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Testing migratory Bogong moth (*Agrotis infusa*) in orientation experiments using an artificial representation of the Milky Way

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Abstract

The Bogong Moth, *Agrotis Infusa*, is a migratory moth native to South-Eastern Australia. These nocturnal insects migrate over more than 1000 km from their breeding grounds to caves in the Australian alps. To find their way during this migration, individuals of the species use several compass cues, both magnetic and celestial. This study aimed to investigate the ability of the moth to navigate after an artificial Milky Way made up of an LED strip. The LED was programmed to give light in different conditions, with two sides and three different widths of lights and was placed on a dome on top of a Mouritsen-Frost flight simulator. The end results did not show a significant directedness, but indicated a preference for the moths to fly away from the light. However, further experiments are needed to confirm this. This study and its methods provide a base for further research.

Introduction

Migrational behaviour among animals is a well-known phenomenon. The most famous examples come from bird species such as the white stork which has been used in the famous tale about where human babies come from (Berthold, 2001; Blas, et al. 2001). However, there are also many other animals that migrate over long distances. Among these some are insects such as the North American Monarch butterfly. This species can migrate over up to 4000 km from its Northernmost habitats in Canada all the way down to Mexico (Berthold, 2001). Another migratory insect species is the Bogong moth (Figure 1) which has been the focus of this study. This tiny animal is a nocturnal migrator and has been shown to migrate more than 1000km from their breeding grounds in Southern Queensland, Western Victoria and Northwestern and Western New South Wales to cave systems in the Australian alps (Figure 2). Once they arrive in the alps, the moths aestivate densely packed on the cave walls. Aestivation is a state in which animals, plants or even unicellular organisms can lower their metabolic rate for periods of time. The Bogongs stay in this state for up to four months before they return back to their breeding grounds and thereby completing a journey of more than 2000 km per individual in a year. (Warrant, et al. 2016)

Figure 1: A male specimen of the Bogong moth *Agrotis infusa*. Credit: Eric Warrant

How these animals find their way while migrating has baffled scientists over the years and many theories have been suggested. One theory that has been tested and shown to be supported is the presence of an internal biological magnetic compass of sorts. The ability to sense the magnetic field and use it as a compass cue has been shown to exist in migratory birds (Chernetsov, 2016). This ability has also been shown to exist in some migratory insects such as the Monarch (Guerra, et al. 2014) and the Bogong moth (Dreyer, et al. 2018). Another theory which has been widely studied is the use of celestial cues such as the sun and stars of the night sky for directionality. The Monarch is a diurnal migrator and it has been found that it uses the disk of the sun to navigate (Mouritsen & Frost, 2002). This has also been found in some birds such as the pied flycatcher which has been suggested to use celestial cues before relying on magnetic cues (Giunchi, et al. 2015). The bogong moth has just recently been shown to use the starry night sky as a navigational cue (Dreyer, et al. 2021a). One potentially useful cue of the night sky is the Milky Way which spans over the night sky creating something of a cloud of light. Interestingly the insect *Scarabaeus satyrus* (a nocturnal Dung beetle) has been shown to use the Milky Way for orientation (e.g. Foster, et al. 2017). In this study we examine whether the Bogong moth also potentially uses the Milky Way as a cue for orientation in navigation.

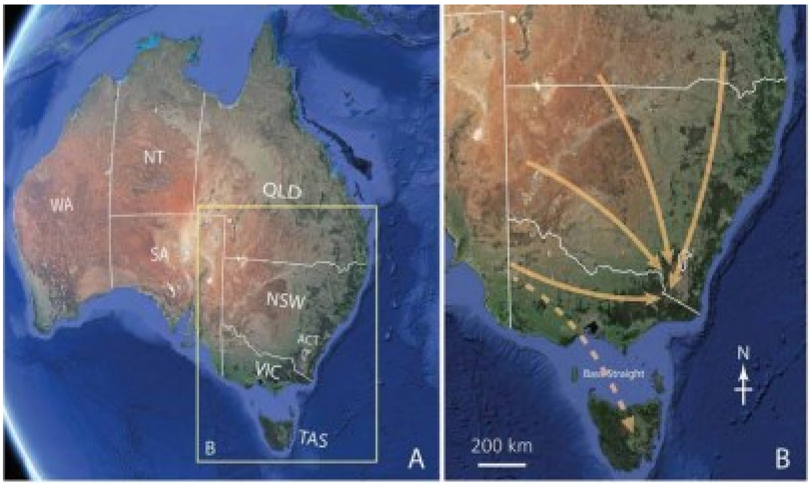

Figure 2: A, the area where Bogong moths can be found. B, The routes of Bogong moth migration in the spring.

Materials and methods

Animals

Wild Bogong moths were captured in South-Eastern Australia in February of 2021 and shipped to Lund University. At the University they were kept in a Percival incubator to retain their aestivation state. The temperature was kept at 6°C and 13°C at night and day respectively with a daylight cycle of 15h light/9h dark with a light spectrum similar to that of natural sunlight. Relative humidity levels were kept at 65-72%. Before being used in experiments animals were placed in a room with a constant temperature of around 16°C with the current outdoors light cycle for at least three days. This was done to accustom them to the natural circadian rhythm of Sweden before experiments were done. The moths were fed with honey solution that contained 20 g/l sugar, 20 g/l eucalyptus honey and 2 g/l ascorbic acid.

Preparation of moths

On the day of experiments the moths were put into individual containers. They were then put into the fridge for 5 minutes to calm them down. They were then placed under a plastic gauze mesh (5 x 5 mm holes), the scales on the dorsal thorax were removed using a small paint brush. Tungsten rods of about 15mm where the end had been bent 90 degrees and then into a circular shape were glued to the dorsal plate using Evo-Stik Impact contact adhesive. These pieces of tungsten rod were then used as tethering stalks. The moths were kept in individual containers and fed with cotton swabs dipped in honey solution. They were then put outside on a slightly elevated position with minimal light pollution 30 minutes before sunset and picked up again 30 minutes after sunset. They were placed in a styrofoam box with a hot water bottle to warm them up though the temperature in the room where experiments were performed sometimes dropped below 0°C.

Experimental setup

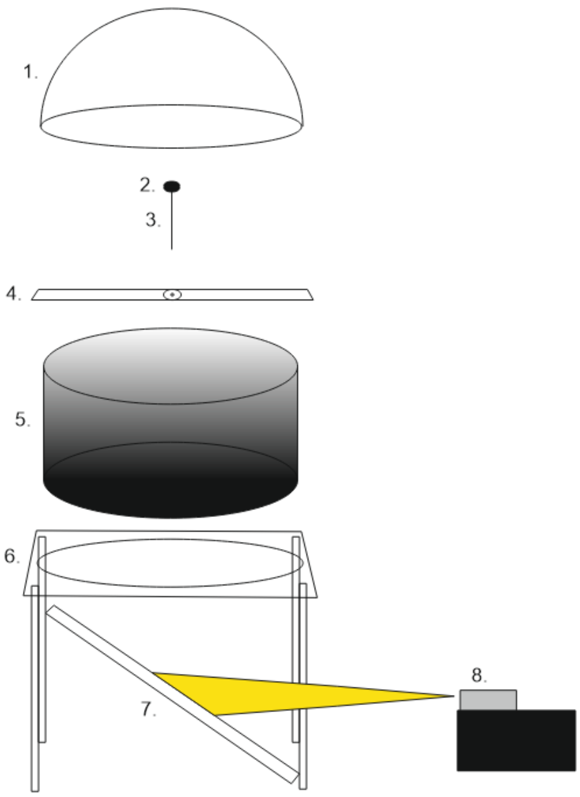

Figure 3: Modified Mouritsen-Frost flight simulator. 1. A semi-transparent plastic dome covered with a programmable LED-stripe. 2. An optical encoder. 3. A tungsten rod to which an experimental animal can be attached. 4. An optical encoder holder. 5. The cylindrical arena covered on the sides in black felt. 6. An aluminium table with a hole the same size as the arena placed at complete level. The hole has been covered with diffuser filters. 7. A mirror placed at 45° under the table. 8. A projector projecting an optic flow onto the mirror and then onto the diffuser filter under the arena.

The setup used (Figure 3) was a modified Mouritsen-Frost flight simulator (Dreyer, et al. 2021b, Mouritsen & Frost, 2002). The simulator consisted of a cylindrical arena of plexiglass which was covered in a homogenous layer of black felt on the inside, to minimize the presence of visual landmarks. The cylinder had a diameter of 50 cm and a height of 35 cm. On top of the arena an encoder holder with an optical encoder was placed. The optical encoder had a tungsten rod (0.5mm diameter and 12 cm length) attached to it and at the bottom of the rod a stalked animal could be attached. The encoder was connected to a computer through a USB-box and the orientation of the moth at a certain time point could thereby be recorded and saved. On top of everything a semi-transparent dome with a programmable LED-strip (Adafruit NeoPixel Digital RGB LED Strip, 60 LED, 1m length, 12.5mm wide, 4mm thick, 5V, 60mA, LED wavelengths: 630nm/530nm/475nm) running across was placed. The LED strip had 23 separate LEDs with the 12th LED placed at the absolute top of the dome and was acting as an artificial Milky Way. Between the LEDs and the dome three layers of Lee Neutral Density filter sheets ND 1.2 were placed (Figure 4). The dome was covered with a layer of black cloth during experiments. All this was placed on top of a level aluminium table (71x71cm)

Figure 4: A picture of the arena and dome of the Mouritsen-Frost flight simulator used.

with a hole covered with a transparent plexiglass disk and diffuser paper. Underneath the table a mirror was placed reflecting a projected image from the projector. The computer recording the orientation of the moth created an image taken from google earth of the ground, in an area close to the common migratory routes of the Bogong in Australia, that was projected under the moth moving in the opposite direction than it was flying. This projection was done to create the illusion of an optic flow, such a flow can stimulate the animal to keep on flying (Preiss, 1987). In front of the projector two pieces of Lee Neutral Density filter sheets ND 1.2 and one sheet of ND 0.3 were added to dim the light intensity. The screen of the laptop used (Dell latitude 7480) was set to minimum brightness and was covered with a red filter.

Experimental procedure

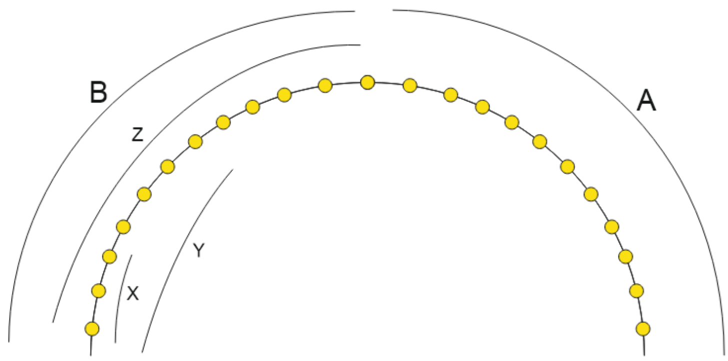

Figure 5: The dome with LED strip that was placed on top of Mouritsen-Frost flight arena. Each yellow dot represents a LED light. A and B are the two sides and X, Y, Z are the three conditions of lights turned on.

During experiments a red LED light was used when removing a tethered moth and fixing a new moth in the arena. The moths were attached to the tungsten rod (3. in Figure 3) using a 1 cm rubber tubing. The animals could then move freely about the yaw-axis. Before each recording the tethered moth was held against magnetic West (which was used as the setup's 0-position throughout the experiments) and the encoder system was recalibrated to the 0-position. The moths were then left for about 20s to get used to the environment before the recordings started. The moth's direction was sampled at a frequency of 5 Hz and everything was saved to the hard-drive. During the experiments the moth's behaviour was continuously monitored by the software (USB1, USB4: US Digital, Vancouver, WA, USA). All trials were of ABA, BAB, AAA or BBB type, the two latter being controls. Meaning that each letter stands for one condition, preferably the animal will perform the same in the second A/B as in the first A/B suggesting that the condition affects the behaviour. The A side was the North-Western side of the LED-strip and the B side on the South-Eastern side (in magnetic terms). Each trial was then performed at one of three different conditions called X, Y and Z. The X condition had 3 LEDs turned on on one side of the arena in each A/B condition, the Y condition had 7 LEDs turned on on each side and the Z condition had 12 LEDs turned on (Figure 5). We chose a light intensity at which the animals seemingly responded to the stimulus. The different light conditions were all programmed in MATLAB. Each experiment lasted for 3 minutes (1 minute on each side) and if the animal was performing well it was used in up to three consecutive experiments (one for each condition). However, if the animal stopped for more than 10 seconds it was kickstarted by a nudge of the arena.

Statistical analysis

All recordings were analyzed using MATLAB scripts and then in the circular statistics software Oriana. The virtual flight tracks were subdivided into 3 parts according to the respective experimental condition (A or B). For example, if the trial was of ABA-type, then it was analyzed as A_1, B and A_2. Then, the mean direction and r-value of each part was established through a prewritten MATLAB script. After this the datasets that filled certain criteria were found to be analyzed separately. The first criterion was that the r-value of each part had to be higher or equal to 0.2 (this threshold has shown to sort out flight tracks which do not show any directed flight) to make sure that the animal was not improperly stalked or very disoriented. The second criterion was that the mean direction of A_2 had to be within 120° (i.e. ±60°) of A_1:s mean value. This particular criterion was chosen since one of the conditions (A or B) was tested twice per experiment which enables to sort out animals which show an inconsistent directedness. The expectation here is that a tested animal would retutn to it's initial flight direction if tested under the given stimulus configuration a second time. The third criterion was that the angle between the mean direction of one condition and the next (A_1 to B or B to A_2) had to be above 60° at least in one condition, this was done to sort out flight tracks of animals which were irresponsive to the stimulus configuration during the entire time of the respective experiment.

The two types of statistical tests used were the Rayleigh test and Moore's modified Rayleigh test (MMRT). Both of them were used to tell how directed an animal or a population of animals was. The difference between them is that MMRT accounts for the r-values of the data and not just the mean direction (Moore, 1980). Both tests rely on the null hypothesis that the choice of direction is evenly distributed around the circumference of a circle and give a significance probability (p-value) that can help in determining the significance (Dreyer, 2021b).

Results and Discussion

Analysis of angles

The first analysis done was between the angular difference from $A_1 \longrightarrow B \longrightarrow A_2$ and $B_1 \longrightarrow A \longrightarrow B_2$ in all experiments. The angle between the mean direction in each condition was calculated in excel (Table 1-3). The mean value of the two angles was then calculated and a histogram was made dividing the angle from 0°-180° in 18 bins (Figure 6A). The graph shows the number of trials within that specific bin of angles. A trendline was added to clarify. The same graph was done for controls to compare (see Table 4) and as can be seen the trendline is here much more normally distributed (Figure 6B). If the moths did not show any reaction to the light, a normally distributed angular difference between the conditions would be expected and that the experiments show a higher number of animals tending to change direction about 90 degrees strongly indicates that the animals somehow respond to the changing stimulus, especially when compared to the controls which were much more normally distributed.

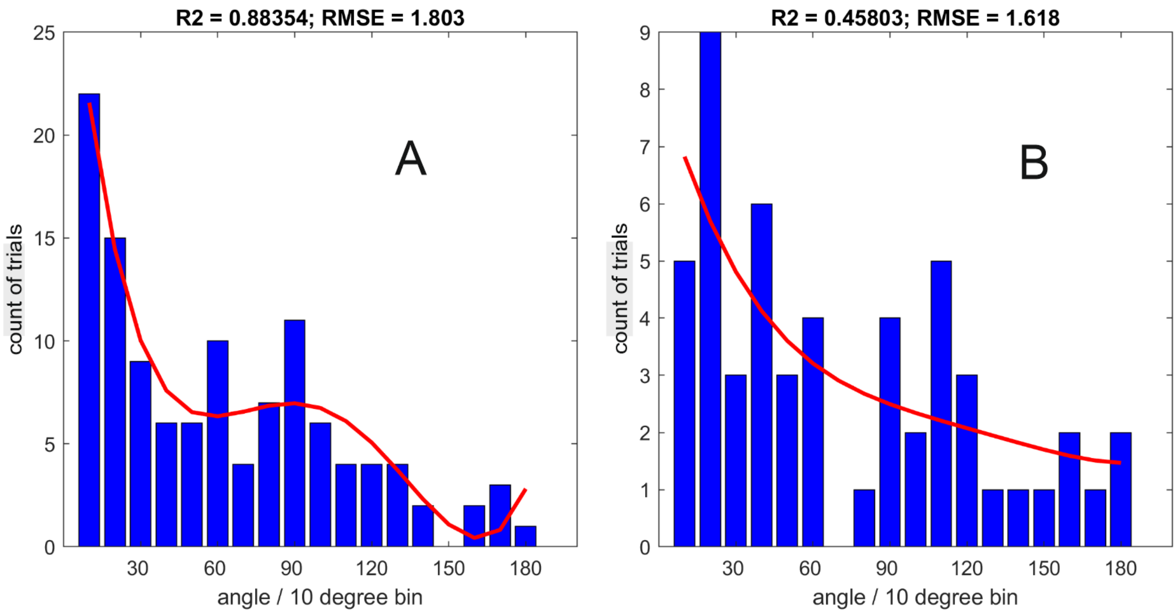

Figure 6: Histogram of the mean angle between two changes of light conditions of Bogong moths in a Mouritsen-Frost flight simulator. In A: all experiments (i.e. of ABA/BAB type). B: all controls (i.e. of AAA/BBB type). The Y-axis shows the amount of experiments and the X-axis the angle in degrees.

The second analysis done took the different conditions X, Y and Z into consideration. Each condition (eg. ABA X) was pooled and mean angles were calculated like in the previous analysis. But in this case put into a histogram with the pooled groups on the X axis and the mean angular difference on the Y axis (Figure 7). As can be seen the ABA Z condition had the highest mean angle and the ABA X the lowest of the ABA conditions. However in the BAB condition this pattern was not the same. Here instead the Y condition had a higher mean angle. This could however be explained by the fact that the same animals were reused and the Z condition was the last in the order of experiments of each animal. In my notes I wrote that many of the moths seemed to have stopped flying during the BAB Z experiments. When further trials are performed this will have to be accounted for by varying the order of conditions. When looking at the controls they differ quite a lot between the AAA and the BBB. The BBBs show a low mean angle overall which is to be expected if the animals fly continuously relative to the light. However the AAA controls show an almost identical pattern to the one in ABA, this is quite unexpected and the reason for this is debatable. One reason could be that the experimenter forgot to change the script from ABA to AAA during some of the AAA controls even though this is unlikely. To find out what the reason could be behind this further AAA controls would have to be done.

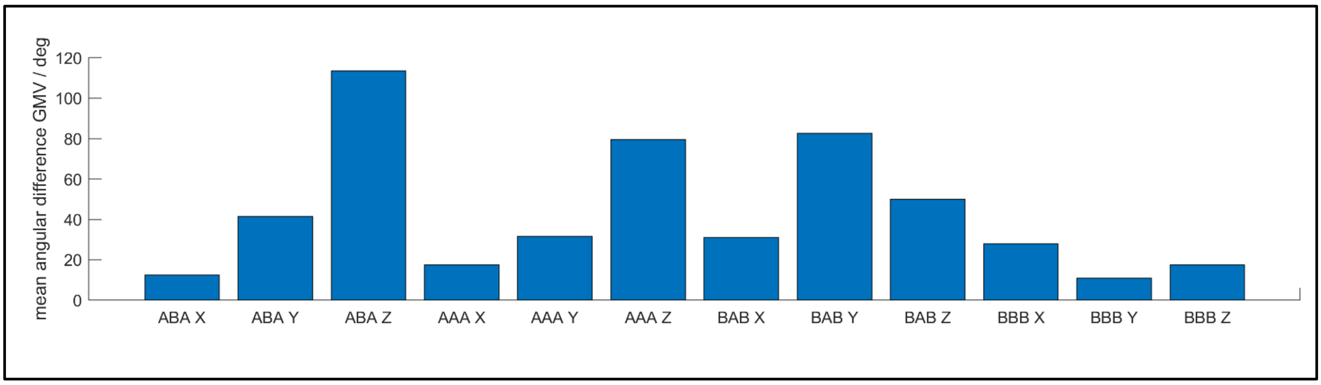

Figure 7: Histogram of the mean angle of moth direction between the two changes of condition pooled into groups.

Analysis of directionality

The third analysis was about finding the mean direction of animals pooled in different populations. This was primarily done on the selected datasets that had fulfilled the criteria (Table 5) described in the methods section. Two examples of such selected files can be seen in the plot of Figure 8. 54B and 55B display a change in mean direction between the conditions while 54A and 55A show a higher resolution of the direction of the moths over time. As can be seen in the higher resolution plots, the moths clearly react to the change of condition by changing direction almost immediately after the condition has changed. The black arrows showcased are pointing towards the magnetic West (setup North) for reference (Figure 8).

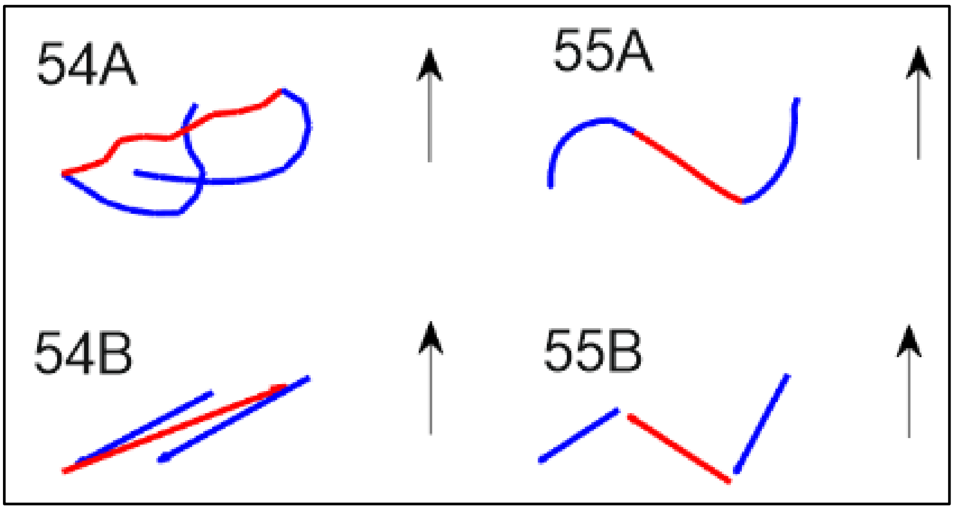

Figure 8: Plots displaying direction of moths during three conditions (blue, red then blue) in different resolutions. The number shows trial number (see Table 5) and the letter describes resolution. A: High resolution that clearly shows the continuous direction during trial. B: Each coloured arrow depicts the mean direction during one of three conditions. Black arrows point towards the magnetic West for reference.

A MMRT test was done on the selected data and the result was plotted using custom MATLAB scripts. First the ABA-condition was done (Figure 9). None of the conditions showed a direction with significance, which can be seen by the red bar not crossing any of the dotted lines showing the R*-value needed for statistical significance (p=0.05, p=0.01, p=0.001). Second the BAB-condition was done (Figure 10) and the direction did not show significance in any of the separate conditions. Even though none of the plots show a significant direction there is still a bias which is to fly away from the light. It should also be mentioned that 0 in all these plots is magnetic West. One quite probable reason that the animals were not significantly oriented is that they were caught in february and that two to three months in captivity has affected their motivation to migrate. Further trials should therefore be done as soon as possible upon delivery of fresh moths.

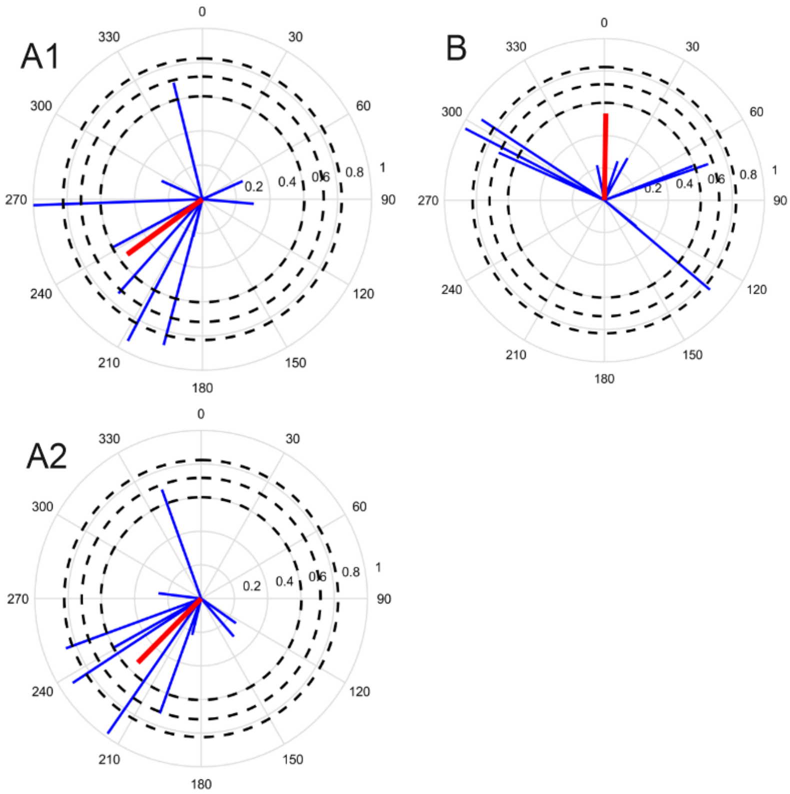

Figure 9: MMRT plots of ABA experiments that fulfilled certain criteria described in the methods section. The dashed circles indicate the R*-values needed for different degrees of statistical significance (p=0.05, p=0.01, p=0.001). The plots divide the ABA experiments into three parts where the first A-condition is named A1 and the second is named A2. 0 in these plots is pointed against magnetic West.

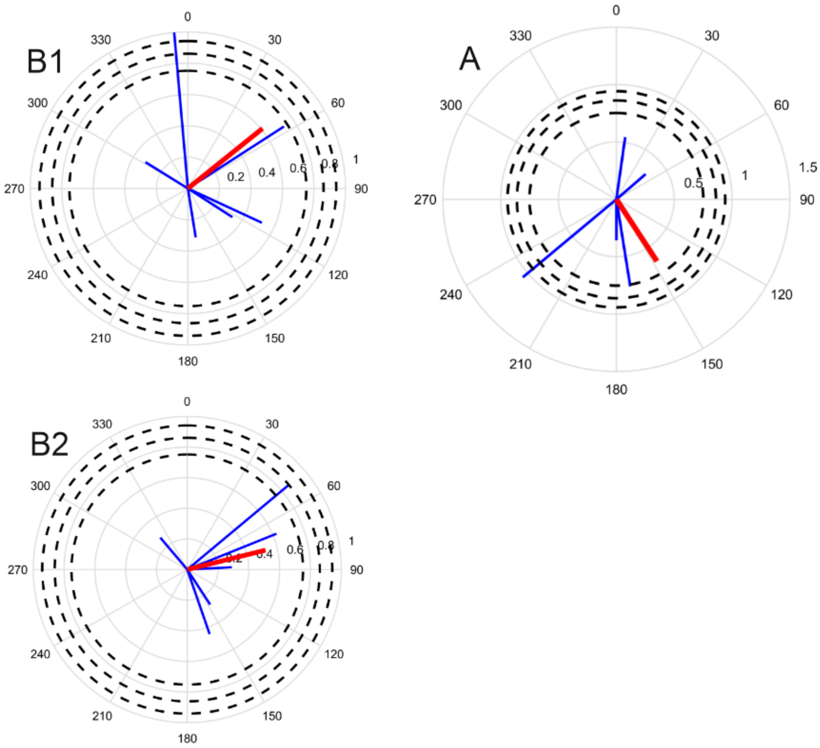

Figure 10: MMRT plots of BAB experiments that fulfilled certain criteria described in the methods section. The dashed circles indicate the R*-values needed for different degrees of statistical significance (p=0.05, p=0.01, p=0.001). The plots divide the BAB experiments into three parts where the first B-condition is named B1 and the second is named B2. 0 in these plots is pointed against magnetic West.

After separating the measurements that fulfilled the criteria mentioned it became clear that three of the datasets that fulfilled the criteria were controls. As can be seen when comparing Figure 8B and 11B they all had the mean direction expected from the reaction to a change in condition in an ABA/BAB experiment. All the black arrows in these plots are pointing against the magnetic West. This was quite unexpected and further analysis was therefore done on these datasets (number 173, 177 and 209). The most probable reason that 177 showed the pattern expected from an actual experiment is that it was kickstarted once, this most probably gave the second change of direction seen in Figure 11 (177A) causing the mean direction of the second condition to change. However unlikely, another possible explanation would be that I forgot to change the script from ABA to BBB. Looking at the xxx pattern for individual 209A (Figure 11), it can be seen that the change in direction occuring in the second condition did not occur directly after the change. This is different from what can be seen in Figure 8 where the change occurs soon after the change of conditions. The change in direction in this case was therefore most likely random. Concerning trial 173, the first change in direction started before the first switch between conditions and the second change in direction happened quite far after the second switch of conditions. Finally, when keeping this in mind and comparing Figure 11A to 8A these are not very clear changes in direction that indicate reaction due to a change in condition.

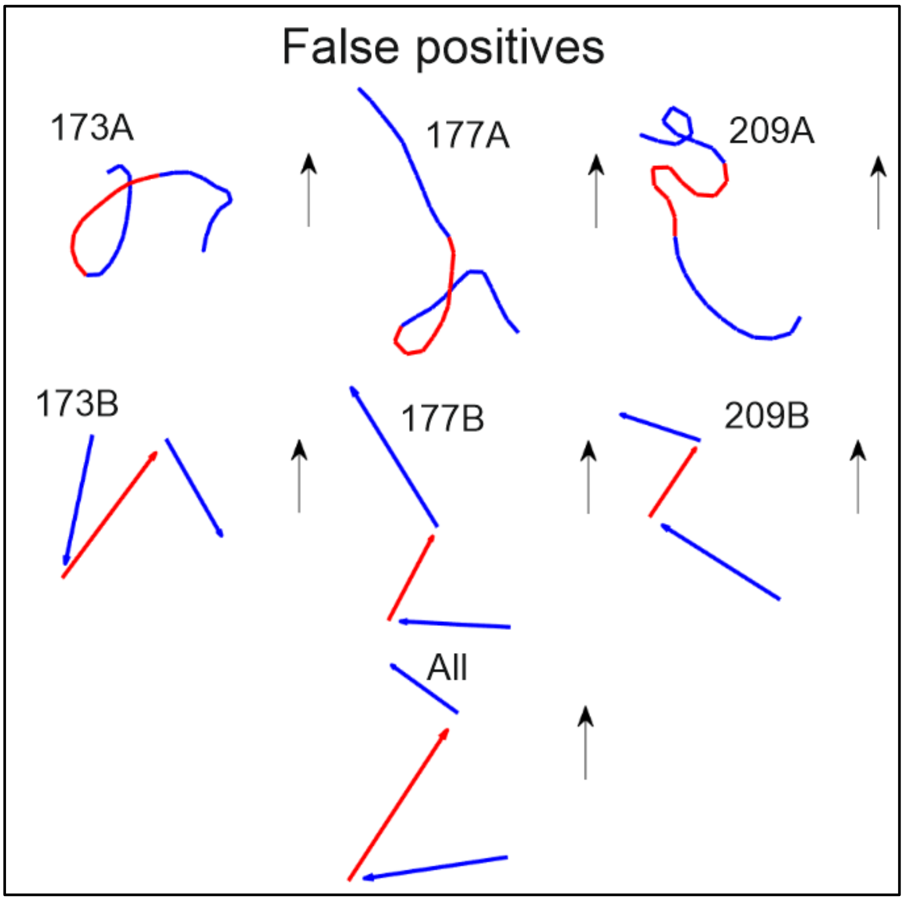

Figure 11: Plots displaying direction of a moth during three conditions (blue, red, blue) in different resolutions. The number shows trial number (see Table 5) and the letter depicts resolution. A: High resolution that clearly shows the continuous direction during trial. B: Each coloured arrow depicts the mean direction during one of three conditions. The plot named All is an average of the three seen above. Black

After removing the false positives from the separated datasets two plots were made for the average directionality of the ABA respectively BAB experiments (Figure 12). As can be seen when compared to Figure 8 the pattern of the plots indicate that some type of reaction to the change of conditions took place. Interestingly we can in Figure 12 just as in Figure 9 and 10 see that the animals seem to have a preference for moving away from the light, considering that the black reference arrows indicate magnetic West. The MMRT and Rayleigh test results used for these plots can be found in table 6 and 7. It can be seen that none of the directions except for the A_2 condition in the ABA experiments had a p-value below 0.05 implying that only this condition showed some statistical significance.

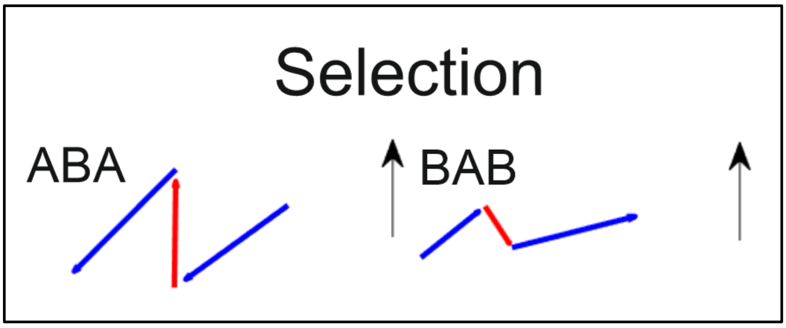

Figure 12: Plots displaying direction of moths during three conditions of ABA- or BAB type (blue, red, blue). The trials in these plots had fulfilled certain criteria described in the methods section. Each coloured arrow depicts the mean direction during one of three conditions. Black arrows point towards the magnetic West for reference.

When analysis of the separated datasets was completed some analysis on all data was done. In Figure 13 through 16 arrow diagrams of all trials separated by ABA-, BAB-, AAA- and BBB- as well as X-, Y- and Z type can be found. The MMRT and Rayleigh test results used for all these plots can be found in Table 8 through 15. The ABA experiments pooled together as seen in Figure 13 do not showcase any clear pattern of change in direction according to stimuli as the one seen in Figure 8. The exception being the Z condition, which if looking at the p-values does not show any statistical significance. One reason for this could as mentioned earlier be that the moths have been held in captivity for a long time which might affect their migratory state and thereby their ability to perform in the arena. When looking at the p-values we see that there is statistical significance in both the MMRT and Rayleigh test only in A_1 of the Y condition. The A_1 of the All plot shows significance in the Rayleigh test but not in the MMRT.

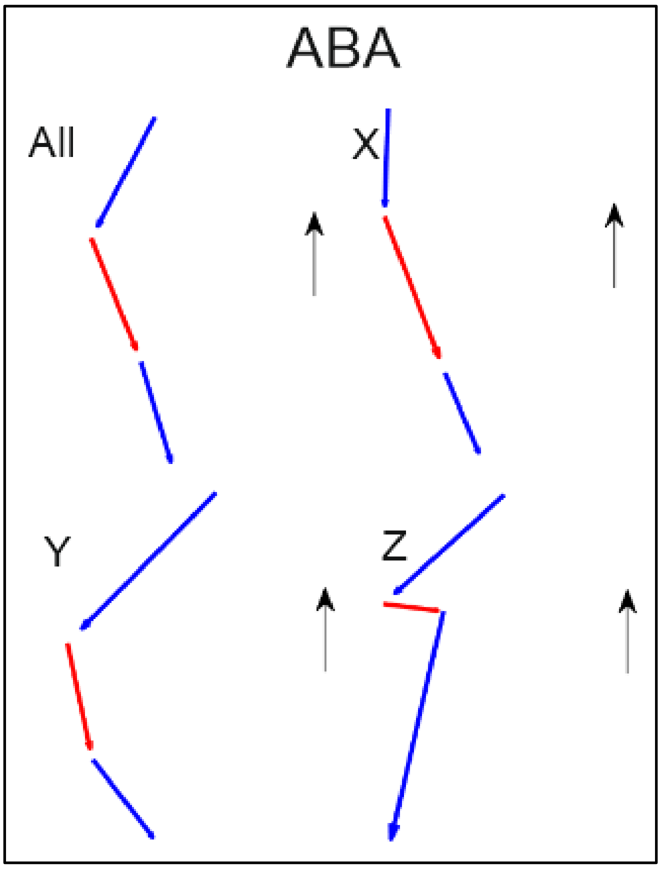

Figure 13: Plots displaying direction of moths during three conditions of ABA type (blue, red, blue) in different resolutions. Each coloured arrow depicts the mean direction during each condition. The letters X, Y and Z describe different light conditions. Black arrows point towards the magnetic West for reference.

None of the BAB plots (Figure 14) show the pattern expected when the moths fly relative to the light as in Figure 8. Again one likely reason for this is the moths' questionable migratory state. The BAB Y B_2 showed statistical significance in the MMRT but not the Rayleigh test.

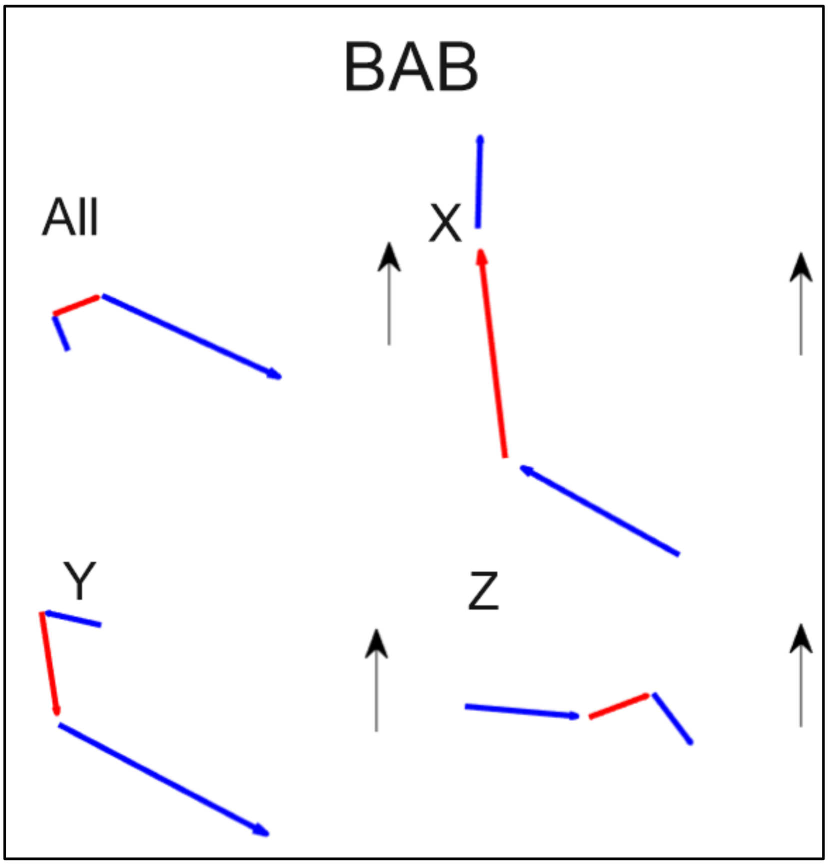

Figure 14: Plots displaying direction of moths during three conditions of BAB type (blue, red, blue) in different resolutions. Each coloured arrow depicts the mean direction during each condition. The letters X, Y and Z describe different light conditions. Black arrows point towards the magnetic West for reference.

All the control plots (Figure 15 and 16) show the expected pattern of flying in a continuous direction except for AAA Z. Why the AAA Z has the pattern expected from actual experiments is hard to say. As discussed before it could have to do with me forgetting to change the script from ABA/BAB to AAA but that I would forget to change the script enough times to affect the the outcome of the plot enough to make it look like a plot for actual experiments is highly unlikely. More controls of the AAA Z type should therefore be made to see what could be behind this pattern.

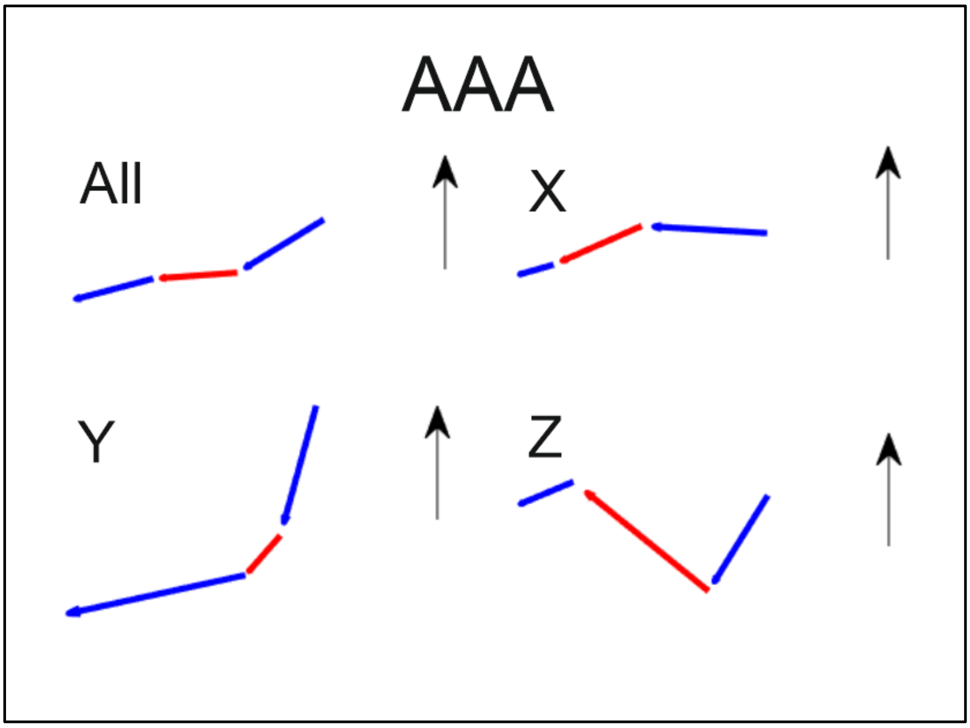

Figure 15: Plots displaying direction of moths during three conditions of AAA type (blue, red, blue) in different resolutions. Each coloured arrow depicts the mean direction during each condition. The letters X, Y and Z describe different light conditions. Black arrows point towards the magnetic West for reference.

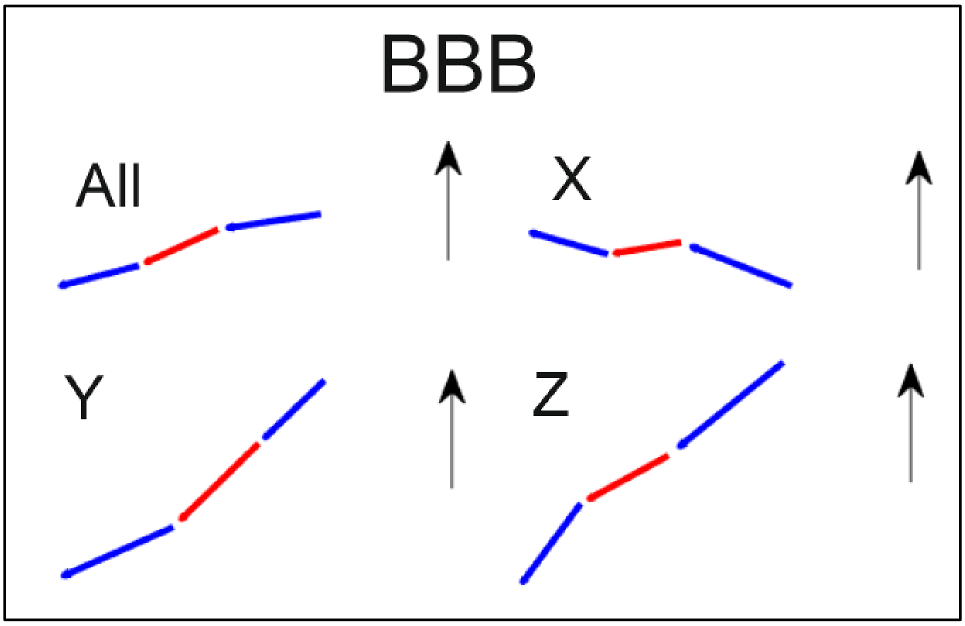

Figure 16: Plots displaying direction of moths during three conditions of AAA type (blue, red, blue) in different resolutions. Each coloured arrow depicts the mean direction during each condition. The letters X, Y and Z describe different light conditions. Black arrows point towards the magnetic West for reference.

Conclusion and outlook

Bogong moths have been shown to use the starry sky as a compass cue (Dreyer, et al. 2021a). At this stage, it is unknown which part of the starry sky is actually used by the moths while they perform flight behaviour in the orientation experiments. A very promising candidate (in terms of brightness, illuminated area and consistency) would be the axis of the Milky way, and indeed, African Dung Beetles have been shown to use the Milky way as an orientation cue (e.g. Foster, et al. 2017).

The main aim of the experiments described above was to test if the Bogong moths which were transferred from Australia to Europe would interpret the presented stimulus (which was supposed to mimic the axis of the Milky way) as an orientation cue. A seasonally appropriate orientation of the tested subsample was not expected for multiple reasons. Some of the more apparent are: 1) The Earth's magnetic field in Sweden is different to the one in Australia. One thing that separates them is the difference in inclination angle or tilt of the magnetic field (Taylor, et al. 2021). 2) The orientation of the axis of the Milky way varies systematically over the time of a respective night, which means that it would be oriented differently relative to the Earth's magnetic field at different times of a respective night. The stimulus which was presented (which was supposed to mimic the Milky way) was stationary. 3) The respective light-intensity of the stimulus was chosen since some of the animals which were tested at the beginning of the experiments showed an instantaneous response to the stimulus. The intensity of our stimulus was surely higher than the natural light intensity of the Milky way. 4) The wavelengths of the LEDs do not match the natural spectrum of the night sky. 5) We don't really know if the animals were in their natural migratory stage. 6) The angle from the moths perspective to the lowest LED was at 40°. In the wild the Milky way would reach the horizon giving a broader reference for navigation.

As described above, there are many variables which make the interpretation of the dataset at hand very difficult. In the following I would like to suggest a few changes in the future experimental design and protocol. One thing that would be interesting is to do the experiments in Australia were the magnetic field is what the animals are used to. It would also be interesting to continously change the orientation of the LED strip according to the milky way orientation on the night sky. Changing the LEDs to lights that represent the same wavelengths as natural lights would also be plausible. The angle from the animal to the first LED should also be minimized. This could be done by building some kind of shelf within the dome where the encoder holder could be placed. As described earlier fresh animals should be used to maximise the possibility of the animals being in a migratory state, this would be easiest done if experiments were done in Australia. One thing that should also be accounted for is to even out the amount of trials of each kind as well as to randomize the order of different conditions. This will minimize the risk of a certain condition being favoured by being run at a night with advantageous conditions such as temperature and weather. Another interesting follow up would be to do the same trials with animals that have been caught on the opposite journey in Australian springtime to see if their direction would change, supposedly they would then have a bias towards flying in the opposite direction.

The method developed for this study has the potential to be used as a base for future studies. The most promising potential for this method would be in behavioural studies. It can be used to see how animals react to light stimuli at different angles and intensities and could thereby potentially lead to some conclusion on different phototaxic behaviour.

References

Berthold, P. 2001. Bird migration : a general survey, *Oxford University Press*.

Blas, J. Salas, R. Flack, A. Torres-Medina, F. Sergio, F. Wikelski, M. Fiedler, W. 2020. Overland and oversea migration of white storks through the water barriers of the straits of Gibraltar. *Scientific reports*, 10(1), 20760. https://doi.org/10.1038/s41598-020-77273-x

Chernetsov, N. (2016). Orientation and navigation of migrating birds. *Biology Bulletin*, 43(8), 788–803. https://doi-org.ludwig.lub.lu.se/10.1134/S1062359016080069

Dreyer, D. Adden, A. Frost, B. Mouritsen, H. Xu, J. Green, K. Whitehouse, M. Chahl, J. Wallace, J., Foster, J. Heinze, S. Warrant, E.J. (2021a) The starry night sky provides true compass information for long-distance nocturnal navigation in the Australian Bogong moth [Submitted for publication].

Dreyer, D. Frost, B. Mouritsen, H. Günther, A. Green, K., Whitehouse, M. Johnsen, S. Heinze, S. & Warrant, E. (2018). The Earth's Magnetic Field and Visual Landmarks Steer Migratory Flight Behavior in the Nocturnal Australian Bogong Moth. *Current Biology*, 28(13), 5–2166. https://doi.org/10.1016/j.cub.2018.05.030

Dreyer D., Frost B., Mouritsen H., Lefèvre A., Menz M., Warrant E.J. (2021b). A guide for using flight simulators to study the sensory basis of long-distance migration in insects [Unpublished manuscript].

Foster, J. J., el Jundi, B., Smolka, J., Khaldy, L., Nilsson, D. E., Byrne, M. J., & Dacke, M. (2017). Stellar performance : Mechanisms underlying milky way orientation in dung beetles. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1717). https://doi.org/10.1098/rstb.2016.0079

Giunchi, D. Vanni, L. Baldaccini, N. Spina, F. & Biondi, F. (2015). New cue-conflict experiments suggest a leading role of visual cues in the migratory orientation of Pied Flycatchers Ficedula hypoleuca. *Journal of Ornithology*, 156(1), 113–121. https://doi.org/10.1007/s10336-014-1107-z

Guerra, P. A. Gegear, R. J. Reppert, S. M. (2014). A magnetic compass aids monarch butterfly migration. *Nature Communications*, 5, 4164. https://doi.org/10.1038/ncomms5164

Moore, B. R. (1980). A modification of the Rayleigh test for vector data. *Biometrika*, 67(1), 175.

Mouritsen, H. Frost, B.J. 2002. Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, 99(15), pp.10162–6.

Preiss, R. (1987). Motion parallax and figural properties of depth control flight speed in an insect. *Biological Cybernetics*, 57(1/2), 1–9.

Taylor, B. K. Lohmann, K. J. Havens, L. T. Lohmann, C. M. F. & Granger, J. (2021).

Long-distance transequatorial navigation using sequential measurements of magnetic inclination angle. *Journal of the Royal Society*, Interface, 18(174), 20200887. https://doi.org/10.1098/rsif.2020.0887
Warrant, E.J. Frost, B. Green, K. Mouritsen, H. Dreyer, D. Adden, A. Brauburger, K. é Heinze, S. 2016. The Australian Bogong Moth Agrotis infusa: A Long-Distance Nocturnal Navigator. *Frontiers in Behavioral Neuroscience*, 10(April), pp.1–17.

Appendix

Table 1: Measurements of moths done under ABA/BAB Z condition.

Trial number	Condition	r-value 1	r-value 2	r-value 3	first direction	second direction	third direction	first angle (°)	second angle (°)
29	ABA	0,528691803	0,543445	0,723317	23	297	223	86	74
30	ABA	0,362176719	0,139227	0,443805	31	31	30	0	1
31	ABA	0,4805775	0,299281	0,779046	314	290	268	24	22
32	ABA	0,432670022	0,832212	0,808324	110	348	303	122	45
35	ABA	0,951379972	0,179836	0,566198	11	309	53	62	104
36	ABA	0,231234421	0,779604	0,335012	35	135	139	100	4
37	ABA	0,997109112	0,996772	0,689402	120	117	115	3	2
38	ABA	0,960548015	0,803935	0,839344	229	160	84	69	76
39	ABA	0,280252251	0,270471	0,449255	331	333	332	2	1
40	ABA	0,672514638	0,972177	0,866453	86	50	47	36	3
44	ABA	0,2977546 6	0,388708	0,44098	95	29	139	66	110
47	ABA	0,482620886	0,274173	0,252675	34	254	317	140	63
50	ABA	0,716083913	0,718821	0,706207	131	140	145	9	5

51	ABA	0,722702074	0,998814	0,405685	222	294	200	72	94
55	ABA	0,915007132	0,996626	0,775186	208	303	237	95	66
58	ABA	0,804563859	0,993199	0,998472	185	193	193	8	0
61	ABA	0,998662493	0,993275	0,975783	353	338	307	15	31
64	ABA	0,997296633	0,999245	0,99962	193	193	196	0	3
67	ABA	0,279077623	0,832589	0,989549	330	126	214	156	88
104	ABA	0,286913667	0,822339	0,930094	290	46	91	116	45
116	ABA	0,580631386	0,66593	0,231085	247	172	107	75	65
119	ABA	0,860546946	0,477639	0,566627	195	130	250	65	120
131	ABA	0,880375451	0,998987	0,996931	167	214	208	47	6
139	ABA	0,975725884	0,992577	0,994145	252	238	237	14	1
144	ABA	0,465117319	0,974997	0,725746	321	328	237	7	91
147	ABA	0,358508816	0,492252	0,892554	274	85	46	171	39
150	ABA	0,354764152	0,999592	0,999712	308	80	80	132	0
155	ABA	0,999529921	0,998142	0,999801	13	15	16	2	1
76	BAB	0,401406722	0,427039	0,55185	115	8	161	107	153
80	BAB	0,47292679	0,542077	0,369701	190	115	119	75	4
85	BAB	0,844904823	0,991491	0,243493	327	315	268	12	47

88	BAB	0,245608756	0,733159	0,681157	302	49	320	107	89
91	BAB	0,997234304	0,761258	0,554265	69	344	276	85	68
94	BAB	0,998487844	0,99958	0,998831	93	101	98	8	3
98	BAB	0,999924182	0,881166	0,915325	177	194	192	17	2
101	BAB	0,562623284	0,67002	0,670098	57	171	68	114	103

Table 2: Measurements of moths done under ABA/BAB X condition.

Trial number	Condition	r-value 1	r-value 2	r-value 3	first direction	second direction	third direction	first angle (°)	second angle (°)
42	ABA	0,0000000000000	0,368609	0,185739	226	332	104	106	132
45	ABA	0,568353455	0,46594	0,303452	185	170	312	15	142
48	ABA	0,422090586	0,367571	0,406629	119	110	116	9	6
53	ABA	0,99850631	0,997785	0,998669	132	132	138	0	6
56	ABA	0,926022348	0,403436	0,909326	51	251	311	160	60
59	ABA	0,759660453	0,142751	0,846797	103	276	127	173	149
62	ABA	0,221351643	0,567783	0,993969	194	348	194	154	154
65	ABA	0,988988285	0,629459	0,592868	110	98	10	12	88
68	ABA	0,975206911	0,986145	0,999703	318	22	32	64	10
102	ABA	0,308889753	0,56167	0,985915	190	210	148	20	62
105	ABA	0,251228743	0,635909	0,92579	267	172	307	95	135

108	ABA	0,57657578	0,955347	0,926254	190	226	268	36	42
109	ABA	0,685736161	0,381901	0,58731	346	71	340	85	91
111	ABA	0,75498114	0,854061	0,990509	250	230	266	20	36
113	ABA	0,996864087	0,999579	0,999231	98	98	101	0	3
114	ABA	0,436629915	0,330585	0,379505	173	235	86	62	149
117	ABA	0,930649109	0,942768	0,910671	289	277	256	12	21
120	ABA	0,973368141	0,985206	0,997248	84	72	73	12	1
121	ABA	0,997830468	0,999375	0,999583	220	218	215	2	3
122	ABA	0,97456408	0,958063	0,268786	268	297	215	29	82
123	ABA	0,999337016	0,999449	0,99856	296	301	313	5	12
124	ABA	0,756538605	0,686927	0,671314	191	178	197	13	19
129	ABA	0,948183539	0,979521	0,985008	187	184	170	3	14
130	ABA	0,845909972	0,983573	0,762803	194	187	263	7	76
132	ABA	0,992607447	0,999703	0,998914	28	41	46	13	5
133	ABA	0,114046459	0,754021	0,978363	197	47	35	150	12
134	ABA	0,81279181	0,925746	0,998639	36	64	99	28	35
135	ABA	0,172991196	0,959751	0,999756	303	269	276	34	7
136	ABA	0,992031261	0,417584	0,980454	259	126	26	133	100

137	ABA	0,689106187	0,581631	0,796434	138	145	121	7	24
142	ABA	0,257227534	0,90736	0,882791	294	129	277	165	148
145	ABA	0,820497271	0,433599	0,483119	329	318	334	11	16
148	ABA	0,254140857	0,855505	0,657142	66	19	125	47	106
152	ABA	0,549995797	0,949549	0,997699	58	55	48	3	7
153	ABA	0,255831671	0,662773	0,905275	207	204	238	3	34
156	ABA	0,999219736	0,99901	0,999842	171	168	168	3	0
158	ABA	0,568090586	0,983825	0,999136	242	202	201	40	1
159	ABA	0,369083777	0,958775	0,999463	343	158	161	175	3
160	ABA	0,584684741	0,994316	0,998109	162	149	133	13	16
161	ABA	0,991991526	0,997699	0,999609	162	136	129	26	7
70	BAB	0,404155411	0,270564	0,190795	197	149	340	48	169
72	BAB	0,02917272	0,781418	0,303144	67	34	38	33	4
74	BAB	0,11441277	0,455003	0,348917	330	37	146	67	109
78	BAB	0,489633717	0,230281	0,284289	243	243	35	0	152
81	BAB	0,291424715	0,688324	0,873807	131	31	223	100	168
83	BAB	0,971001105	0,95043	0,483499	280	272	261	8	11
86	BAB	0,208612501	0,820773	0,445042	350	180	194	170	14

89	BAB	0,269615674	0,303289	0,764755	135	4	22	131	18
92	BAB	0,612520146	0,560178	0,913512	4	13	68	9	55
96	BAB	0,154128375	0,215342	0,267755	318	276	227	42	49
99	BAB	0,612110304	0,579485	0,778766	341	339	344	2	5

Table 3: Measurements of moths done under ABA/BAB Y condition.

Trial number	Condition	r-value 1	r-value 2	r-value 3	first direction	second direction	third direction	first angle (°)	second angle (°)
43	ABA	0,634767287	0,550843	0,4325	59	48	87	11	39
46	ABA	0,253948554	0,365706	0,335066	252	146	93	106	53
49	ABA	0,453660384	0,682956	0,640543	162	159	138	3	21
54	ABA	0,591075785	0,91935	0,66085	242	69	241	173	172
57	ABA	0,612229367	0,842398	0,969318	242	9	36	127	27
60	ABA	0,98819913	0,370731	0,995256	315	333	76	18	103
63	ABA	0,564417366	0,560632	0,323718	247	214	312	33	98
103	ABA	0,329373168	0,61919	0,463475	136	167	248	31	81
106	ABA	0,966674419	0,754342	0,119069	280	328	278	48	50
112	ABA	0,960397273	0,772769	0,972826	188	177	58	11	119
115	ABA	0,285499232	0,216993	0,469847	28	186	272	158	86
118	ABA	0,628730135	0,881961	0,16678	258	284	297	26	13

125	ABA	0,881965906	0,967768	0,997141	193	169	181	24	12
128	ABA	0,834449186	0,867722	0,896102	150	187	148	37	39
138	ABA	0,864483123	0,952396	0,997673	173	116	158	57	42
143	ABA	0,47576325	0,600098	0,806062	219	287	294	68	7
146	ABA	0,085296564	0,384957	0,53401	299	331	308	32	23
149	ABA	0,593401427	0,322428	0,106321	330	74	140	104	66
154	ABA	0,373701173	0,994433	0,995496	208	187	160	21	27
69	BAB	0,752652864	0,60492	0,251591	267	249	255	18	6
75	BAB	0,245387961	0,268954	0,491885	171	8	147	163	139
79	BAB	0,340955858	0,934996	0,839328	337	31	69	54	38
82	BAB	0,437372787	0,975821	0,969333	273	184	169	89	15
87	BAB	0,261030053	0,690389	0,423031	123	180	87	57	93
90	BAB	0,634803469	0,99779	0,99975	153	134	131	19	3
93	BAB	0,858779806	0,838133	0,99947	344	15	85	31	70
97	BAB	0,728886551	0,824892	0,905588	172	189	184	17	5
100	BAB	0,774207826	0,535331	0,900432	355	230	50	125	180

Table 4: Control measurements of moths done under AAA/BBB X/Y/Z conditions.

Trial number	Condition	r-value 1	r-value 2	r-value 3	first direction	second direction	third direction	first angle (°)	second angle (°)
164	AAA Z	0,028886015	0,184338	0,020185	128	328	88	160	120
176	AAA Z	0,212521017	0,892342	0,982995	136	300	287	164	13
171	AAA Z	0,986551657	0,974102	0,978293	187	180	189	7	9
185	AAA Z	0,683992782	0,622566	0,946559	198	305	70	107	125
190	AAA Z	0,474451471	0,429713	0,462364	51	356	249	55	107
196	AAA Z	0,464829775	0,803091	0,995215	55	113	108	58	5
201	AAA Z	0,639966534	0,066376	0,604516	213	107	136	106	29
207	AAA Z	0,930382778	0,995484	0,999065	262	286	282	24	4
214	AAA Z	0,778033179	0,927656	0,894201	359	359	329	0	30
169	AAA X	0,822561421	0,372971	0,699285	298	88	121	150	33
162	AAA X	0,107553986	0,262273	0,388967	294	248	321	46	73
174	AAA X	0,551314827	0,382875	0,732264	26	42	48	16	6
180	AAA X	0,424804533	0,534017	0,343414	243	219	226	24	7
187	AAA X	0,956512575	0,998843	0,978247	212	243	222	31	21
188	AAA X	0,948574013	0,132842	0,277701	299	176	342	123	166
194	AAA X	0,328103402	0,392837	0,424144	201	202	61	1	141
205	AAA X	0,403734293	0,693316	0,508822	331	291	285	40	6

212	AAA X	0,763813871	0,728285	0,863021	252	261	246	9	15
170	AAA Y	0,917544519	0,908078	0,962181	173	182	200	9	18
213	AAA Y	0,492476901	0,858604	0,852742	80	82	48	2	34
206	AAA Y	0,610711232	0,223449	0,936255	284	272	266	12	6
200	AAA Y	0,070524994	0,093121	0,585721	111	324	216	147	108
195	AAA Y	0,740059387	0,661474	0,625221	2	355	7	7	12
189	AAA Y	0,245973625	0,368232	0,920101	91	82	304	9	138
181	AAA Y	0,366767532	0,396395	0,940356	195	249	236	54	13
186	AAA Y	1	1	1	183	183	183	0	0
175	AAA Y	0,281076606	0,934625	0,966809	321	359	343	38	16
163	AAA Y	0,92865395	0,981729	0,279186	215	248	191	33	57
173	BBB Z	0,676869543	0,805598	0,573959	192	37	150	155	113
182	BBB Z	0,81892871	0,789505	0,928712	207	248	226	41	22
193	BBB Z	0,86145889	0,941837	0,349603	265	240	95	25	145
168	BBB Z	1	0,996249	0,950362	225	223	224	2	1
211	BBB Z	0,542036486	0,355811	0,788207	274	226	260	48	34
177	BBB X	0,672840559	0,575852	0,989752	273	28	328	115	60
178	BBB X	0,361234054	0,122142	0,113577	25	149	60	124	89

172	BBB X	0,437079958	0,803323	0,754777	189	244	284	55	40
165	BBB X	0,948070743	0,823005	1	292	52	42	120	10
166	BBB X	0,490212063	0,511215	0,157286	310	284	174	26	110
184	BBB X	0,100982124	0,438247	0,497159	351	228	197	123	31
191	BBB X	0,493430555	0,85958	0,835792	227	252	257	25	5
197	BBB X	0,651308985	1	1	275	282	282	7	0
198	BBB X	0,550062939	0,666895	0,316376	358	217	248	141	31
202	BBB X	0,355323455	0,572559	0,296229	220	243	280	23	37
203	BBB X	0,345011516	0,130624	0,35759	297	236	252	61	16
208	BBB X	0,990163404	0,374606	0,15177	311	188	148	123	40
209	BBB X	0,773395453	0,457746	0,465691	302	34	288	92	106
167	BBB Y	0,295422566	0,210878	0,309365	217	274	151	57	123
192	BBB Y	0,824754568	0,84307	0,77169	247	238	296	9	58
179	BBB Y	0,987967149	0,999977	1	190	189	189	1	0
183	BBB Y	0,724090024	0,969302	0,671873	280	268	240	12	28
199	BBB Y	1	0,999946	0,506837	81	81	353	0	88
204	BBB Y	0,242766913	0,571697	0,218239	246	268	295	22	27
210	BBB Y	0,306285631	0,236988	0,50898	356	106	243	110	137

Table 5: Selection amongst all measurements done according to description in methods section.

Trial number	Condition	r-value 1	r-value 2	r-value 3	first direction	second direction	third direction	first angle (°)	second angle (°)
44	ABA Z	0,29775466	0,388708	0,44098	95	29	139	66	110
51	ABA Z	0,722702074	0,998814	0,405685	222	294	200	72	94
54	ABA Y	0,591075785	0,91935	0,66085	242	69	241	173	172
55	ABA Z	0,915007132	0,996626	0,775186	208	303	237	95	66
62	ABA X	0,221351643	0,567783	0,993969	194	348	194	154	154
75	BAB Y	0,245387961	0,268954	0,491885	171	8	147	163	139
76	BAB Z	0,401406722	0,427039	0,55185	115	8	161	107	153
87	BAB Y	0,261030053	0,690389	0,423031	123	180	87	57	93
88	BAB Z	0,245608756	0,733159	0,681157	302	49	320	107	89
100	BAB Y	0,774207826	0,535331	0,900432	355	230	50	125	180
101	BAB Z	0,562623284	0,67002	0,670098	57	171	68	114	103
109	ABA X	0,685736161	0,381901	0,58731	346	71	340	85	91
119	ABA Z	0,860546946	0,477639	0,566627	195	130	250	65	120
122	ABA X	0,97456408	0,958063	0,268786	268	297	215	29	82
142	ABA X	0,257227534	0,90736	0,882791	294	129	277	165	148
148	ABA X	0,254140857	0,855505	0,657142	66	19	125	47	106

173	BBB Z	0,676869543	0,805598	0,573959	192	37	150	155	113
177	BBB X	0,672840559	0,575852	0,989752	273	28	328	115	60
209	BBB X	0,773395453	0,457746	0,465691	302	34	288	92	106

Table 6: MMRT and Rayleigh test results for ABA trials in Table 5.

	A_1	B	A_2
Rayleigh Test (Z)	1,328	1,498	3,254
Rayleigh Test (p)	0,272	0,228	0,034
MMRT (R*)	0,931	0,68	1,018
MMRT (p)	$0.5 > p > 0.1$	$0.5 > p > 0.1$	$0.1 > p > 0.05$

Table 7: MMRT and Rayleigh test results for BAB trials in Table 5.

	B_1	A	B_2
Rayleigh Test (Z)	0,545	0,03	1,421
Rayleigh Test (p)	0,6	0,973	0,251
MMRT (R*)	0,674	0,35	0,641
MMRT (p)	$0.5 > p > 0.1$	$0.9 > p > 0.5$	$0.5 > p > 0.1$

Table 8: MMRT and Rayleigh test results for ABA trials in Table 1-3.

	A_1	B	A_2
Rayleigh Test (Z)	3,202	1,427	1,15
Rayleigh Test (p)	0,041	0,24	0,317
MMRT (R*)	0,944	0,879	0,843
MMRT (p)	$0.1 > p > 0.05$	$0.5 > p > 0.1$	$0.5 > p > 0.1$

Table 9: MMRT and Rayleigh test results for BAB trials in Table 1-3.

	B_1	A	B_2
Rayleigh Test (Z)	0,097	0,172	0,855
Rayleigh Test (p)	0,909	0,844	0,429
MMRT (R*)	0,133	0,22	0,907
MMRT (p)	$0.975 > p > 0.950$	$0.9 > p > 0.5$	$0.1 > p > 0.05$

Table 10: MMRT and Rayleigh test results for ABA Z trials in Table 1.

	A_1	B	A_2
Rayleigh Test (Z)	0,36	0,1	0,449
Rayleigh Test (p)	0,701	0,907	0,642
MMRT (R*)	0,305	0,053	0,384
MMRT (p)	$0.9 > p > 0.5$	$0.995 > p > 0.990$	$0.9 > p > 0.5$

Table 11: MMRT and Rayleigh test results for BAB Z trials in Table 1.

	B_1	A	B_2
Rayleigh Test (Z)	0,589	0,436	0,233
Rayleigh Test (p)	0,57	0,661	0,802
MMRT (R*)	0,637	0,263	0,31
MMRT (p)	$0.5 > p > 0.1$	$0.9 > p > 0.5$	$0.9 > p > 0.5$

Table 12: MMRT and Rayleigh test results for ABA X trials in Table 2.

	A_1	B	A_2
Rayleigh Test (Z)	2,29	2,047	0,538
Rayleigh Test (p)	0,101	0,129	0,587
MMRT (R*)	0,625	0,876	0,639
MMRT (p)	$0.5 > p > 0.1$	$0.5 > p > 0.1$	$0.5 > p > 0.1$

Table 13: MMRT and Rayleigh test results for BAB X trials in Table 2.

	B_1	A	B_2
Rayleigh Test (Z)	0,667	0,994	0,193
Rayleigh Test (p)	0,524	0,379	0,831
MMRT (R*)	0,552	0,595	0,193
MMRT (p)	$0.5 > p > 0.1$	$0.5 > p > 0.1$	$0.95 > p > 0.90$

Table 14: MMRT and Rayleigh test results for ABA Y trials in Table 3.

	A_1	B	A_2
Rayleigh Test (Z)	3,537	0,686	0,253
Rayleigh Test (p)	0,027	0,51	0,781
MMRT (R*)	1,032	0,625	0,572
MMRT (p)	< 0.05	$0.5 > p > 0.1$	$0.5 > p > 0.1$

Table 15: MMRT and Rayleigh test results for BAB Y trials in Table 3.

	B_1	A	B_2
Rayleigh Test (Z)	0,182	0,397	2,659
Rayleigh Test (p)	0,841	0,684	0,066
MMRT (R*)	0,459	0,523	1,13
MMRT (p)	$0.9 > p > 0.5$	$0.9 > p > 0.5$	< 0.05