Magnetotaxis and orientation of chitons *Mopalia muscosa*

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Abstract:

Previous studies on magnetotaxis behavior have found a diverse range of taxa, such as reptiles, birds, and even bacteria, that use magnetite to gather information about their geographic position based on gradients in the earth's magnetic field. Among the polyplacophorans, it appears that the specific chiton species, *Mopalia muscosa* (Polyplacophora) also contain magnetite in the lateral teeth of their radula. The purpose of this study is to examine as to whether if magnetite bearing chitons exhibit magnetotactic behavior. This study was conducted in two parts: an artificial ambient magnetic field was manipulated in a laboratory to test for any magnetotactic behavioral responses. Through field observations, we observed the head orientation of chiton (vertical pitch and positional degree) which was documented in their natural habitat. Our artificial ambient magnetic field results indicate differences in head orientation between control group and experimental group and were statistically significant with a p-value <0.02. The chitons' heads orientation found in the three outdoor sites were not significantly significant but the mean vector in the raw data plot in each site pointed in the same direction. Our data suggests that chiton have an ability to detect the ambient magnetic field, which can influence their orientation.

Introduction:

Organisms use a variety of methods to orientate themselves to where they settle and where they need to travel. Specifically, behavioral studies have shown that a diversity of species use magnetotaxis while migrating, homing, or moving though their habitats, wherein they orient themselves using the Earth's magnetic field (Cain, 2006). To date, most magnetotactic studies have concentrated on vertebrate systems such as honey bees, homing pigeons, and dolphins in different morphological structures. Findings show that bacteria, honey bees, and pigeons have a tendency to detect the direction of the Earth's magnetic field, magnetotaxis (Kirschvink1981), to orientate their direction and navigation. In contrast, little is known about magnetotaxis invertebrates that show some magnetic properties or can be moved by a magnet (Ratner 1976). In 1962, Dr. Lowenstam of the California Institute of Technology found x-ray diffraction patterns of magnetite, or possible maghemite material found in the dentical capping in chiton radula. (Plylum Mollucs:Polyplacophora) (Lowenstam 1962) which has largely led to a speculation of magnetic navigational properties within the species.

It has long been known that chiton synthesize magnetic iron particles to harden teeth to scrape and file algae off rock (Kirschvink, 1981). To date, behavioral studies focusing on other aspects of magnet use within teeth have inconclusively shown that particular species of chiton demonstrate navigational homing behavior. (Chelazzi et al 1982;Mielczarek 2000; Smith 1975). Chitons magnetic teeth may not be purely a feeding adaptation, but may also serve as an newly evolved sensory cue.

Through previous (Mollusca:Polyplacophora) comparative movement patterns between species studies, the directional cue and orientation of the varied species of chiton is highly unknown due to the research methodological shortcomings, (non-individual recording, small sample size, short study period, and inadequate statistical analysis) (Chelazzi et al 1983). Though studies have little data, scientists are presently looking at the possible homing behavior of *M. muscosa* (Murray 1997), the journey of dispersing from rock to feed and return to the same rock they left along a consistent pathway (Chelazzi et al 1983; Smith 1975). But additionally, there is no scientific explanation of this homing behavior due to the lack of data and methodology. However, the possible mechanism remains unexplained since *M. muscosa* have poor sensory apertures, with neither eyes nor aesthetes leading to an abstruseness of orientation.

Our study focuses on the direction and orientation of *M. muscosa* in relation to the ambient magnetic field. Working with an indoor rotated ambient magnetic field using a Merritt coil and outdoor natural habitat ambient magnetic field, we seek results supporting one hypothesis: the ferromagnetic mineral magnetite within chiton teeth acts as a functional navigation system, directing chitons towards the ambient magnetic field rotated. Therefore, we predict that chitons' heads may be orientated towards the ambient field at resting periods.

Methods and Materials

Two complementary sites were used for research, all chitons used within each complementary site were individually marked with yellow numbered paper.

Manipulated Magnetic Field

Tank set up

Mopalia muscosa were collected from Argile Creek, Friday Harbor, Washington, were separated into two groups, and held at Friday Harbor Labs. The experimental and control group each consisted of fifteen chitons, ranging in length from three to five centimeters. Both groups were allowed to acclimate for one day in captivity before being separated. Individual acclimated chiton were randomly picked and put into separate circular base-containers (diameter 8.5 cm height 4.5 cm). Fifteen circular-containers were within the same water tank (70cm x 43cm x 20 cm) surrounded by an magnetic coil. Water temperature was kept at a constant temperature of 12.5 C with a low water input flow.

Magnetic coil device set up

An imposed magnetic stimulus was generated by a magnetic coil system (4 square coils, each 1.02m on a side) and powered by BK Precision 1760 Triple output DC power supplies. (Cain, 2006). The magnetic coil system was activated by a computer-controlled relay. Constant current was introduced and flowed through the circular wire. This created a magnetic field around the coiled wire magnetizing the metal as if it were a permanent magnet. The intensity of current is controlled by resistor, which exposed organisms to a magnetic field intensity of 100,000 nT which is the same intensity of ambient field. Due to vector addition, different direction of an ambient magnetic field can be generated by changing the current intensity of different coils. We rotated the horizontal component of the ambient magnetic field 90 degrees clockwise meaning that the artificial ambient field points west for our experimental group.

Experimental trial

Each chiton had same initial position with their head pointed 150 degrees related to the north in their own container at the start of each trial. Pictures were taken every 30 mins to document orientation. Chiton head orientation was measured by a compass between the times of three hours for each stable chiton. In order to have minimum variable factors other than different magnetic field direction, both control and experimental groups were tested in the same tank. Each trial was replaced with new specimens. After each trial, experimental and control groups were released back to Argyle Creek fifteen meters away from the collection site.

Natural Magnetic Field

M. muscosa were found and anteriorly marked with yellow numbered paper glued with cyanoacrylate instant glue in three sites at Friday Harbor Labs, Friday Harbor, WA, and variation, such as water flow, were recorded. Chitons were aggregated in three sites. Each site had a different water flow condition. At site one, there was an obvious water current. At site two, it had a steady water current at low tide. At site three, which was located at the exit of the water outflow from Friday Harbor Labs, made the site exposed to constant water flow. Individual lengths were measured, anterior to posterior, with a ruler. We determined body orientation relative to north using a compass, and used a level to determine the angular rotation of each chiton above horizontal.

Data analysis methods

The average length of sample size was calculated by Excel for the manipulated ambient magnetic field experiment. The average length and vertical angle of chiton sample size for the natural magnetic field was calculated by Excel.

In the manipulated ambient magnetic field experiment, the angular orientation of individual chiton were measured by compass in degree relative to the north. Using pictures we recorded during the experiment, we marked the "stable stage" of each chiton which was defined as stationary at the spot for over an hour. For the chitons that did not reach a stable stage during the experiment time period, we used their position in last picture (time=3hr) as their final position. We recorded the angle between chitons' final position to the north for further analysis.

Oriana is a program that was used to calculate the special forms of sample statistic that required circular data which is ideal for analyzing our data. All data were keyed in a uni-directional angle between 0 to 360 degrees to the north. We separated the data into two columns, control and experimental group. With our data, we ran the program to generate a circular-linear plot for each group and used the Mardia-Watson-Wheeler test and Watson's U^2 test (Multisampled test) to generate a U^2 value and p- value to find statistical significance.

Result:

There are 75 total samples for both experimental group and control group (n=75.) The size of the samples were similar between the two sample groups. The average length for control group was 3.712 cm with a sample standard deviation of 0.487. The average length for experimental group was 3.697 cm with a sample standard deviation of 0.512.

The raw data plot from control group showed no statistically significant cluster of chiton in the angle between 0 to 360 degrees. The 95 % confidence interval fell between 225 to 45 degrees that covered the northwest area of circular plot with mean vector of 319.344 degrees . However, lack of data cluster showed no statistically significant in neither confidence interval nor mean vector (Fig.2.) The raw-data plot from the artificial ambient magnetic fields was clustered significantly. The 95 % confidence interval fell between the southeast area on the circular plot, with the mean vector of 128.587 degrees, which is opposite compared to the data from control group (Fig.3.)

We used two mutisamples tests, Watson U^2 test and Mardia-Watson-Wheeler test to evaluate whether two samples had statistically significant different means and variances. With Watson U^2 test which yielded: U^2= 0.253 with p-value < 0.02.h (Table.1.), and the Mardia-Watson-Wheelwe test which yielded: W=7.843 with p-value < 0.02.

The data from the outdoor experiment was analyzed by Orianan. Looking at the raw data plot, only site one showed statistically significant data cluster within a 95% confidence interval. However, among these three graphs, all mean vectors are at the position between 30 to 60 degrees to the north. The sample sizes and the length of chitons varied in each site.The average length in site one was 7.032cm (sample S.D. =1.069) with 28 samples within the group. The average length in site two was 6.161cm (sample S.D.=1.147) with 22 samples within the group. The average length in site three was 4.388cm (sample S.D.= 1.108) with 8 samples within the group. For angular rotation of each chiton, data was greater than zero if chitons' heads position was above horizontal, and negative value if head was below horizontal level. The average in site one was 2.39 degree (S.D.= 11.070); the average in site two was 3 degree (S.D= 7.831), and the average in site three was 3.662 (S.D.= 14.08.)

Discussion:

Manipulated ambient magnetic field

The main findings of our study was that the differences in chiton head orientation between control group and experimental group were statistically significant with a p-value <0.02. The mean vector of the experimental group was on the opposite side of the mean raw data vector of control group. This finding was significant since it indicated that the rotation of the ambient magnetic field had influenced and changed the orientation of chiton. This shows that there is a possibility that chiton have the ability to detect the ambient magnetic field.

However, due to the lack of trials with the rotated ambient magnetic field, there was no statistical evidence to show any correlation between the angular change of chitons' heads and the angular rotation of the ambient magnetic field. Our trials rotated the ambient magnetic field 90 degrees clockwise, and the outcome showed that chitons changed their head orientation almost 180 degrees, instead of anticipated 90 degree angle. Therefore, results did not fully support our hypothesis: the ferromagnetic mineral magnetite within chiton teeth acts as a functional navigation system, directing chitons towards the ambient magnetic field. *M. muscosa* had a 180 degree reverse orientation vs. the control group when they were exposed to rotated ambient magnetic field; however, the pattern of their orientation is still unknown and needs further studies.

Natural magnetic field result

The chitons' heads orientation found in the three outdoor sites was not significantly significant but the mean vector in the raw data plot in each site pointed in the same direction. We interpreted these results concluding that the environmental variables were too distracting (water flow, varied water temperatures, and varied water cover) taking priority over chitons' heads orientation than orienting themselves towards the Earth's magnetic field. The only interpretation of the common mean vector in the raw data plot in each site could possibly be related to the tide. All chiton found during low tide in all three sites experienced high tide recently before. Though the data is not significantly significant, their mean vector orientation may of been related to an orientation towards the ocean. For each site, a very small sample size was statistically analyzed in Oriana that may lead to a non-statistically significant result yielding a high p-value.

Artificial ambient magnetic field

The three out of five artificial ambient magnetic field trials had two chiton spawn indicating stress related behavior. Deprived of environmental stimuli (constant water temperature and water flow), and only experiencing a stressful environment, navigational behavior to migrate might have been triggered. In most mobile invertebrates, when stressed or threatened, organisms move away from the threat as quickly as possible (Watts 508). If the experimental chiton felt the need to move away from the stressful environment but had no influencing environmental factor to guide them, following the ambient magnetic field might have been necessary for navigational guidance.

Throughout our data collection within the three outdoor sites, the debated homing behavior within the species was not observed in the field. Chiton found within the three sites were marked, along with their corresponding rocks. Marked chitons were not observed again days later. We assume that chitons traveled to a new spot with no homing behavior or markers were lost.

Results to the rotated ambient magnetic field may have biased results due to the starting initial position of chiton in each trial. Chitons' heads were positioned at the start of each trial at 150 degrees related to the north. Chitons did not have to move very far to remain stable at their 180 degree sable resting period for measurement. In future studies, the initial head orientation starting position in each trial should be randomized.

The conclusion to our study is that *M. muscosa* possibly demonstrates magnetotaxis behavior using the ferromagnetic mineral magnetite within their teeth but lack any type of homing behavior. The ability to detect and orientate themselves to the rotated ambient magnetic field was apparent, but disproved our predicted orientation. The speculated homing behavior in our specific species was not demonstrated as marked chitons disappeared off their homing rocks and never returned.

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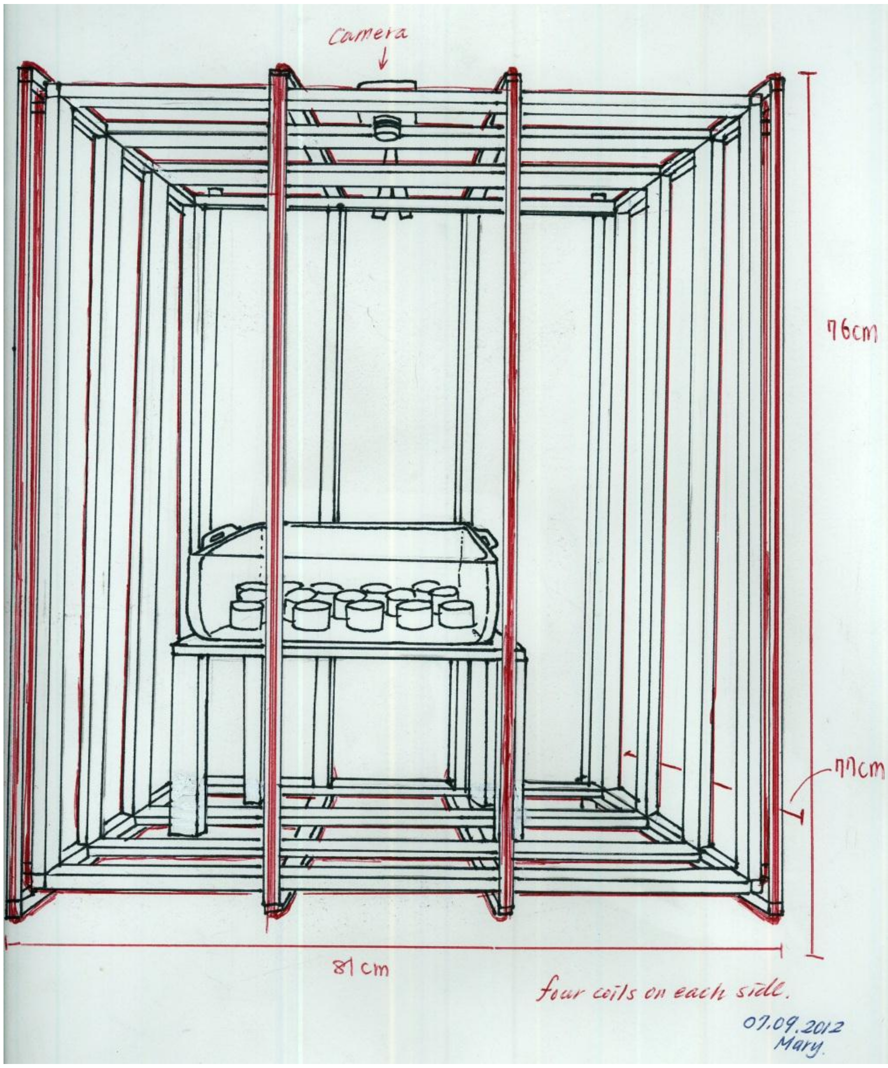

Fig. 1. Magnetic stimulus was generated by a magnetic coil system (4 square coils, each 1.02m on a side). Constant current was introduced and flowed through the circular wire. This created a magnetic field around the coiled wire magnetizing the metal as if it were a permanent magnet. Organisms were exposed to a magnetic field intensity of 100,000 nT which is the same intensity of ambient field.

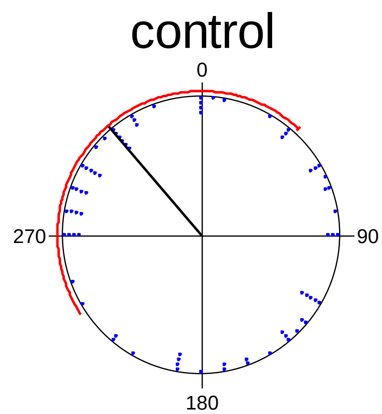

Fig. 2. The raw data plot of control group. The data from the control group was not significantly clustered which made the 95% confidence interval very broad and not statistically significant. Each blue dot represents the frequency of chiton.

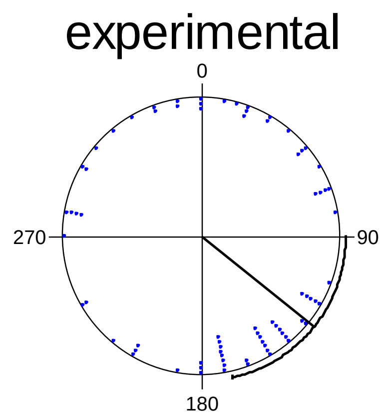

Fig. 3. The raw data plot of experimental group. The data from the artificial ambient magnetic field was significantly clustered within the 95% confidence interval at the opposite side of control group. Eath blue dot represents the frequency of chiton.

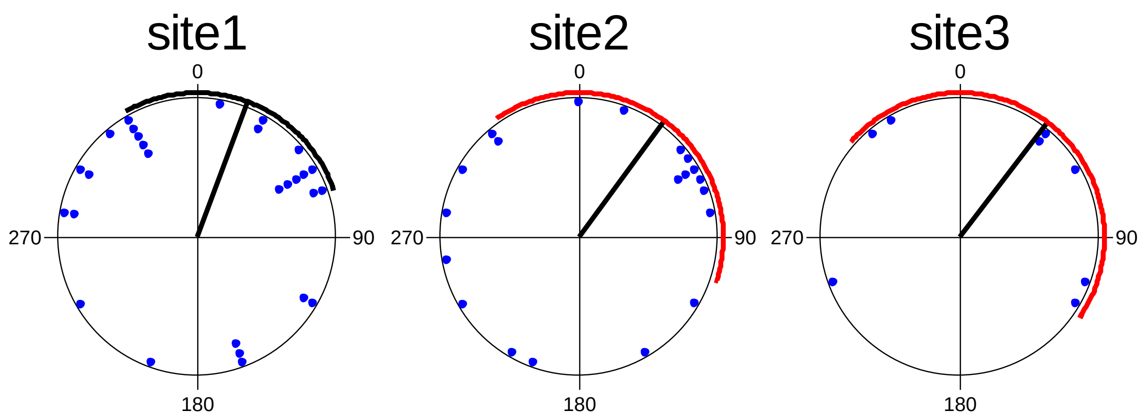

Fig. 4. Raw data plot of each individual site in the natural ambient magnetic field. Site one showed statistically significant cluster of chitons. Site two and three has no statistical significant cluster of chitons.

Table 1. The result from Welson's U^2 mutisample test comparing data between experimental and control groups. Test shows statistically significant values between the control vs. experimental group supported by p value <0.02.

Variable (& observations)	**U^2**	**p**	**d.f.**	**d.f.2**
Control group & experimental group	0.253	<0.02	75	75