Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Mari Landsverk

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Supervisor: Basil el Jundi
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# Abstract

Monarch butterflies (*Danaus plexippus*) make an impressive long-distance migration from Canada and the northern United States to their overwintering sites in central Mexico. To do so, they use the sun as a compass. However, because the sun is not always visible due to weather conditions, the Earth's magnetic field has been suggested as a backup mechanism. The Earth's magnetic field is a known orientation cue that migratory and non-migratory animals use. Research indicates that the butterflies are sensitive to the magnetic field, but little is known about the Earth's magnetic field's role in non-migratory Monarch butterflies. The non-migratory Monarch butterflies do not fly long distances to feed and breed but stay in a more confined area. Here, I investigated whether non-migratory Monarch butterflies can use Earth's magnetic field to keep a flight direction. For this, I performed behavioural experiments on tethered butterflies in a flight simulator, with the magnetic field being manipulated by a three-dimensional Helmholtz coil system. On a group level, no results showed that the butterflies used the magnetic field. However, on the individual level, one butterfly changed its flight direction precisely with the magnetic manipulations. This indicates that the non-migrating Monarch butterfly can use the magnetic field for flight direction, but more research needs to be done on the matter. This study opens the possibility of using non-migratory Monarch butterflies to study magnetic orientation to further understand Earth's magnetic field's impact on animals today.

# Sammendrag

Monarch sommerfuglen (*Danaus plexippus*) er kjent for å migrere årlig fra Canada og nordlige deler av USA, til deres overvintrings sted i Mexico. For å finne frem på denne lange reisen, bruker sommerfuglene blant annet sola som et kompass. Siden været ikke alltid tillater at sola er synlig, så har jordas magnetfelt blitt foreslått som ett reserve kompass for sommerfuglene. Det er kjent at jordas magnetfelt blir brukt av migrerende og ikke migrerende dyr for orientering. Tidligere forskning gjort på Monarch sommerfuglene viser at de er sensitive til det magnetiske feltet, men lite er visst om hvordan jordas magnetiske felt påvirker de ikke-migrerende Monarch sommerfuglene. Ikke-migrerende Monarch sommerfugler beveger seg i mer avgrensede områder for å avle og finne mat. I denne studien, undersøker jeg om ikke-migrerende Monarch sommerfugler kan bruke jordas magnetiske felt til å holde en ønsket fly retning. For å teste dette, gjennomførte jeg adferds eksperimenter på festede sommerfugler i en flysimulator. Det magnetiske feltet ble manipulert ved bruk av et tredimensjonalt Helmholtz spole system. På gruppe nivå, fant jeg ingen resultater som tilsier at sommerfuglene bruker jordas magnetiske felt. Derimot, på individ nivå, var det en sommerfugl som endret sin fly retning nøyaktig med det magnetiske feltet. Derfor virker det som at de ikke-migrerende sommerfuglene kan bruke jordas magnetiske felt for holde en retning, men mer forskning burde bli gjort for tydeligere resultater. Dette prosjektet åpner for muligheten til å bruke ikke-migrerende Monarch sommerfugler til å forstå hvordan disse, og andre dyr bruker jordas magnetiske felt for orientering.

# Preface

## Acknowledgements

Firstly, I want to thank my wonderful supervisors, Basil el Jundi and Robin Grob, and the whole el Jundi lab group for making me feel welcome from the start. Thank you, Basil, for providing me with this opportunity and always being helpful and supportive during my journey. I also want to thank Robin for all your help and support. Thank you for always being available and putting off time to answer all my questions. It has been a true pleasure getting to learn from you. I would also like to thank Fredrik Hanslin for being my IT support whenever I needed it. Thank you for always being positive and willing to help. Lastly, I would like to thank my family and friends for their encouragement and support over the last 5 years.

## Thesis' relevance for the teaching profession

With my degree, I will become a science teacher in middle school and high school. By doing my master's thesis, I have learned much more about what lies in the work of a scientist. Problem solving has been a big part of this journey, which I believe is one of the most valuable skills I get to teach my students. I have failed, tried again and learned a lot in the process, which I believe is an important lesson all students should experience, no matter the subject. In 2020, a new school reform was set in place in Norway, called Kunnskapsløftet 2020 (LK20). In LK20, sustainable development was added as a core curriculum. This means sustainable development shall be a fundamental approach in all pedagogical practices in lower and secondary education in Norway (Kunnskapsdepartementet, 2020a). An important part of sustainable development is learning how to protect life on earth (Kunnskapsdepartementet, 2020b). To protect life on Earth, we need to understand the different species and how they live. By studying the Monarch butterfly, I developed a better understanding of how these insects use aspects of nature to their advantage. This is only a small part of life on Earth, but we need to understand all aspects to teach our future generations how to take care of Earth and all its life.

# Table of Contents

# 1. Introduction

## 1.1 Animal migration

Animal migration is a natural phenomenon that has fascinated humans for centuries. How migratory birds, sea turtles and insects find their way over thousands of kilometres is remarkable (Mouritsen, 2018). Migratory animals depend on visual and internal cues to guide them towards their goal, and one cue used by both insects and birds is the sky (Mouritsen, 2018). Insects adjust the sun's position after their circadian clock to keep their desired flight direction throughout the day (Perez et al., 1997), while nocturnal migratory songbirds need to learn the star pattern to use as a compass (Emlen, 1975). Celestial cues are weather and time dependent, meaning they are not always suitable as orientation cues. An optimal orientation cue should always be available, regardless of time and space. One such cue would be the Earth's magnetic field (Wiltschko, 1980).

## 1.2 Earth's magnetic field

The Earth's magnetic field is constantly available on any location on Earth throughout day and night, seasons, and weather, making it an ideal navigational cue for long-distance migration (Johnsen et al., 2020). It can be visualised as a giant bar magnet's dipole field, where the field lines radiate from the southern hemisphere, reaching around the globe before re-entering in the northern hemisphere (Fig. 1a) (Johnsen & Lohmann, 2005). The intensity of the magnetic field varies across the globe from 25 μT up to 65 μT, being weakest at the magnetic equator and strongest at the magnetic poles (Fig. 1a) (Fleischmann et al., 2020; Lohmann et al., 2022). The angle between the geographic north and the horizontal component of the magnetic field (magnetic north) is called the declination, while the inclination angle is the angle between the magnetic field lines and the Earth's surface (Fig. 1b) (Skiles, 1985). The inclination is 0° at the magnetic equator, where the field lines are parallel to the globe's surface before gradually changing to + 90° or − 90° at the magnetic poles (Fleischmann et al., 2020). In theory, if animals can detect some of the different aspects of the magnetic field, it can give them valuable cues they can use for directional or positional information (Clites & Pierce, 2017).

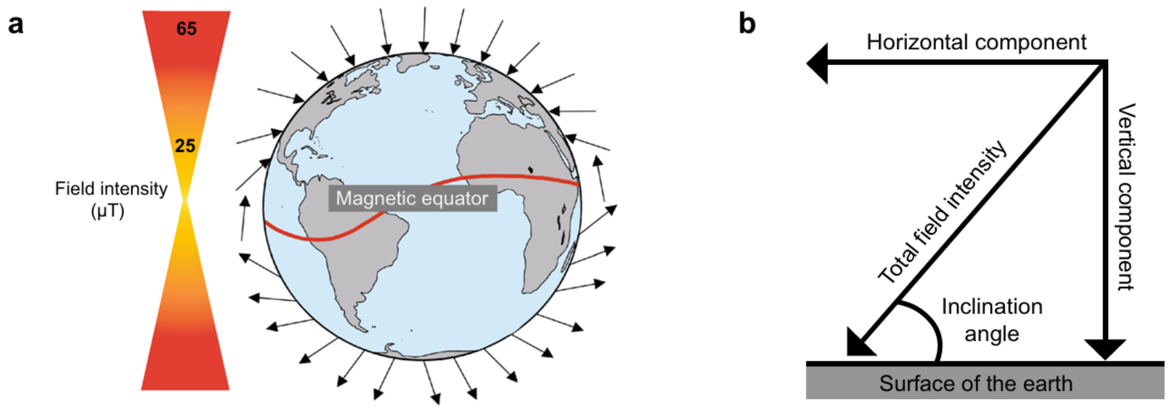


**Figure 1:** Earth's magnetic field. **a)** An illustration of the magnetic field that shows how the field lines (black arrows) leave the surface in the southern hemisphere, moving around the globe before returning in the northern hemisphere. The field lines are parallel to the Earth's surface at the magnetic equator (red line). Field intensity varies from the weakest at the magnetic equator before it increases to the strongest at the magnetic poles (colour gradient). **b)** An illustration of the four magnetic field components that provide orientation information to animals. The magnetic field

comprises the horizontal and vertical components, resolving in the total field intensity and inclination angle. Figures adapted from Lohmann et al. (2022).

Animals can use Earth's magnetic field as a map to assess their geographic location (Lohmann et al., 2022). Species with this ability are known to have a "magnetic map", which can be learned or inherited, and serve various purposes (Lohmann et al., 2022; Lohmann et al., 2007). Both the inclination and intensity of the magnetic field provide information used in a magnetic map (Lohmann et al., 2007). Research has shown that some sea turtles, fish, and birds utilise a magnetic map (Chernetsov et al., 2008; Lohmann et al., 2004; Putman et al., 2014). However, using a magnetic compass is more common among animals (Wiltschko & Wiltschko, 2005).

A magnetic compass provides animals with directional information that allows them to choose and maintain a heading, such as distinguishing north from south (Lohmann et al., 2022). Animals can use the polarity and inclination of the magnetic field for directional information (Solov'yov & Greiner, 2009). A study conducted on European Robins (*Erithacus rubecula*) tested different variations of turning both the horizontal and the vertical component of the magnetic field while observing the direction the bird was flying in. The results showed that they chose their direction by interpreting the inclination angle of the magnetic field (Wiltschko & Wiltschko, 1972). Similarly, also non-migratory birds, like pigeons, use an inclination compass for homing (Walcott & Green, 1974).

While magnetic orientation has been predominantly studied in vertebrates (Lohmann, 1991; Walcott & Green, 1974; Wiltschko & Wiltschko, 1972), there is growing evidence for its use by insects (Dreyer et al., 2018; Fleischmann et al., 2018; Guerra et al., 2014). A study conducted on the migrating nocturnal Bogong moth (*Agrotis infusa*) investigated the species' utilization of visual and magnetic cues (Dreyer et al., 2018). The study revealed that the animals headed in a predictable direction when visual landmarks and magnetic cues corresponded. However, when the cues conflicted, the animals became disoriented. Dreyer et al. (2018) emphasise that the conflict was detected after 2-3 minutes, revealing that the calibration mechanism between the visual landmarks and magnetic cues appears to be periodic. The study concludes that migratory Bogong moths can use a magnetic sense (Dreyer et al., 2018). Other migratory insects, such as the Monarch butterfly, have been suggested to use a similar magnetic sense (Mouritsen, 2018).

## 1.3 Monarch butterfly

Each fall, millions of Monarch butterflies (*Danaus plexippus*) from Canada and the northern United States, embark on a striking long-distance migration to reach their overwintering sites in Mexico (Reppert & de Roode, 2018). Monarchs migrate up to 4000 km to specific oyamel fir trees (*Abeies religiosa*) in Central Mexico (Reppert et al., 2010). This is a part of the species annual multi-generational migration cycle (Culbertson et al., 2022), which allows the butterflies to avoid cold temperatures and dying host plants in the winter months (Reppert & de Roode, 2018). As spring approaches with warmer temperatures, the overwintering butterflies become reproductive before they mate and start their journey northwards. The Monarchs will reproduce along the way, and new generations will continue to travel back north. Finally, the butterflies reach their northern breeding ground, where a new migratory generation will return to Mexico in the fall (Reppert & de Roode, 2018).

Monarch butterflies use different cues, such as the skylight, when orienting towards their overwintering sites (Mouritsen, 2018). It is known that the butterflies use a time-compensated sun compass, where they use the sun's position, adjusted for the time of day, to keep a steady heading in the desired direction (Mouritsen & Frost, 2002; Perez et al., 1997; Reppert et al., 2010). Research on Monarch butterflies has mostly focused on migrating populations, but there are populations that do not migrate. Non-migratory Monarch butterflies find food and breed in more confined areas, but the need to spatially orientate is still present (Franzke et al., 2020). Franzke et al. (2022) performed indoor behaviour experiments on tethered flying non-migrating butterflies, which showed that the butterflies maintained a steady direction when exposed to a single green LED, used to simulate the sun. The study revealed that regardless of their migratory or internal state, Monarch butterflies can keep a steady direction based on a simulated sun (Franzke et al., 2022). Making the non-migratory Monarch butterflies a valuable research subject for both migratory and non-migratory populations. The research done on migratory Monarch butterflies has shown that the butterflies manage to continue their migratory flight and remain oriented in the desired direction, even when the sky is overcast (Freas & Cheng, 2022). Since the sun compass is not always reliable, the Earth's magnetic field has been suggested as a backup mechanism for the butterflies (Reppert & de Roode, 2018).

Research on migratory Monarch butterflies has shown that they most likely use magnetic cues as a compass, not as a map (Guerra et al., 2014; Guerra et al., 2022; Mouritsen et al., 2013). Guerra et al. (2014) placed a magnetic coil system around a flight simulator to alternate the inclination angle of the magnetic field. By changing the inclination angle from + 45° to − 45°, the study showed that the animals interpret this as a 180° change in direction, confirming that they use an inclination compass for oriantation. The study also showed that the compass is light-dependent, utilising ultraviolet-A/blue light between 380 and 420 nm (Guerra et al., 2014). By measuring wing beat frequency, Wan et al. (2021) were able to show that also lab-raised animals, i.e. non-migratory animals, are sensitive to a change in magnetic inclination dependent on the availability of light in the UV-A/blue spectrum. In this study, the butterflies were unable to move in the horizontal plane, revealing that the butterflies had a higher motivation to fly in the presence of the Earth's magnetic field, but it provided no insights into the use of the magnetic field as a cue for orientation. In addition, they could show that cryptochrome 1 is essential for magneto sensation in Monarch butterflies. However, whether non-migratory Monarch butterflies can use the magnetic field to keep a direction during dispersal has not yet been studied.

## 1.4 Aim of study

This study aims to examine and further understand the role of Earth's magnetic field in non-migratory Monarch butterflies' orientation. The main aim is to examine non-migratory Monarch butterflies' ability to use the magnetic field as a compass, as no research shows this today. Another aim is to see if the Monarch prefer a simulated sun cue (green LED) or a magnetic cue, when presented both. Non-migrating Monarch butterflies were tethered in a flight simulator, surrounded by a three-dimensional Helmholtz coil system. By manipulating the magnetic field using the Helmholtz coils, I observed how the changes in the magnetic field affected the flight direction of the butterflies. Single green LEDs inside the flight simulator were used as a simulated sun cue. I hypothesise that if non-migratory Monarch butterflies use the magnetic field as a compass, they will follow the directional change of the magnetic cue when this is the only orientation cue being presented. When both cues are presented, I hypothesise that the non-migratory Monarch butterfly will follow the green LED since the sun is the most well-known cue that the migratory Monarch butterflies use today. Understanding if non-migrating Monarch butterflies use magnetic cues to orientate will supply more insights into the impact the Earth's magnetic field has on the orientation system of these butterflies, as well as the migratory Monarch butterflies and possibly other insects. My results open the possibility of using non-migratory and lab-raised Monarch butterflies to study magnetic orientation, from the behaviour to the brain and, ultimately, the receptor level.

# 2. Materials and methods

## 2.1 Experimental animals

Monarch butterfly (*Danaus plexippus*) pupae were obtained from Costa Rica Entomological Supply (butterflyfarm.co.cr) and kept in the Animal facility of the NTNU Trondheim. The animals were kept in an incubator (HPP 110, Memmert GmbH & Co. KG, Schwabach, Germany) at 25°C, 80% relative humidity, with a 12h:12h light: dark cycle, until eclosed. Adult butterflies were transferred into a flight cage at 25°C, with a 12h:12h light: dark cycle. Males and females were separated into different cages. At all times, feeders containing 15% sucrose solution were available for the animals in the flight cages.

## 2.2 Preparation of animals

To prepare the animals for experiments, they were placed on a table with their wings open and their thorax accessible (Fig. 2a). The hair and scales on the Monarch's thorax were removed using cotton pads and tape. A small amount of instant contact adhesive glue (multi-purpose impact instant contact adhesive, EVO-STIK, Bostik Ltd, Stafford, UK) was placed on the butterfly's thorax and on a tungsten stalk (0.508 x 152.4 mm, Science Product, GmbH, Hofheim, Germany). After allowing the glue to pre-dry for three minutes, the tungsten stalk was placed on the animal's thorax (Fig. 2b). After letting the glue completely dry for 15 minutes, the butterfly was put into a plastic cup for at least two hours in a dark environment before participating in experiments. The Monarchs had access to 15 % sugar water in the plastic cups.

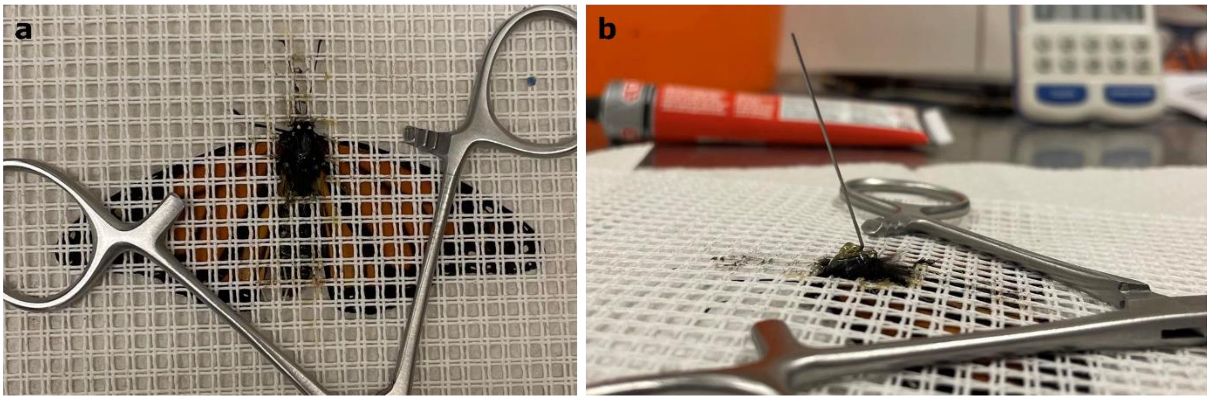


**Figure 2:** Stalking of Monarch butterflies for flight simulator experiments. **a)** A butterfly was placed with its wings open and thorax accessible under a mesh with weights to immobilise it. **b)** A tungsten stalk was glued to a butterfly thorax.

## 2.3 Experimental setup

A three-dimensional Helmholtz coil (HHS 3D 5213-50, Schwarzbeck Mess-Elektronik, Schönau, Germany) was placed inside a Faraday cage to present the butterfly with precise and homogeneous manipulated magnetic fields. A power supply (HMP4020, Programmable power supply 381 W, Rhode&Schwarz, Munich, Germany) provided current to the coils, which was programmed using an HMEscript (HMExplorer 1.7, SCPI Terminal 2.1) to change the current automatically throughout the experiments. Before and after each day of experiments, the magnetic field inside the coils was measured using a magnetometer (FVM-400, Vector magnetometer, Macintyre Electronic Design Associates, Dulles, VA, USA) to control the direction and strength of the field. The strength of the magnetic field was set to about 41 µT, which was the natural intensity found in Trondheim during the experiments. All experiments were conducted between 9 am and 5 pm.

Inside the Helmholtz coils, the butterflies were placed in the centre of a flight simulator, similar to the ones previously described (Dreyer et al., 2018; Mouritsen & Frost, 2002). All the inner surface of the simulator was covered in black fabric to avoid light reflections. In geomagnetic south, a green LED (Emission peak = 520 nm; LZ1-00G102, Osram, San Jose, CA, USA) simulating the sun (Edrich et al., 1979; el Jundi et al., 2015) was attached to the flight simulator. To displace the artificial sun by - 120° another green LED was placed in the North-East (Fig. 3c). The light intensity of both LEDs was adjusted to $3.7 \times 10^{14}$ photons/cm$^2$/s.

Previous studies have shown that magnetoreception in Monarch butterflies is light-dependent (Guerra et al., 2014; Wan et al., 2021). To give the butterflies the right light conditions during the experiments, I mounted 18 LEDs evenly on an aluminium plate (Fig. 3b). Six white LEDs (Emission peak = 5500 K; LZ1-10CW02-0055, Osram, San Jose, CA, USA), and two different UV LEDs: six blue UVs (Emission peak = 410; LZ4-V0UBH0-00U8, Osram, San Jose, CA, USA) and six violet UVs (Emission peak = 365 nm; LZ1-10UV00, Osram, San Jose, CA, USA). The plate was attached to the ceiling of the Faraday cage, simulating the sky. A diffusion paper was placed in front of the lights to distribute the light in the simulator. The light intensity from all skylight LEDs was set to $1.5 \times 10^{15}$ photons/cm$^2$/s (see Appendix 1), similar to the intensity used in previous studies (Guerra et al., 2014; Wan et al., 2021). All the LEDs were powered by power supplies (HMP2020, Programmable power supply 188W, Rhode&Schwarz, Munich, Germany). A dark garment was drawn around the flight simulator to avoid reflection (Fig. 3a).

To record the heading direction of the butterfly, the tungsten stalk on the animal was connected to an optical encoder (E4T miniature Optical Kit Encoder, US Digital, Vancouver, WA, USA) (Fig. 3d). The direction was recorded with an angular resolution of three degrees and a temporal resolution of 200 ms using a data acquisition device (USB4 Encoder Data Acquisition USB Device, US Digital, Vancouver, WA, USA) and a computer with the corresponding software (USB1, USB4: US Digital, Vancouver, WA, USA). A camera (USB Camera Module Megapixel USB Camera, ELP, Guangdong, China) was placed below the flight simulator to record and observe if the butterflies were constantly flying. All animals that stopped more than three times in an experiment were excluded from the study.

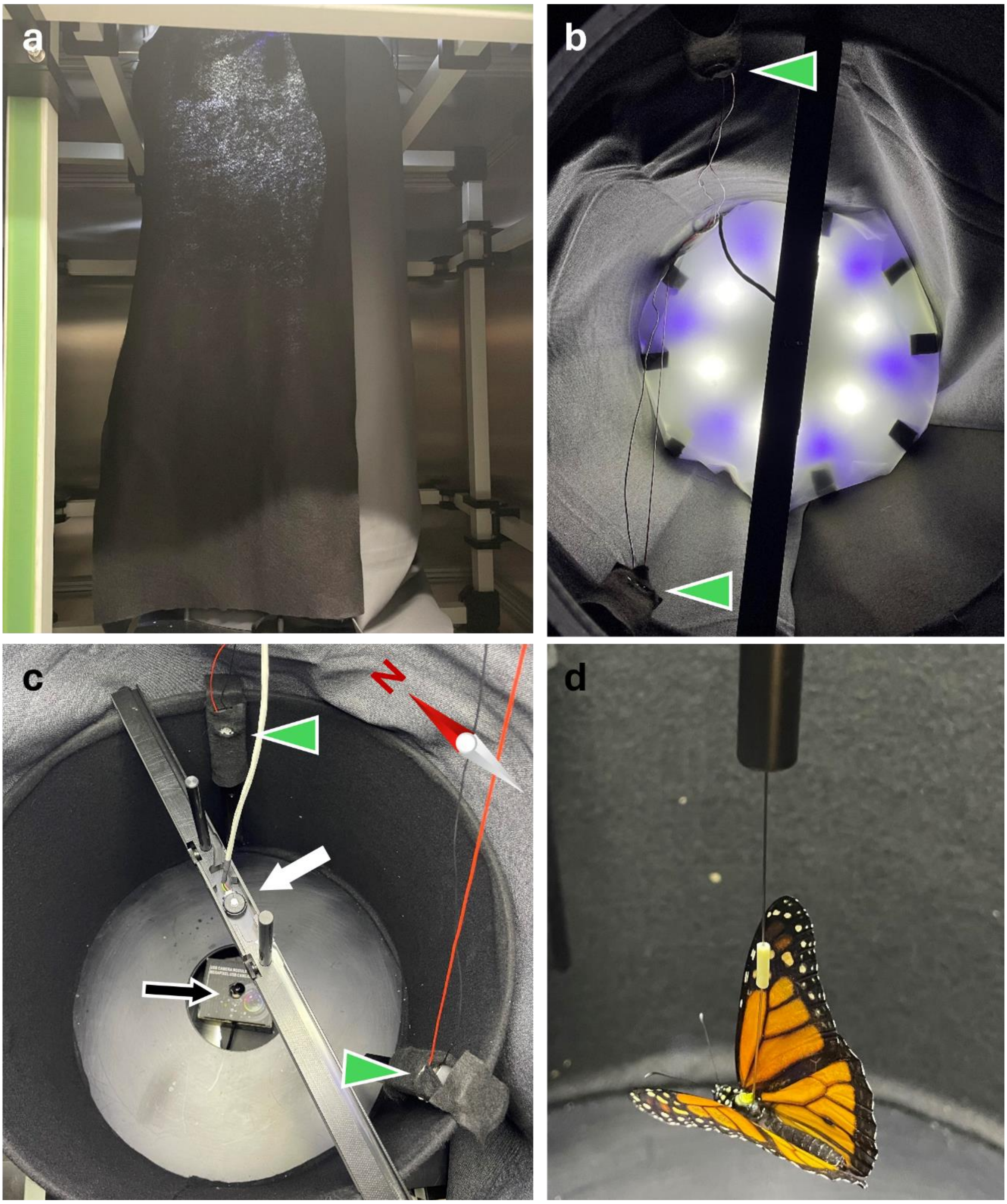

**Figure 3:** Flight simulator setup in the 3D-Helmholtz coil. **a)** The flight simulator was covered by dark fabric to keep ambient light out and avoid reflections. **b)** Two green LEDs (green arrowheads) acted as artificial suns in the setup. The flight simulator was illuminated from above with white, blue and UV LEDs behind a diffusion paper (for details see text). **c)** A camera (black arrow) was placed centred under the digital encoder (white arrow) to record the flying animal. One artificial sun (green LED (green arrowhead)) was placed in geomagnetic south, and another one was placed - 120°, to the north-east. **d)** A Monarch butterfly was attached through a tungsten wire, which has been glued to the animal's thorax, to the digital encoder.

## 2.4 Performance of experiment

### *2.4.1 Magnetic field as an orientation cue experiment*

The experiment comprised eight phases, each lasting for 90 seconds. In the first two phases (Phase 1 and Phase 2), the butterfly was presented with both cues: a green LED in magnetic south and the natural magnetic field (Fig. 4). When the butterfly was given natural magnetic conditions, the Helmholtz coil was turned off. In Phase 3, the green LED was displaced by - 120°. By turning the Helmholtz coil on and powering the two coil pairs manipulating the x- and y-axis, I also turned the horizontal component of the magnetic field by - 120°. This turned both the declination and the inclination of the magnetic field. Thus, despite being displaced, the green LED remained in magnetic south. In Phase 4 and Phase 5, the light cue was turned off, and the butterfly was only given natural magnetic information, i.e. turned back to the original position. In Phase 6 and Phase 7, I turned the horizontal component of the magnetic field by - 120°. Finally, in Phase 8, the green LED was turned back to its original position (Phase 1), while the Helmholtz coil was turned off, presenting the butterfly with natural magnetic conditions.

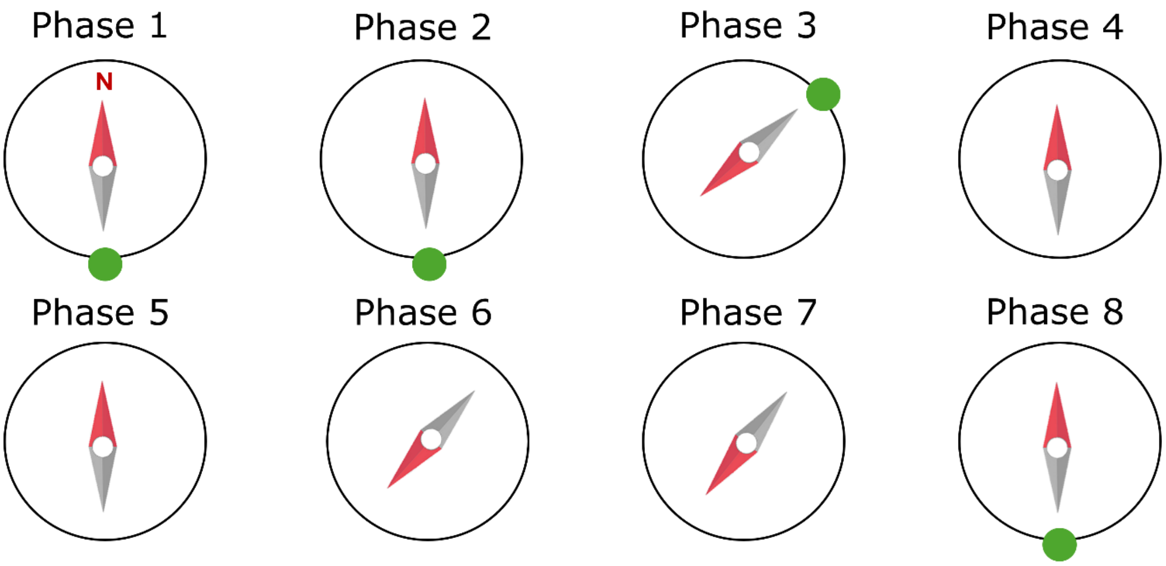


**Figure 4:** Experimental conditions during the phases of the magnetic field as an orientation cue experiment. The green circle illustrates the green LED, and the magnetic needle shows how the magnetic field was turned, with the red part pointing to magnetic north (N). In phases 1 and 2, the green LED was positioned in magnetic south, and the butterfly was given natural magnetic conditions. In Phase 3, both cues were turned by - 120°, thus being displaced, the cues were still positioned in magnetic south. In phases 4 and 5, the animals were only provided with natural magnetic information. In phases 6 and 7, the magnetic cue was turned by - 120°. The artificial sun cue and magnetic cue were directed back to their original position in Phase 8.

### *2.4.2 Conflict experiment*

A conflict experiment was conducted to see if the non-migratory Monarch butterflies have a preferred orientation cue. The experiment consisted of three phases, each lasting 90 seconds. In phases 1 and 2, butterflies were presented with an artificial sun cue (the green LED) placed in geomagnetic south and natural magnetic conditions, i.e. Helmholtz coil turned off (Fig. 5). In Phase 3, the two cues were set in conflict, with the green LED displaced by - 120° and the horizontal component of the magnetic field was turned by + 120°.

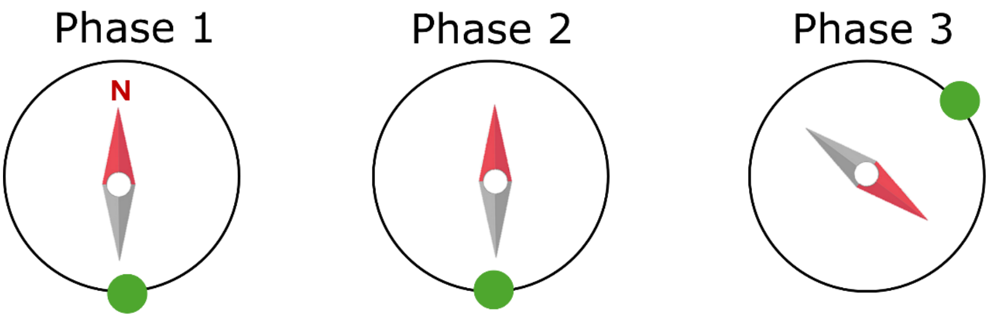


**Figure 5**: Experimental conditions during the phases of the conflict experiment. The green circle illustrates the green LED placement. The compass needle shows how the magnetic field was directed, with the red part pointing towards magnetic north (N). In phases 1 and 2, the green LED was positioned in magnetic south, and the butterfly was given natural magnetic conditions. In Phase 3, the artificial sun cue was displaced by - 120°, while the horizontal component of the magnetic field was turned by + 120°.

## 2.5 Data analysis

The flight directions were calculated by importing the data into MATLAB (Version R2023b, MathWorks, Natick, MA, USA) and analysed using the CircStat toolbox. In the first experiment, only the animals with a stronger mean vector strength (r) than 0.2, in Phase 2 and Phase 3, were used for the analysis. It is established that animals with a higher r-value than 0.2 are directed (Dreyer et al., 2021; Franzke et al., 2020), which is the measurement that will be used in this study. It's known that the non-migratory Monarch butterflies can use a simulated sun cue to keep a flight direction (Franzke et al., 2022; Mouritsen & Frost, 2002). Therefore, in phases 2 and 3, a simulated sun cue was used to test if the butterflies reacted to the given cues. Only the animals that used the sun cue by turning their flight direction by 60° or more between Phase 2 and Phase 3 were used for the analysis. These conditions discarded 3 out of 10 animals that completed the experiment. In the conflict experiment, all animals that flew through the experiment were used in the analysis. Because of the small sample size, I was not able to do all the planned statistics.

The angular velocity data was analysed and presented in R 4.2.1. A mean was calculated with all the animals every 200ms and plotted in R. A violin plot was used to plot the last ten seconds of Phase 3 and Phase 5 and the first ten seconds of Phase 4 and Phase 6. Mann-Whitney U test was used to check if there was a difference between the two phases. The circular statistics were performed with Oriana 3. A Rayleigh test was used to check if the data was randomly distributed or directed, with a significance level of $\alpha = 0.05$. If the data was directed, a 95% confidence interval is stated. For the individual butterfly, A Mardia Watson Wheeler test was performed in Oriana to calculate if the data in each phase were different.

It is established that some insects take longer to register changes in the magnetic field (Dreyer et al., 2018). That is why Phase 4 and Phase 6, in the magnetic field as an orientation cue experiment (Fig. 4), are used as transition phases, giving the butterflies more time to detect the magnetic cues. Therefore, only the data from phases 5 and 7 will be presented and used in the analysis.

# 3. Results

## 3.1 Magnetic field as an orientation cue experiment

To investigate whether the non-migratory Monarch butterflies use the Earth's magnetic field to orient by, I recorded their flight direction while tethered at the centre of a flight simulator. In total, 14 Monarch butterflies participated in the experiment.

### *3.1.1 The angular velocity*

The butterflies decrease their angular velocity by - 0.18 deg/s ($p < 0.001$, Linear regression) throughout the experiment (Fig. 6). Phase 1 and Phase 2 consist of the same conditions, but the angular velocity is much higher in Phase 1 (Phase 1: $\mu = 217.66$ deg/s, Phase 2: $\mu = 130.85$). This indicates that Phase 1 is an acclimation phase for the animals to get used to the flight simulator. Therefore, Phase 2 will be used as the starting phase of the experiment. A small peak in the mean angular velocity can be observed between phases 3 and 4 (Fig. 6). After phase 4, the mean velocity of the animals slowly declines throughout the experiment, with no apparent visual instant reaction to the changing cues (Fig. 6).

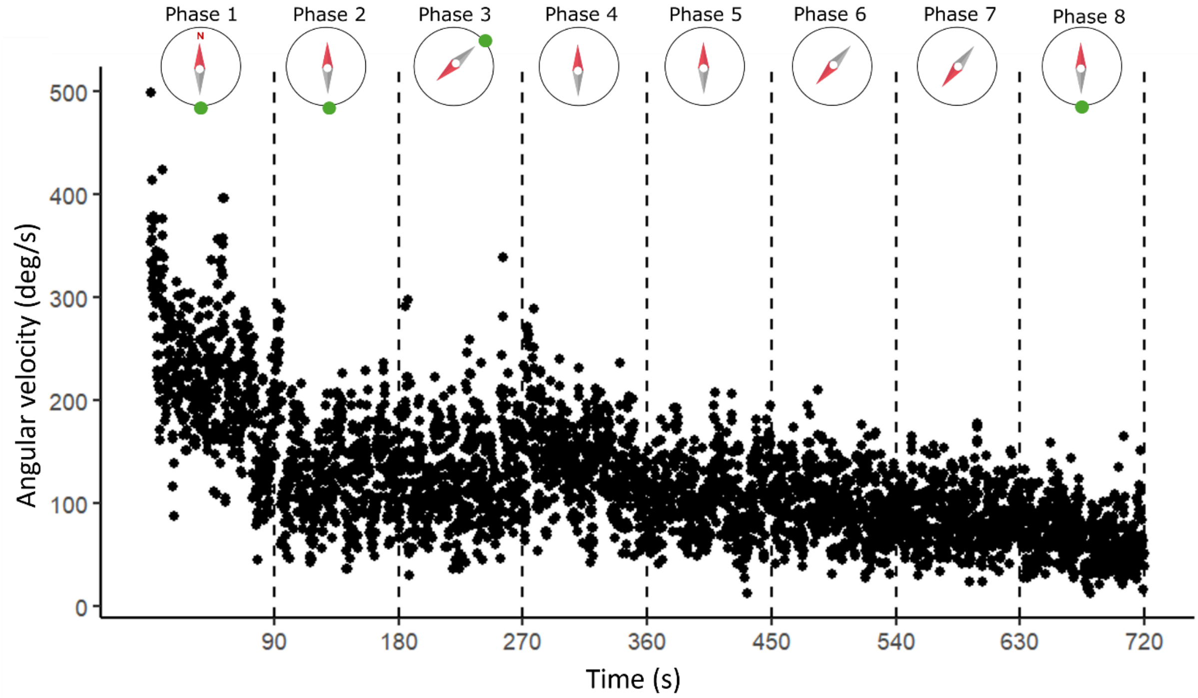


**Figure 6:** Change in angular velocity during magnetic manipulations ($n = 7$). One black dot represents the mean angular velocity for 7 animals, every 200 ms throughout the experiment. The icons at the top show the conditions in each phase, with the dashed vertical lines displaying when the phases change. The mean angular velocity appears to be highest in Phase 1 before gradually declining throughout the experiment ($\beta = -0.18$ deg/s, $p < 0.001$, Linear regression). Between phases 3 and 4, a small peak in the angular velocity is observed.

The last ten seconds of Phase 3 and the first ten seconds of Phase 4 were analysed further to investigate the observed peak in mean angular velocity between the two phases (Fig. 7a). A significant difference was found, with higher angular velocity at the beginning of Phase 4, than at the end of Phase 3 (Mann-Whitney U test: $n_{Phase\ 3} = 7$, $n_{Phase\ 4} = 7$, $p = 1.77e\text{-}05$, $\chi^2 = 18.42$; last ten s of Phase 3: $\mu = 131.3°/s$, first ten s of Phase 4: $\mu = 176.6°/s$). To see if there was an instant change in the butterflies' angular velocity between phases 5 and 6, where only the magnetic field cue was changed, the first and last ten seconds of the two phases were analysed (Fig. 7b). A significant difference between the two phases was observed, with more activity at the end of Phase 5 than at the beginning of Phase 6 (last ten s of Phase 5: mean vector μ = 117.9°/s, first ten s of Phase 6: μ = 99.5°/s, Mann-Whitney U test: $n_{Phase\ 5}$ = 7, $n_{Phase\ 6}$ = 7, p = 0.046, $\chi^2$ = 3.99).

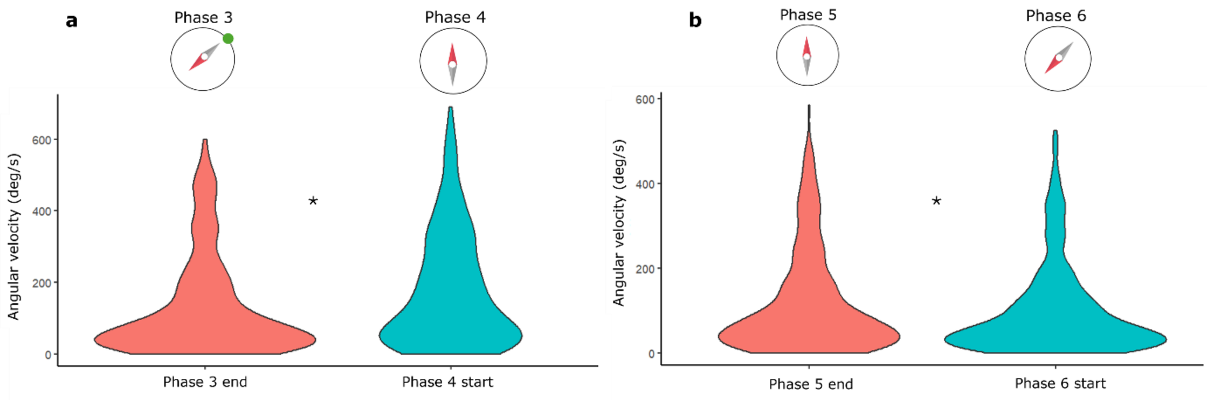


**Figure 7:** Change in angular velocity for ten seconds of specific phases in magnetic orientation as an orientation cue experiment, plotted as a violin plot (*n* = 7). **a)** Change in angular velocity for the last ten seconds of Phase 3 and the first ten seconds of Phase 4. The angular velocity is higher in Phase 4, than in Phase 3, indicating that the butterflies turn faster at the beginning of Phase 4 (last ten s of Phase 3: μ = 131.3°/s, first ten s of Phase 4: μ = 176.6°/s). A significant difference was found between phases 3 and 4 (Mann-Whitney U test: $n_{Phase\ 3}$ = 7, $n_{Phase\ 4}$ = 7, p = 1.77e-05, $\chi^2$ = 18.42). **b)** Change in angular velocity for the last ten seconds of Phase 5 and the first ten seconds of Phase 6. The angular velocity is higher in Phase 5 than in Phase 6, meaning they turn slower in Phase 6 (last ten s of Phase 5: mean vector μ = 117.9°/s, first ten s of Phase 6: μ = 99.5°/s). A significant difference was found between the two phases (Mann-Whitney U test: $n_{Phase\ 5}$ = 7, $n_{Phase\ 6}$ = 7, p = 0.046, $\chi^2$ = 3.99).

### *3.1.2 Flight direction*

The experiment started and ended with the same conditions (Phase 2 and Phase 8). Comparing these two phases showed that 71% (5 out of 7) of the butterflies kept a similar heading direction in both phases (Fig. 8), revealing that the butterflies interpreted the given cues similarly and were motivated throughout the experiment.

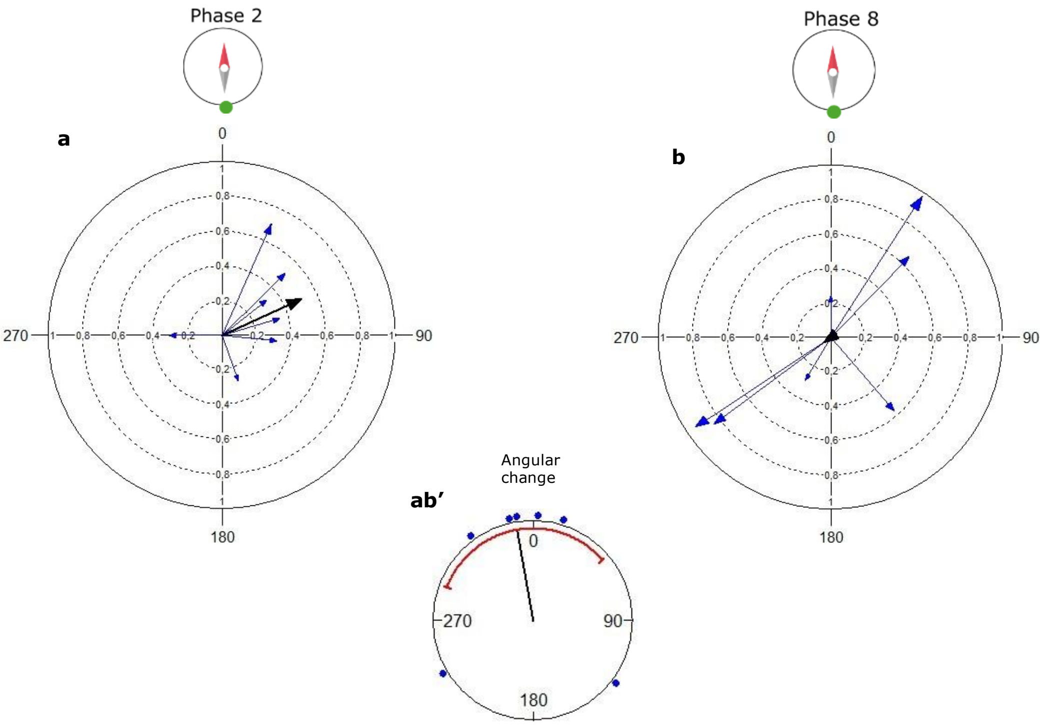


**Figure 8:** Change in flight direction in Phase 2 compared to Phase 8 *(n* = 7). Blue arrows show the individual butterfly's mean flight direction and its directedness (mean vector strength r). The black arrow represents the group's mean vector strength and direction. The vector strength can vary from 0 (disorientated) to 1 (perfectly orientated), and the inner dashed line shows r = 0.2. **a)** The flight direction of the animals during Phase 2 (Rayleigh test of uniformity: p = 0.175, Z = 1.759, μ = 65.3°, Length of mean vector: r = 0.501). **b)** The animal's flight direction during Phase 8 (Rayleigh test: p = 0.985, Z = 0.016, μ = 233.7°, r = 0.048). **ab')** On a group level, the Monarchs did not change their mean flight direction in Phase 2 and Phase 8 (Rayleigh test: p = 0.147, Z = 1.924, μ = 349.9°, r = 0.524, 95% Confidence Interval (-/+): 291.0°/48.7°).

Phase 2 is considered the starting phase of the experiment. During Phase 2, most animals kept a directed flight direction, but chose different headings (Rayleigh test: p = 0.175, Z = 1.759, μ = 65.3°, r = 0.501, Fig. 9a). All animals had a higher directedness, represented by the vector strength r, higher than 0.2, suggesting that all animals were directed (see Appendix 2). In Phase 3, the green LED and the horizontal component of the magnetic field were turned by - 120°. As a group, they changed their direction by 120° or more between Phase 2 and Phase 3 (Rayleigh test: p = 0.02, Z = 3.628, μ = 152.6°, r = 0.72, 95% Confidence Interval (-/+): 109.1°/196.2°, Fig. 9ab').

In Phase 5, the simulated sun (green LED) is turned off, and the butterfly is only given the natural magnetic field as an orientation cue. As a group, the animals did not have a common flight direction in Phase 5 (Fig. 9c) and did not show a groupwide change in direction (Rayleigh test: p = 0.603, Z = 0.534, μ = 91.4°, r = 0.276, Fig. 9bc'). Neither in Phase 7, when the magnetic cue was turned by - 120°, did the butterflies keep a common flight direction (Rayleigh test: p = 0.591, Z = 0.556, r = 0.282, Fig. 9d). Only one animal changed its flight direction as expected by about - 120°, between phases 5 and 7 (Fig. 9cd').

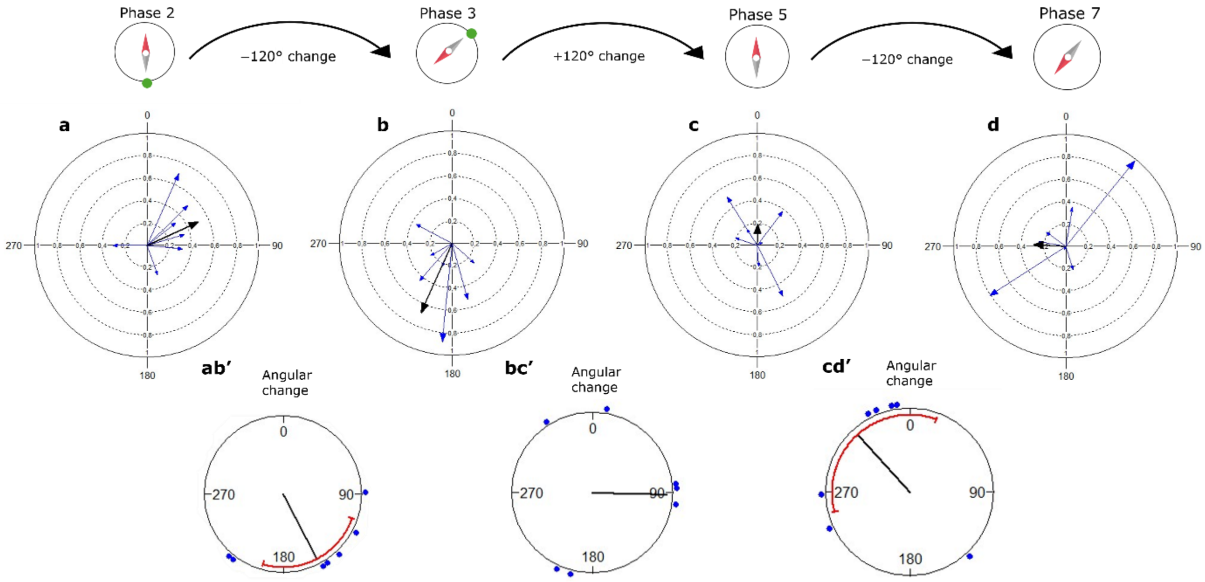


**Figure 9:** Change in flight direction during magnetic manipulations ($n$ = 7). **a)** The flight direction of the butterflies in Phase 2 (Rayleigh test: p = 0.175, Z = 1.759, μ = 65.3°, r = 0.501). **b)** The flight direction of the butterflies in Phase 3 (Rayleigh test: p = 0.036, Z = 3.166, μ = 203.7°, r = 0.672, 95% Confidence Interval (-/+): 163.9°/243.5°). **ab')** The angular change between phases 2 and 3 (Rayleigh test: p = 0.02, Z = 3.628, μ = 152.6°, r = 0.72, 95% Confidence Interval (-/+): 109.1°/196.2°). **c)** The flight direction of the Monarchs in Phase 5 (Rayleigh test: p = 0.798, Z = 0.241, μ = 1.4°, r = 0.185). **bc')** The angular change between Phase 3 and Phase 5 (Rayleigh test: p = 0.603, Z = 0.534, μ = 91.4°, r = 0.276). **d)** The flight direction of the butterflies in Phase 7 (Rayleigh test: p = 0.591, Z = 0.556, μ = 273.2°, r = 0.282). **cd')** On a group level, the butterflies did not change their mean flight direction between Phase 5 and Phase 7 (Rayleigh test: p = 0.17, Z = 1.791, μ = 317.7°, r = 0.506, 95% Confidence Interval (-/+): 255.5°/19.8°). For figure conventions, see Fig. 8.

### *3.1.3 Individual Monarch butterfly*

While the group data ($n$ = 7) did not provide very clear results, the magnetic field might be used by some animals, and it is more visible at the individual level. One Monarch butterfly out of the seven tested, number 48, followed the changing magnetic cues nearly as expected (Fig. 10). When provided with a simulated sun cue and natural magnetic conditions (Phase 2), the butterfly was clearly directed (Rayleigh test: p < 1E-12, Z = 219.64, μ = 23.9°, r = 0.699, 95% Confidence Interval (-/+): 19.4°/28.3°, Fig. 10b). In Phase 3, the butterfly changed its mean flight direction (see statistics in Fig. 10c) making a clear change in direction from Phase 2 ($n$ = 28, p < 1E-12, W = 436.346, Mardia Watson Wheeler test). When the green LED was turned off, the animal was only given the natural magnetic field as a cue (Phase 4 and Phase 5). The butterfly's mean flight direction changed between Phase 3 and Phase 4 ($n$ = 28, p < 1E-12, W = 232.622, Mardia Watson Wheeler test). Interestingly, when the sun stimulus was withheld, and the magnetic field was turned by + 120° (between phases 3 and 4), the animal turned its mean heading by about + 110° (see statistics in Fig. 10c & 10d). The butterfly flew in the same direction in phases 4 and 5 (see statistics in Fig. 10d & 10e). Next, the horizontal component of the magnetic field was turned by - 120° (phases 6 and 7). In Phase 6, the butterfly changes its mean flight direction by about 50° from Phase 5, before turning additionally 40° in Phase 7 (Fig. 10f & 10g). The butterfly flew in the same direction in Phase 3 and Phase 7 ($n$ = 28, p = 0.495, W = 542.784, Mardia Watson Wheeler test, Fig.

10c & 10g). Finally, in Phase 8, the butterfly kept its flight direction from Phase 7 ($n$ = 28, p = 0.745, W = 0.106, Mardia Watson Wheeler test, Fig. 10g & 10h).

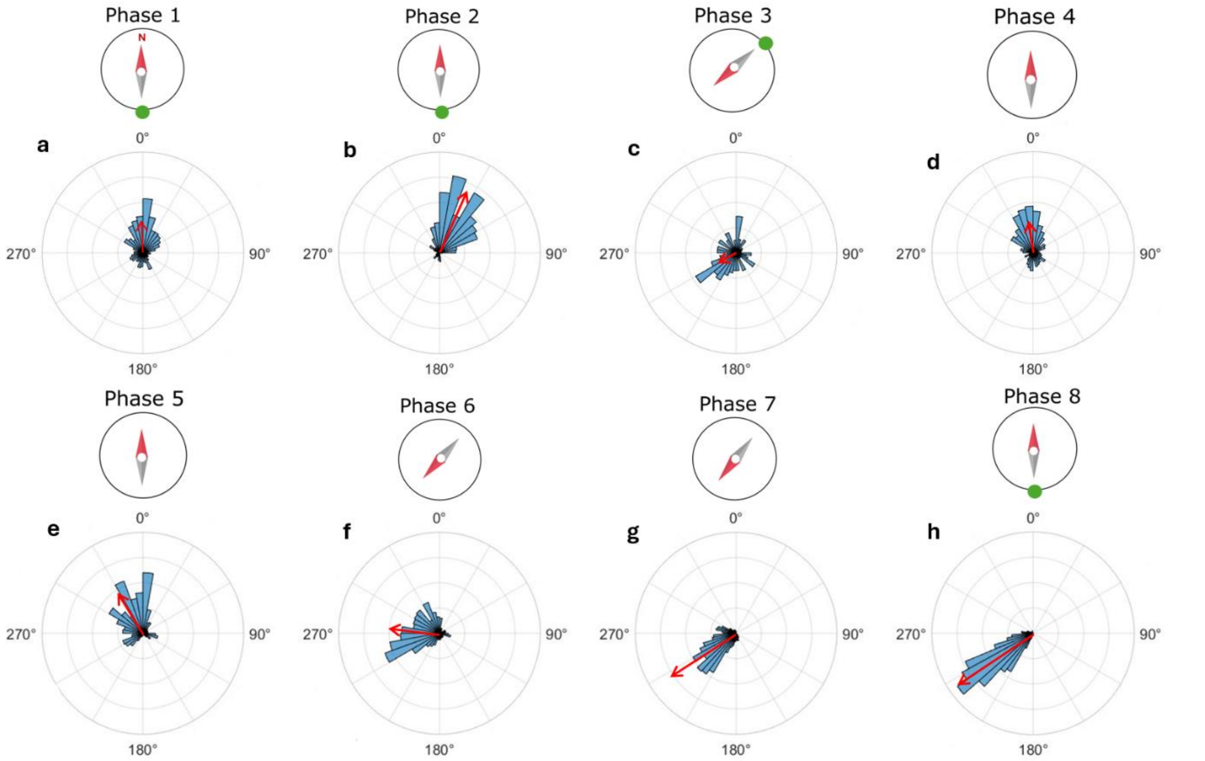


**Figure 10:** The flight direction of Monarch butterfly number 48 during magnetic manipulations. The blue bins comprise 10° and the outer circle represents 80 data points. The red arrow displays the mean vector strength for each phase, with the outer circle having a vector strength of 1. Each phase comprises 450 data points. **a)** The flight direction of an individual butterfly in Phase 1 (Rayleigh test: p < 1E-12, Z = 50.239, μ = 356.6°, r = 0.334, 95% Confidence Interval (-/+): 345.8°/7.5°). **b)** The flight direction of an individual butterfly in Phase 2 (Rayleigh test: p < 1E-12, Z = 219.64, μ = 23.9°, r = 0.699, 95% Confidence Interval (-/+): 19.4°/28.3°). **c)** The flight direction of an individual butterfly in Phase 3 (Rayleigh test: p = 1.73E-10, Z = 22.477, μ = 240.9°, r = 0.223, 95% Confidence Interval (-/+): 224.4°/257.5°). **d)** The flight direction of an individual butterfly in Phase 4 (Rayleigh test: p < 1E-12, Z = 50.921, μ = 351.3°, r = 0.336, 95% Confidence Interval (-/+): 340.5°/2.1°). **e)** The flight direction of an individual butterfly in Phase 5 (Rayleigh test: p < 1E-12, Z = 116.393, μ = 328.4°, r = 0.509, 95% Confidence Interval (-/+): 321.6°/335.3°). **f)** The flight direction of an individual butterfly in Phase 6 (Rayleigh test: p < 1E-12, Z = 128.521, μ = 277.1°, r = 0.534, 95% Confidence Interval (-/+): 270.6°/283.5°). **g)** The flight direction of an individual butterfly in Phase 7 (Rayleigh test: p < 1E-12, Z = 295.073, μ = 237.1°, r = 0.81, 95% Confidence Interval (-/+): 233.7°/240.5°). **h)** The flight direction of an individual butterfly in Phase 8 (Rayleigh test: p < 1E-12, Z = 398.027, μ = 236.5°, r = 0.94, 95% Confidence Interval (-/+): 234.6°/238.3°).

## 3.2 Conflict experiment

A conflict experiment was performed to see if the animals preferred visual cues over magnetic cues. All animals that flew throughout the experiment were used in the analysis ($n$ = 13). In Phase 2, the butterflies were exposed to a simulated sun cue placed in geographic south and natural magnetic conditions (Rayleigh test: p = 1.42E-4, Z = 7.561, μ = 71.0°, r = 0.763, 95% Confidence Interval (-/+): 45.4°/96.7°, Fig. 11a). Only 5 animals were directed in phase 2 (see Appendix 3), meaning 62% of the animals were disoriented in this phase (Fig. 11a). In phase 3, the sun cue was displaced by -120°, while the horizontal component of the magnetic field was changed by + 120°. The overall individual directedness of the butterflies was higher in Phase 3, 62% had a stronger r value than 0.2 (see Appendix 3, Fig. 11b). On a group level, the mean flight direction was higher in Phase 2 than in Phase 3 (Phase 2: r = 0.763, Phase 3: r = 0.263). Phase 2 and Phase 3 are significantly different (p = 0.002, W = 12.279, Mardia Watson Wheeler test). On a group level, the butterflies changed their flight direction by about 180° (Fig. 11ab').

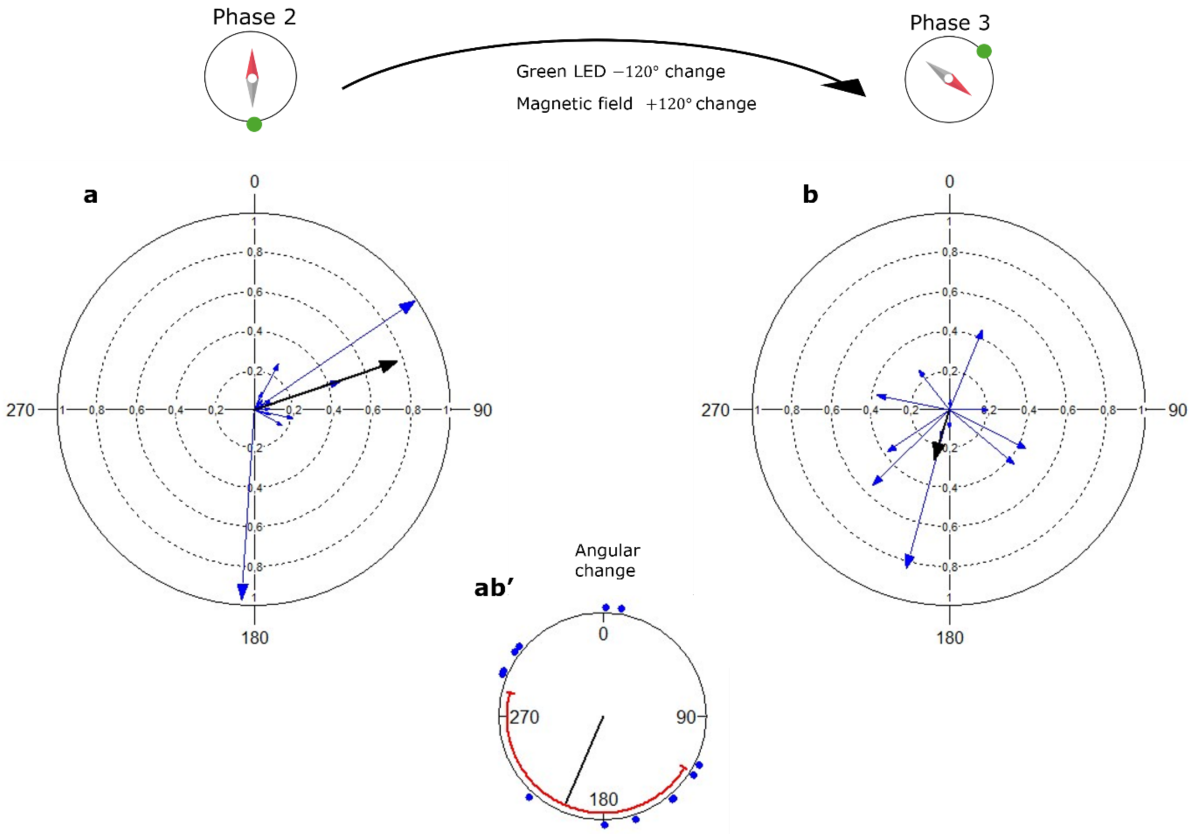


**Figure 11:** Change in flight direction when exposed to a conflict between cues (*n* = 13). **a**) The flight direction of Monarch butterflies in phase 2 (Rayleigh test: p = 1.42E-4, Z = 7.561, μ = 71.0°, r = 0.763, 95% Confidence Interval (-/+): 45.4°/96.7°). **b)** The flight direction of the butterflies in Phase 3, when the two cues are set in conflict (Rayleigh test: p = 0.416, Z = 0.898, μ = 196.3°, r = 0.263). **ab')** The angular change between Phase 2 and Phase 3 (Rayleigh test: p = 0.884, Z = 0.128, μ = 203.5°, r = 0.099). For figure conventions, see Fig. 8.

# 4. Discussion

## 4.1 Flight behaviour over time

The observation that the butterflies had a higher angular velocity in Phase 1 (Fig. 6) supports using this phase as an acclimation phase, which is consistent with a previous study indicating that the butterflies need time to adapt to the experimental setup (Franzke et al., 2020). The decrease in angular velocity observed from Phase 2 onwards (Fig. 6) indicates that the butterflies can keep a more stable flight direction once acclimated.

## 4.2 Sun compass orientation in the flight simulator

My results show that the butterflies were able to keep a constant direction towards the green LED (Phase 2, Fig 9a). The animals do not show phototactic behaviour, i.e. flying directly to the light, but keep an arbitrary direction. Such behaviour is called menotaxis (Grob et al., 2021). This behaviour is also seen in Franzke et al. (2022) on non-migratory Monarch butterflies. On a group level, the non-migratory Monarch butterflies changed their flight direction between Phase 2 and Phase 3, following the cue changes (Fig. 9a, 9b, 9ab'). In these phases, a simulated sun cue and a magnetic cue were given. This behaviour is part of my selection criteria for the analysis since the literature shows that Monarch butterflies can follow a sun cue (Mouritsen & Frost, 2002; Perez et al., 1997) and an artificial sun cue (Franzke et al., 2022).

I found a peak in the mean angular velocity between Phase 3 and Phase 4 (Fig. 6 & 7a) when the magnetic field changed, and the sun cue was turned off. This peak is likely due to the lack of a simulated sun cue. It is known that both migratory (Perez et al., 1997) and non-migratory (Franzke et al., 2022) Monarch butterflies use the sun or a simulated sun as an orientation cue. When the visual sun cue disappears, the Monarchs become less directed (Mouritsen & Frost, 2002). When the sun is excluded from the animal's view, they can fall back on other compass cues like the panoramic skyline, as found in non-migratory Monarch butterflies, but they are less directed over time than when using the sun cue (Franzke et al., 2020). Taken together, my sun compass orientation results are in line with previous studies and show that the newly developed setup is suitable for studying compass orientation in Monarch butterflies.

## 4.3 Magnetic compass orientation

### *4.3.1 Reaction to magnetic changes*

Throughout the experiment, the animals turned less, with no visual obvious behavioural reaction to the changing magnetic cues (Fig. 6 & 7b), indicating that the Monarch butterfly is not sensitive to changes in the magnetic field. This does not support Wan et al. (2021) findings, where they measured the wing beat frequency. Both measurements reflect the butterflies' responsiveness to environmental cues, suggesting that changes in angular velocity could indicate behavioural adjustments like those observed in wing beat frequency. If the butterflies were sensitive to changes in the magnetic field, I would assume we would see increased turning when the magnetic cue changes, as between phases 5 and 6. The results showed that the butterflies were more active at the end of Phase 5 than at the beginning of Phase 6 (Fig. 7b), not supporting my assumption. However, differences can be found between our studies. Wan et al. (2021) changes the inclination angle of the magnetic field, as done by Guerra et al. (2014), while I change the direction of the polarity and inclination of the magnetic field. The non-migratory Monarch butterflies I used are from a population in Costa Rica that never migrates, while the butterflies used by Wan et al. (2021) are a lab-raised population of Monarchs found in the United States. Since the lab-raised population from the United States originates from the migratory ones, their genetics might make them more sensitive to detecting the magnetic field. Since the magnetic field is a global cue, I would assume that the migratory butterflies need to use this cue more than the non-migratory populations. The consequences of not keeping the direction are far worse for the migratory populations than for the non-migratory butterflies. Given that the animals I used and Wan et al. (2021) used are from different populations, their need and ability to use the magnetic field might differ.

*4.3.2 Compass orientation*

On a group level, the non-migratory Monarch butterflies changed their flight direction with the given cues, between Phase 2 and Phase 3 (Fig. 9ab'). In these phases, a simulated sun cue and a magnetic cue were given. Whether or not the butterflies use both cues or only one cannot be determined based on these two phases. To test this, I only presented the animals with the magnetic cue in phases 4-7. In Phase 5, the butterflies were given natural magnetic conditions, and in Phase 7, the magnetic cue was turned by - 120° (Fig. 4). On a group level, I did not find results showing that the non-migratory Monarch butterflies use the magnetic field as a compass (Fig. 9bc' & 9cd'). This does not support Guerra et al. (2014) findings on migrating Monarch butterflies. However, in that study, they found that the butterflies used an inclination compass to orientate when they changed the inclination angle from + 45° to − 45°, which the butterflies interpreted as a 180° change in direction (Guerra et al., 2014). In my experiment, I changed the horizontal component of the magnetic field. By doing this, the magnetic field's direction of both inclination and polarity changes (Fleischmann et al., 2020), not the inclination angle, as in Guerra et al. (2014). Consequently, we do not know which parameters the non-migratory Monarch butterfly might use. This was irrelevant to this experiment since I only aimed to establish whether they used the magnetic field for compass orientation. However, by doing the change in direction, I get to test more parameters of the magnetic field than they do in Guerra et al. (2014). If the Monarch butterflies can detect the change in inclination angle, they should also be able to detect the change in the direction of the inclination. Taken together, this could mean that non-migratory, lab-reared Monarch butterflies do not have the ability to use Earth's magnetic field, such as the migratory ones do.

There is evidence that lab-reared animals can lose their ability to sense the Earth's magnetic field (Riveros et al., 2014). An interesting study done on leaf-cutter ants (*Atta colombica*) compared the effect of turning the horizontal component of the magnetic field by 90° on ants exposed to soil and ants in the lab not exposed to soil (Riveros et al., 2014). The study found that the ants, not exposed to soil, did not detect the change in the magnetic field and oriented towards their home. However, the soil-exposed ants changed their direction somewhere between their true and subjective home when the magnetic field was turned (Riveros et al., 2014). The same could be true for the difference between wild Monarch butterflies and my results on lab-reared ones. However, the ants are expected to have a particle-based magnetic sense (Riveros et al., 2014), while the Monarch butterflies are expected to have a radical-pair-based magnetic sense, formed by the photoexcitation of cryptochrome proteins (Mouritsen, 2018; Wan et al., 2021). The particle-based magnetic sense theory is based on the presence of magnetite particles in animals, that could act as compass needles (Mouritsen, 2018). In Riveros et al. (2014) they hypothesised that the leaf-cutter ants obtain these magnetic particles from the soil. The radical-pair-based magnetic sense theory is based on the quantum mechanics of electron spins, initiated by light specialized photoreceptors, that could form the basis of a magnetic compass sense (Mouritsen, 2018; Wan et al., 2021). The cryptochrome protein have been proposed as a light-dependent magnetic detector, because of their photoreceptive function (Wan et al., 2021). The cryptochrome protein is not something the Monarchs can obtain from the environment, which is why this makes the ants and the Monarch butterflies and how their magnetic sense works, most likely, very different. However, interestingly, the study on ants found a difference in the magnetic sense of the same species but exposed to different environments. Comparing the non-migratory Monarch butterflies I have used to the migratory ones used in Guerra et al. (2014) it appears that their ability to detect and use the magnetic field differs, as seen with the leaf-cutter ants (Riveros et al., 2014). However, on the individual level, it seems that the non-migratory Monarch butterflies can use the magnetic field for flight direction (Fig. 10). This could mean that the non-migratory butterflies have the ability to sense or use the magnetic field, but they don’t do it to the same extent as the migratory ones.

### *4.3.3 Compass orientation on the individual level*

One individual changed its flight direction as expected with the cues, indicating that the non-migrating butterfly can use the magnetic field for flight direction (Fig. 10). A study done on Bogong moths found that they have a magnetic sense that appears to be periodic (Dreyer et al., 2018). Because of this, the butterflies were given three minutes of only the magnetic cue (phases 4-5) before the cue turned by - 120°, and the butterfly had another three minutes with the new turned magnetic cue (phases 6-7). However, with this individual, the butterfly seemed to detect the magnetic cue change fast (Fig. 10). Meaning that the Monarch's magnetic compass is most likely not periodic like it appears to be in the Bogong moth.

In Phase 8, the animal kept its flight direction from phase 7 (Fig. 10h), which could be because the animal was getting tired. Twelve minutes is long for non-migratory butterflies to fly continuously in a laboratory setting. Other studies using the same animals have let the animals fly for a maximum of eight minutes (Franzke et al., 2020). Another reason could be that the animal does not care for the changed cues in the end. In Phase 8, the simulated sun cue is turned back on, and the butterfly is given natural magnetic conditions. The previous group results proved that the animals use the simulated sun cue for flight direction (Fig. 9a, 9b, 9ab’). However, in Phase 8, the animal does not care for the sun cue either. Alternatively, it might have used idiothetic cues. Idiothetic cues are not accounted for in this study, but it is known that insects receive different idiothetic cues to help steer their flight direction (Beetz et al., 2022).

In Dreyer et al. (2018) they investigated the Bogong moths’ magnetic sense in combination with visual landmark cues and found that their magnetic sense was periodic. They did not test the insects' use and response to only the magnetic field, as I did. The insects' cue hierarchy might differ when multiple cues are available, in contrast to when only one is available. Studying these cue conflict situations can provide much needed insight into orientation cue hierarchy. I, therefore, performed a similar conflict experiment with a visual sun cue.

## 4.4 Sun compass versus magnetic compass

Monarch butterflies are known to use the sun (or an artificial sun) as an orientation cue (Franzke et al., 2022; Perez et al., 1997) however, their use of Earth's magnetic field has not been established to the same extent (Guerra et al., 2014; Reppert & de Roode, 2018; Wan et al., 2021). The conflict experiment aimed to further understand the use of both cues, and which cue the animals prefer when given both. Because of the low sample size, all animals that completed the experiment were analysed, as well as the individuals that were not directed. 62 % (8 out of 13) of the animals in Phase 2 were disoriented, making the control results weak (Fig. 11). In Phase 3, the individual animals were more directed, which questions if the animals needed more time in the experimental setup to acclimate.

On a group level, the butterflies changed their flight direction by about + 180° (Fig. 11a & 11b), meaning that no clear preferred cue was found. The angular change for all individuals shows that some Monarchs change their direction by about + 120° (Fig. 11ab'), indicating that these butterflies used the magnetic field. Some animals did not change their direction at all (Fig. 11ab'). This can mean that they got confused by the conflict and did not know which direction to go, or that they did not care for either cue. There might be idiothetic cues they rather follow. Three individual butterflies changed their flight direction by about - 60°, while one butterfly switch its direction by about - 130° (Fig. 11ab'). This indicates that these Monarch butterflies rely more on the sun cue. The individual variations seen in these results indicate that the preference for cues can vary on an individual level. However, since the directedness of the animals in Phase 2 was low, it is difficult to trust the individual butterfly's directions in Phase 3 (Fig. 11a & 11b). Because of the low directedness in Phase 2 and the general low sample size, the animals' preferred use of cues needs more investigation. An interesting next step would be to test migratory Monarch butterflies in the same experimental setup, to study if their preferred cue would differ from the non-migratory butterflies.

## 4.5 Outlook

Previous studies have found that the magnetic sense in Monarch butterflies is UV-A/blue light-dependent (Guerra et al., 2014; Wan et al., 2021), which is why I built the artificial skylight. The intensity of these LEDs was set similar to the intensity used in previous studies (Guerra et al., 2014; Wan et al., 2021), and they were on at all times during the experiment. Different light conditions were not tested, which is why I cannot state their effect on the experiment. However, my experimental setup is perfectly designed to test if the magnetic compass is wavelength dependent, since I can turn off specific wavelengths while keeping the overall light intensity the same. This would have been an interesting development of my study and should be tested in future experiments.

This study investigated if non-migratory, lab-reared Monarch butterfly can use the magnetic field as a compass orientation cue. To my knowledge, this has never been tested before. Tethered in a flight simulator, non-migratory Monarch butterflies were presented with different light cues and magnetic conditions. On a group level, no clear evidence was found stating that the non-migratory Monarch butterflies' sense or use the magnetic field. Because of the low sample size, it's difficult to state clear results, so more animals should be tested. However, one butterfly did fly in the expected direction with the changing magnetic cues throughout the experiment. From the flight direction of this animal, it appears that the non-migratory Monarch butterflies can use the Earth’s magnetic field for orientation, making this an interesting field of study for the future.

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# Appendix

## Appendix 1:

The irradiance curve of the light conditions from the LEDs in the ceiling during experiments (Fig. A1). This includes six white LEDs (Emission peak = 5500 K; LZ1-10CW02-0055, Osram, San Jose,CA, USA), six blue UVs (Emission peak = 410; LZ4-V0UBH0-00U8, Osram, San Jose, CA, USA) and six violet UVs (Emission peak = 365 nm; LZ1-10UV00, Osram, San Jose, CA, USA).

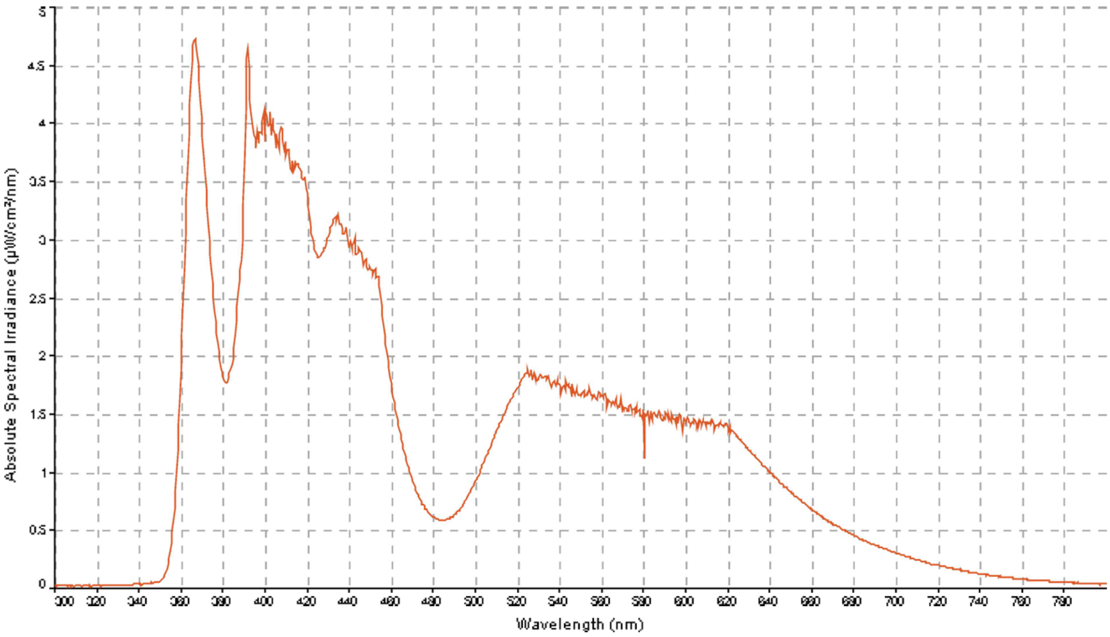


**Figure A1**: The irradiance curve of the light conditions from the skylight LEDs used in the experiments. The absolute spectral Irradiance (µW/cm$^2$/nm) is plotted against wavelength (nm).

## Appendix 2:

The mean direction angle and length of the mean vector (r) for each animal participating in the analysis in each experiment phase (Fig. A2). The data is for the magnetic field as an orientation cue experiment.

| | Phase 1 | | Phase 2 | | Phase 3 | | Phase 4 | | Phase 5 | | Phase 6 | | Phase 7 | | Phase 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value |
| 48 | 356,63 | 0,334 | 23,852 | 0,699 | 240,91 | 0,223 | 351,28 | 0,336 | 328,41 | 0,509 | 277,05 | 0,534 | 237,12 | 0,81 | 236,48 | 0,94 |
| 56 | 121,34 | 0,077 | 160,88 | 0,277 | 297,88 | 0,363 | 76,127 | 0,333 | 36,873 | 0,38 | 44,483 | 0,473 | 9,734 | 0,356 | 139,19 | 0,563 |
| 33 | 41,057 | 0,357 | 96,176 | 0,316 | 185,58 | 0,879 | 166,36 | 0,274 | 153,3 | 0,506 | 143,07 | 0,458 | 38,645 | 0,982 | 32,787 | 0,974 |
| 58 | 35,796 | 0,106 | 52,13 | 0,328 | 203,51 | 0,225 | 182,77 | 0,089 | 287,64 | 0,204 | 191,16 | 0,125 | 280,29 | 0,247 | 0,028 | 0,247 |
| 51 | 56,343 | 0,202 | 45,519 | 0,508 | 164,36 | 0,518 | 185,84 | 0,285 | 175,2 | 0,189 | 221,49 | 0,117 | 163,5 | 0,22 | 43,89 | 0,653 |
| 32 | 229,75 | 0,428 | 270,45 | 0,306 | 131,5 | 0,265 | 332,8 | 0,084 | 327,06 | 0,172 | 332,16 | 0,238 | 305,47 | 0,217 | 233,49 | 0,847 |
| 37 | 83,559 | 0,139 | 73,735 | 0,345 | 221,09 | 0,441 | 132,49 | 0,116 | 66,833 | 0,053 | 142,74 | 0,118 | 204,1 | 0,036 | 211,32 | 0,293 |
| Mean | 132,068 | 0,235 | 103,249 | 0,397 | 206,404 | 0,416 | 203,952 | 0,217 | 196,474 | 0,288 | 193,165 | 0,295 | 176,980 | 0,410 | 128,169 | 0,645 |

**Figure A2:** The mean direction and length of the mean vector for all participating animals in the analysis. The first column is the different animals, followed by the mean direction and the mean vector length (r) in all 8 phases. The last row contains the mean value of the mean direction and the r-value for each phase.

# Appendix 3:

The mean direction and length of the mean vector (r) for each animal participating in the analysis in each experiment phase (Fig. A3). The data is for the conflict experiment.

| | Phase 1 | | Phase 2 | | Phase 3 | |
|---|---|---|---|---|---|---|
| ID | Angle (°) | r-value | Angle (°) | r-value | Angle (°) | r-value |
| 33 | 43,949 | 0,465 | 55,658 | 0,988 | 195,3 | 0,837 |
| 48 | 348 | 0,219 | 27,472 | 0,268 | 322,74 | 0,253 |
| 49 | 257,62 | 0,285 | 118,39 | 0,166 | 236,06 | 0,38 |
| 51 | 345,56 | 0,238 | 22,239 | 0,099 | 201,87 | 0,015 |
| 52 | 359,68 | 0,185 | 70,376 | 0,088 | 4,163 | 0,054 |
| 38 | 60,888 | 0,031 | 105,83 | 0,091 | 116,76 | 0,435 |
| 37 | 39,69 | 0,144 | 36,331 | 0,042 | 198,65 | 0,145 |
| 46 | 76,65 | 0,261 | 71,746 | 0,463 | 22,605 | 0,438 |
| 58 | 336,04 | 0,128 | 38,451 | 0,071 | 179,45 | 0,097 |
| 57 | 11,371 | 0,206 | 87,314 | 0,08 | 90,123 | 0,199 |
| 45 | 334,8 | 0,136 | 58,769 | 0,095 | 281,5 | 0,378 |
| 56 | 345,3 | 0,213 | 102,33 | 0,202 | 225,4 | 0,551 |
| 32 | 160,85 | 0,335 | 183,86 | 0,972 | 129,96 | 0,433 |
| Mean | 209,261 | 0,219 | 75,290 | 0,279 | 169,583 | 0,324 |

**Figure 3A:** The mean direction and length of the mean vector for all participating animals. The first column is the different animals, followed by the mean direction and the mean vector length for all 3 phases. The last row contains the mean value of the mean direction and the r-value for each phase.

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