to avoid this issue.

Verifying clock-shift

In order to be sure that the bias we are introducing in these experiments is what we expect, we must have an accurate method to measure the circadian rhythm of the pigeons to verify the clock-shift procedure. Without this, any overall direction chosen by the pigeons between the presumed manipulated sun-compass direction and the direction of other unmanipulated cues could be a result of cue integration, incomplete clock-shift, or both. The measurements of circadian rhythm in chapter 4 indicate that, although clock-shift is taking place and there is a difference between the 1h and 3h treatments, the shift may be incomplete. Ideally, a method of measuring circadian rhythm would always be used alongside clock-shifting to validate the results of clock-shift flights, in terms of the actual shift applied to each bird. Although activity levels are the easiest measure, it is possible that they are not fully representative of circadian rhythm, so more invasive methods such as an internal temperature probe could be applied at the same time to confirm whether activity levels are indeed accurate. It may also be necessary to adjust clock-shift procedures to reflect natural conditions more accurately.

For example, the artificial light regimes used in clock-shift lofts do not reflect the normal changes in natural daylight. Ideal light conditions would account for dawn and dusk with dimming light, and changes in intensity and changing wavelengths of light over the day. Wavelengths of light may be particularly important, given evidence that certain wavelengths of light may influence melatonin production (Brainard et al., 1984). The spectral composition of natural light varies throughout the day, with shorter (blue-green) wavelengths scattered at dawn and dusk, as the low angle of the sun means that the light must pass through a thicker layer of atmosphere (Wyszecki and Stiles, 1982). Shorter wavelengths of light reduce night-time production of melatonin, and birds can be entrained to a cycle of blue (day) and red (night) light instead of using only light-dark entrainment (Csernus et al., 1999; Malik et al., 2004; Yadav et al., 2015). Therefore, controlling light wavelengths to mimic natural conditions may help to produce an accurate clock-shift. Additionally, feeding schedules should also be shifted (e.g. always fed 3h after subjective sunrise), to account for food access acting as an additional zeitgeber (Hau and Gwinner, 1996).

Although all of these conditions may be impossible to achieve under experimental limitations, reporting procedure more fully, measuring circadian rhythm and adjusting procedure where possible may help to improve the efficacy of clock-shift. Future experiments could be designed to test the impact of these different factors (light intensities and wavelengths, feeding schedule, soundproofing etc.) on the effectiveness of clock-shift procedure, trialling different methods of circadian rhythm measurement to find a balance between accuracy and invasiveness.

The effect of location

The difference seen in response to clock-shift at our two release sites in chapter 3 suggests a possible location effect, most likely due to the difference in visual landmark cues between the locations, as other navigational factors should have been consistent. A reduced percentage of sun-compass direction under clock-shift at one site suggests that other cues are being weighted more highly, and thus the integration with the conflicting sun information is drawn less towards the sun direction (example figure 2).

Although controversial in the past and arguably still so, the importance of visual landmark cues when navigating in the familiar area has been demonstrated upon many occasions (Burt et al., 1997; Holland, 2003; Biro et al., 2007; Vyssotski et al., 2009; Gagliardo et al., 2020). Multiple route recapitulation experiments have identified that birds will learn specific non-linear routes (i.e. not maximally efficient paths) and can return to these routes when displaced a short distance, rather than taking a direct heading towards the home loft (Biro et al., 2004; Meade et al., 2005; Biro et al., 2006; Guilford and Biro, 2014; Martinho et al., 2015). Additionally, many experiments have identified instances of landscape-following, e.g. following major roads or boundaries such as coast lines, as well as indirect evidence of landscape use (Mora et al., 2012; Guilford and Biro, 2014; Mann et al., 2014). For example, clock-shift experiments conducted at different locations producing different amounts of deflection, likely due to the differing availability of salient landmark cues (Filannino et al., 2014).

The analysis of route recapitulation data in chapter two found distinct navigational strategies among the birds, including evidence of route joining, parallel routes, direct paths, and coast-following behaviour (Griffiths et al., 2021). Each bird was not limited to a single strategy, with multiple different birds showing a different strategy at the second off-route release site compared to the first. This highlights the importance of repeating experiments, as a single strategy chosen on one flight is likely not representative of all of the navigational strategies available. Additionally, the presence of multiple strategies suggests that there may not be one optimal strategy to use based on the available information, and that individual birds may have different preferences when it comes to cue use. Whether this is a conscious decision, or an automatic choice based on differences in experience that we cannot not observe is a difficult question to answer.

Birds in the repeated releases experiment had the same level of training from the repeated release site but were divided into two age groups: one group of one year-old birds, and a second group of birds with at least ten years of flight experience. Birds from the older group produced more efficient routes earlier in the training process, and more idiosyncratic routes overall. However, there was not a significant distribution of off-route release strategy choice between the age groups. Idiosyncrasy in

itself is suggestive of local landmark usage, as the tightly followed routes are not completely straight (Biro et al., 2006). The higher levels of idiosyncrasy in the older birds may indicate that visual cues become more important in navigation as overall experience increases. However, we still see a variation in off-route strategy from the old birds, including some parallel routes, indicative of use of a learnt compass heading. Therefore, this suggests that birds are able to switch between navigational strategies dependent on local conditions, although the conditions which lead to this and what causes the variation between individuals remains unclear.

Despite high individual variation found in field experiments, experimental releases can tell us some very important things about homing strategies which would not be possible from studying navigation in a controlled setting. The clock-shift releases in chapter three showed a distinct difference between our two release sites in terms of response to clock-shift. Despite being fairly close geographically (around 11km apart), distinct landmark differences seem to have had differing influences the weighting of the sun compass in the cue integration process. At the Menai Strait site where the presence of the coastline is a distinct and followable cue, the birds may have been more able to detect the conflict between cues when clock-shift was introduced. Although it might be possible to introduce differing visual cues in a controlled setting to look for an effect, real-world flights are required to see where this behaviour occurs naturally.

Field vs arena approaches

The level of individual variation found in tracks from experimental releases is a source of both frustration and intrigue. Variation adds noise when averaging track features and begs the question whether tracks should be averaged at all. Is the variation between individuals simply a combination of natural variance in the cue availabilities and abiotic or biotic environmental influences, or are there differing navigational strategies being masked? If two different strategies look very similar, would it be possible to tell the difference? In reality, statistical methods require us to average tracks, in order to mitigate the impact of external factors which may cause variation. Although we can attempt to carry out experimental releases on the same day or close together, limitations of equipment and time can cause difficulties, and we cannot account for factors which may be changing along the several kilometres of the pigeons' journeys home.

Therefore, it is important to consider both controlled arena and experimental flight approaches to the study of cue use in pigeons. The first method allows us to address fundamental questions concerning cue use without interference from unexpected variables, and the second lets us see how those fundamentals play out in reality. The ability to navigate did not evolve within a controlled arena, so the navigational system of homing pigeons must be able to cope with the variations of the real world,

and how it does this is what is really important to understand about the system. If there are individual variations in navigational strategies used, it is in real-world experiments where such strategies would be revealed.

In the future, further investigations of cue integration in homing pigeons should utilise both approaches. Application of optimal integration theory in homing pigeon navigation can be investigated in an arena setting by testing the specific predictions as outlined above. Once we know more about whether or not this method of cue integration is being applied, we can analyse experimental releases through the lens of cue integration, and consider the impact of cue availability, variation, and learned reliabilities from past experience on the outcomes of individual flights.

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