Contents

Abstract

Research was carried out to investigate the effect of the direction of incident light on the behavioural response of Calliphora larvae. They were centrally placed onto a piece of white paper marked with positive, negative and neutral in a circular chart. White light was shone onto the maggot from one direction and the subsequent direction and speed of movement was measured.
Results suggest that the directional movement of the Calliph ora is influenced by the angle of light and that the maggots showed negative phototaxis behaviour. This supports work which was carried out by previous researchers. This response of moving away from the incident light may offer the Calliphora larvae increa sed chance of survival.

Introduction

Larvae of the Calliphora species demonstrated negative phototaxis when illuminated with incident light radiation. Taxis is an orientation movement towards or away from a light stimulus. Negative phototaxis is the mo vement away from a light stimulus. In this investigation the maggots were kept in the same conditions and were placed individually into the experimental area. The maggots were then illuminated in light from only one direction and there response was observe d. The vast majority of the maggots (80%) moved away from the light, exhibiting negative phototaxis behaviour. Calliphora larvae have a limited protection from UV radiation, the negative phototaxis behaviour shown will take them away from the harmful radi ation will improve their chance of survival.

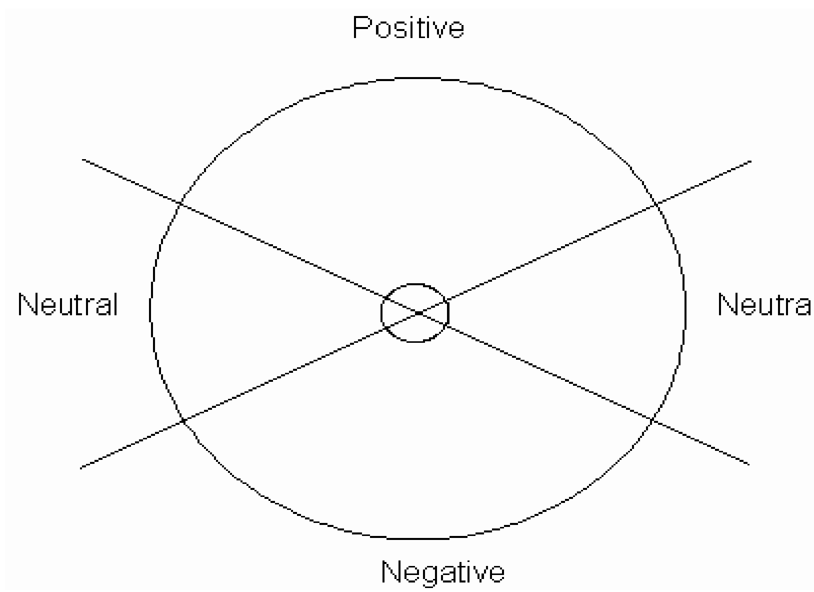
Implementing

Modifications to the design

After my preliminary work I found a few problems which would cause my investigation to not be as accurate as possible. These problems are:

- The investigation in my plan was only measuring the rate of movement for the maggot. From my preliminary work I found that this wasn't enough to get the
- results as accurate as I wanted them, I would now like to introduce a new criteria then I will be measuring, this is the direction of the maggot moves in relation to the light shining on them. This will be quite easy to instigate and the method will not have to be changed drastically. To measure the direction the maggots will move I will use a piece of paper with a chart (below) on, this will allow me to see which part of the chart the maggots go into and therefore show weather the maggots show positive, negative or no phototaxis behaviour.
- My null hypothesis has to be change because of the new criteria:

There is no relationship between the distance the maggot moves and the distance the maggot is away from the light. As there is the same angle for each of the three sectors an equal amount of maggots will leave the paper by each sector. 33.3% of the maggots should leave from each sector.



(Each of the positive and negative sectors should have angles of 120° and each of the neutral sectors should have angles of 60°. The sector marked positive should be the closest to the light.)

- The maggots move a lot faster than I had anticipated so I will use 2-second intervals instead of 5 seconds.
- The only other problem I had was the effect of chemical traces made by the pencil to track the maggot's movement. This time I have decided to use a piece of OHP paper over a plastic box this way the light will still be able to get through the box and the maggots will not be affected by the chemicals from the pen at all.

Experimental results

To measured the rate of movement in cm/second this meant having to measure the distance moved and the time taken for each 2 second interval and using the following equation

$$\text{Speed} = \frac{\text{Distance}}{\text{Time}}$$

Distance (cm) From Lamp at each 2 second interval

Maggot	2	4	6	8	10	Overall Direction
1	20.6	22.6	24.4	25.5	26.4	-
2	21.6	23.5	25.0	25.5		-
3	20.6	22.5	24.3	25.9		-
4	22.8	23.5	22.0	23.0		Neutral
5	21.7	23.7	25.8			-
6	22.0	24.4	25.4			-
7	20.9	23.7	25.2	26.1		-
8	22.0	23.5	23.0	23.0	23.5	Neutral
9	21.5	23.0	24.0	25.3	25.9	-
10	22.2	23.7	24.0	24.5	24.8	Neutral
11	21.4	22.9	24.9	25.9	26.5	-
12	21.7	23.5	24.5	26.2	26.0	-
13	21.9	24.4	25.6	25.8		-
14	21.8	23.0	24.6	25.6		-
15	21.5	24.5	26.1			-
16	20.9	22.7	23.7	25.1		-
17	21.7	23.9	26.1			-
18	15.5	14.0	12.0			+
19	21.5	20.5	21.0			Neutral
20	21.3	23.1	25.1	26.5		-

Speed Of Maggot (cm/second)

Maggot	2	4	6	8	10	Overall Direction
1	0.8	1.0	0.9	0.8	0.7	-
2	1.2	1.1	0.9	0.9		-
3	0.7	1.0	0.9	0.8		-
4	1.5	1.1	1.2	0.6		Neutral
5	1.3	1.0	1.1			-
6	1.5	1.2	0.5			-
7	0.8	1.4	0.9	1.0		-
8	1.2	0.8	0.8	1.0	0.8	Neutral
9	1.0	0.8	0.5	0.5	0.6	-
10	1.5	0.8	0.8	0.7	0.5	Neutral
11	1.0	0.8	1.0	0.6	0.8	-
12	1.2	0.9	0.5	0.9	0.9	-
13	1.3	1.3	0.8	0.8		-
14	1.3	0.6	0.8	0.5		-
15	1.1	1.5	0.8			-
16	0.9	0.9	0.5	0.7		-
17	1.3	1.1	1.1			-
18	1.1	1.0	1.3			+
19	1.3	1.1	1.0			Neutral
20	1.0	0.9	1.0	0.7		-

Associated results

The associated results are measurements taken to ensure that the variables of this investigation were being co ntrolled. These variables needed to be controlled in order to improve result reliability.

Measurement	1st reading	2nd reading	3rd reading	Average
Lab temperature	21 c	21 c	21 c	21 c
Light intensity	2.950 flux	2.920 flux	2.930 flux	2.933 flux
Maggot mass	0.070g	0.080g	0.100g	0.083g

Precautions

These are the factors that would have rendered my investigation invalid, and the steps I took to minimise them

Heat pollution and Variations in temperature

Description: If there is a significant amount of I. R. radiation emitted from the source of light or any other stationary source, it is possible that the maggots respond to this rather than the visible light stimulus. Also the temperature in the lab varies from day to day, and throughout the day.

Steps taken to minimise it: Measured the temperature every 10 minutes to check whether it varies. I also made sure I completed the experiment on the same day as the temperature could have changed drastically from one day to the next.

Light flooding

Description: If stray light of considerable intensity reaches the maggots, they may respond to this rather than the lamp I am using. This could cause the results to be invalid, as they would not be affected by the lamp I am using and so the negative or positive phototaxis I have recorded will be wrong if my light isn't the main one in the room.

Steps taken to minimise it: I avoided this problem by working in a darkroom and shielded the light from any other experiments that were happening in the room.

Pupating

Description: if the maggots get old enough, they may start to develop into the pre - pupal stage and their behaviour may begin to change.

Steps taken to minimise it: I kept the maggots in the fridge and only took them out for as long as necessary. They were bought fresh near the time of execution of the experiment.

Small number of maggots causing fluctuations

Description: The more samples are taken, the less the extent to which random results affect the averages.

Steps taken to minimise it: The effect of this was kept to a minimal by keeping the number of maggots the same. I used a different larva each time, which increased the chances of having random results but decreases their affect as the other larva in the same experiment show it to be anomalous.

Maggot injury

Description: If a maggot is handled carelessly or are used for many consecutive experiments, it may become damaged and no longer act in a manner typical of other maggots.

Steps taken to minimise it: I used a scoop, a different maggot for each experiment and tried not to drop any of them, if this did happen I would not use that maggot in the experiment.

Reliability of data

I believe that the data I have produced is of a very high standard, the precautions I took made the experiment contain the least amount of errors possible and so in my opinion my data is reliable. As the responses are innate (instinctive nervous responses rather than conditioned/learned), they would not vary between maggots. I think that, despite the differences between the natural and tested mediums, the conclusion is valid, as a negative phototaxis would only be more strongly demonstrated in an environment where it has evolved to move. Therefore it is safe to conclude that this study is representative of all Blowfly Larvae.

Analysing evidence and drawing conclusions

The data I have collected shows me that the maggots move away from the light as soon as they were placed in the plastic container. The maggot's behaviour is therefore negative phototaxis. 15 of the 20 maggots left the outer circle through the negative sector, showing that the maggots moved away from the light. The Chi ^{2} test is shown below.

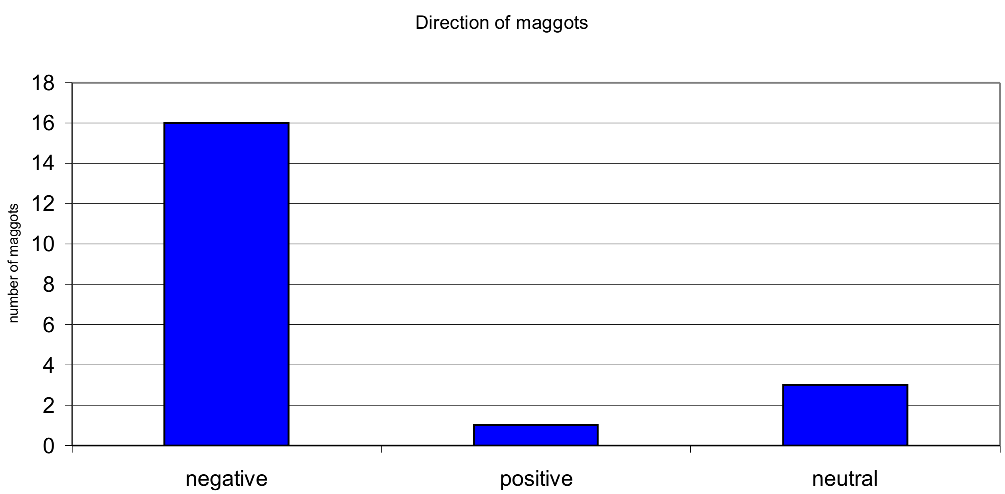
Direction	Observed	Expected	O-E	(O-E) ^{2} /E
Positive	1	6.67	-5.67	-4.81
Negative	16	6.67	9.33	13.04
Neutral	3	6.67	-3.67	-2.01
			Total	**6.21**

As the total is larger than 3.841 I can be 95% certain that there is a significant difference between these results and my null hypothesis in which I stated, there is no relationship between the distance the maggot moves and the distance the maggot is away from the light. As there is the same angle for each of the three sectors an equal amount of maggots will leave the paper by each sector. 33.3% of the maggots should leave from each sector; therefore I can reject my null hypothesis. The maggots in my experiment show negative phototaxis the movement of the whole organism away from light, for example maggot number 17 moves away from the light at a speed of 1.3 cm/s, by the time it had reached the outer sector it had slowed down to only 1.1 cm/ second. Fifteen of the twenty maggots showed the same behaviour and left by the negative sector.

Conclusion

I found that the blowfly larvae demonstrated an innate negative phototactic response .

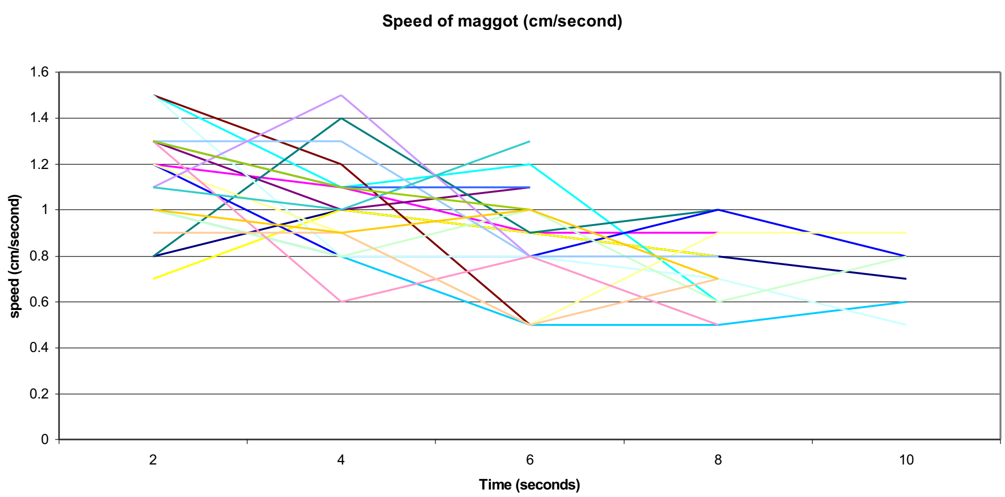
Graph 1

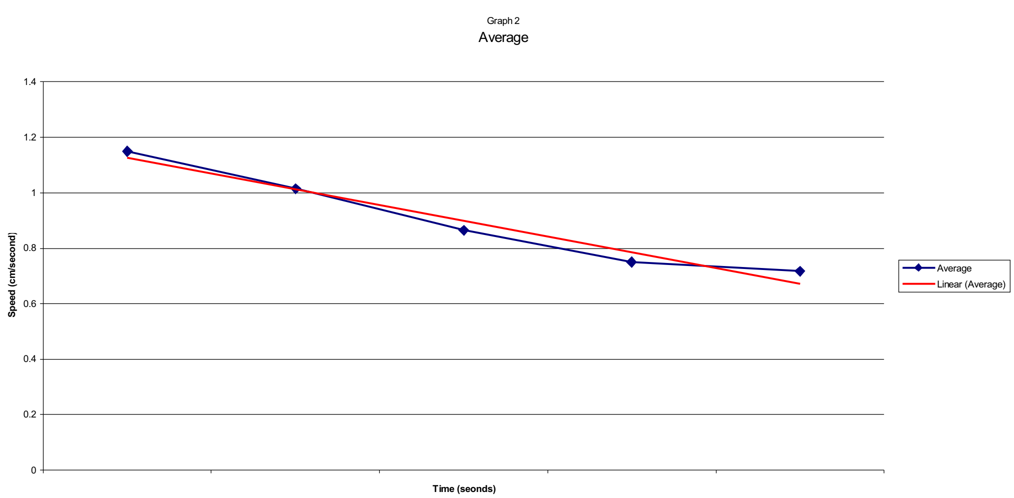


The graph of the overall position of the maggot, which sector (positive, negative or neutral) it left the circle by. It shows that the Majority of the maggots left the circle by the negative sector, 80% of the maggots were showing a negative photot actic response.

There was only one maggot that didn't show the same response, number 18. Maggots have light sensors on either side of their head, by moving their head they can sense where the light is coming from, if the maggot detects the light is stro nger on the left side it will move to the right to get away from the light. This is because the U.V rays will harm the maggot, moving away from the light will increase the maggot's chance of survival. If the intensity of light is same on both sides the m aggot will move in a straight line. This could explain why maggot 18 headed towards the light, when it was placed in the tray it may have been pointing towards the light and so was receiving equal light on both receptors. The position of the maggot was c hosen by spinning a pencil so it was random and couldn't be helped.

Graph 2 to 3





The graph of the speed of movement against the distance from the lamp and the average speed of movement shows a negative correlation between the time the maggot has been moving and the speed of the maggot. It shows that as the maggots get further away from the light their speed decreases. This could mean the maggots are showing photokinesis, more experiments would need to be done to confirm this. Kinesis is a reaction to the intensity of the stimulus and not the direction so photokinesis could be tested by using light that the intensity could be changed for example a light with a variable resistor.

Evaluating evidence and procedures

Limitations of apparatus

Humidity
This is something that could not have been controlled unless I had access to high - quality expensive labs and equipment, which could let me keep a constant humidity, but this would be very unlikely. To keep the humidity change to a minimum I used the same room on the same day and tried to keep my experiment as short as possible.

Temperature
The temperature couldn't have been changed but I used the same room on the same day to stop drastic changes to the tem perature and I also used a water tub in front of the light to absorb as much heat as possible. I also had a thermometer to keep a check on the temperature but even if I had have noticed a change I couldn't have done anything about it.

Light intensity
I controlled the intensity of the light using a variable resistor that was kept on the same level throughout the experiment; this would have been all I could do to keep the intensity the same. If I had to do my experiment on different days I would have made sure I used the same light and variable resistor so the intensity would be exactly the same.

Chemical traces
I used a different method to my preliminary work for keeping the chemical traces away from the maggots. I used a plastic tray and OHP paper, whi ch meant the chemicals could not reach the maggot but the light could still get though the box. This seemed to work very well as in my preliminary experiments the maggot would sometimes be seen to move away from the pen no matter where the light was and this affected my results. I don't think I could have done much more to stop chemical traces affecting the maggot's behaviour and I was pleased with the end method.

Surface gradient
I used a hard board base to keep the level constant and used a spirit leve l to ensure there wasn't a slope on the table. This worked well and I don't believe it affected my results at all.

Age of maggot
It would be impossible to tell the age of the maggots unless I had bred them myself, this meant that the stage of development could not have been identified. It will be assumed that all the maggots from the same batch are at the same stage of development.

Colour of the maggot
I chose maggots of similar colour and the maggots were all taken from the same batch and by doing rep eats the results are made more accurate.

Positions the maggots are placed in
I chose to put the maggots in a random starting direction although they all started in the same place in the circle. I spun a pencil around and the direction the pencil was

facing would be the one the maggot would be placed in. This meant that the direction was unbiased and the direction the maggot was placed in wasn't affecting the overall position of the maggot.

Colour of light

The same light box and light were used for the entire experiment to reduce the risk of the light changing colour, this wouldn't have a huge effect on the maggot as the colour would only change slightly but by using the same equipment I hoped to decrease this risk.

The conditions the maggots are kept in

The maggots were all placed in a beaker covered with black paper and stored at room temperature before the experiment to get them used to the new conditions. I made sure all of the maggots were subjected to the same conditions throughout the experiment and so they would have all been affected in the same way if the conditions did cause them to change behaviour.

Direction of light

The direction of the light was kept the same throughout the experiment and the dark room made sure that there was no other light from any other sources. I did have the problem of light from other people's experiments in my preliminary experiments but I chose to use the dork room on my own to stop this affecting my results at all.

Limitations of techniques

Time inaccuracies are unlikely due to the stop watch and are most probable from the reaction time of myself which could not have been helped this would not affect the results a lot.
Percentage errors were fairly high, as the measurements were only to the nearest centimetre and second.
The maggots were a large limitation as there was no telling how old they were and so in which stage of development they were in. If I chose maggot's from the same stage I would know that there behaviour has something to do with the condition s I have put them in, but because they were different ages their behaviour could have something to do with the stage of development or the conditions. The only way I could overcome this would be to breed them myself and this would be very impractical.

Effects of these limitations on the data collected

Limitation	Possible effect on results
Age of maggot	The maggot's reaction to the light may vary during the different stages of its life cycle. It is unlikely that the UV light will e any more harmful to the maggot in the differing stages.
Humidity	The humidity I have found out does have an effect of the maggots behaviour, but the changes in humidity during the experiment will be minimal and the maggots behaviour wouldn't be effects very much
Temperature	The temperature does chang e the rate of activity in the maggots, the change in

	temperature was kept to a minimal and there wasn't a huge change in temperature during the whole experiment this shouldn't have an effect on the maggots behaviour. If there is a change in temperature the effect on the data will be quite small.
Light intensity	I controlled the intensity of the light using a variable resistor that was kept on the same level throughout the experiment , the intensity of the light does affect the behaviour of the maggots but because of the equipment I used it shouldn't have affected the data.
Chemical traces	Although I had problems with this in my preliminary work, I found that there were no visible effects from the chemical traces, if there were it would only be a small amount this wouldn't have affected my results.
Surface gradient	The surface of the table should have been close to flat, this was one of the harder things to control but I found the maggots were moving in a direction away from the light rather than moving in the direction that was slightly down hill.
Percentage errors, time inaccuracies	These were very things that I was unable to stop I don't feel they effected the data very much.

Effects on conclusion

I believe that the conclusion I have come to is reliable and valid. The limitations I discovered through out this investigation I feel wouldn't have had a big effect on my results and so my conclusion will be ok. I had some results from an investigation similar to mine and the results match mine very closely and from this I can see that my conclusion is dependable. Every experiment has its limitations especially when using the limited resources and equipment that was available to me but I do not feel that the conclusion I have come to would have changed if I had have used expensive equipment and more reliable methods.

Synthesis of principles and concepts

Scientific knowledge

The housefly (mucus domestica) is in the order Diptera (flies and mosquitoes). The general characteristics of the Diptera order are that the members have only one pair of membranous wings, structural mouth parts with no mandibles and apodous (without legs) larva[1]. Other examples of this order include Drosophila (fruit files), Tabanus (horse flies) and Calliphora Vicina (bluebottles). Female house flies lay cylindrical eggs about 1mm in length onto decomposing matter. They have a four stage life (metamorphosis) cycle, consisting of an egg stage, a larvae stage, a pupal stage and an adult stag e.

The eggs, approximately 3 days after being laid, hatch into larvae, which are about 1cm in length. The larvae metamorphose into the pupal, which are about 8mm in length. The adult (imago) hatches to form the pupal after about a 4 day period assuming external conditions such as temperature are right, there maybe several generations in one year. As soon as the larva has emerged from the egg its immediate purpose is to feed. In this way the larva gains the energy it requires for the metamorphosis during the non feeding pupal stage. The eggs are laid in decaying organic matter that the maggot can feed on, therefore the maggot does not need to locate food when it hatches, instead it has to stay on the food and avoid hazardous conditions. For a maggot the ma in hazards include ultra violet radiation and predators. Over time maggots have evolved methods of behaviour that have ensured the continuation of the species.

The way in which animals respond to external factors is known as behaviour, i nnate behaviour, or instinct, is generally taken to be pattern of behaviour elicited by specific stimuli and fulfilling vital needs of an organism. It is demonstrated in its purest form by many lower animals including insects as larger animals tend to have more complex nervous and hormonal systems. The expression or types of behaviour include taxis and kinesis these are both examples of innate behaviour. This means that this behaviour is not learned or a result of experience, it is inherited, inflexible and is not changed by the environment that the organism is inhabiting at the time. Most organisms of the same species will exhibit the same behaviour if it is innate to that species. Innate behaviour is also known as 'Fixed action pattern' behaviour.

Taxis is an orientation movement of a whole organism towards or away from a directional stimulus. Positive phototaxis is the movement towards a light stimulus; negative phototaxis is the movement away from a light stimulus. Maggots exhibit negative phototaxis, so they move away from a light stimulus. This behaviour has survival value for the maggot as bright light include ultra violet rays (UV). UV rays are high frequencies, short wavelength radiation, and are highly damaging to maggots. The pale skin of the maggot appears to hav e less pigmentation and is likely to be susceptible to the damage caused by light radiation perhaps from the UV range of light radiation. This could explain why the vast majority of the maggots (80%) in this investigation moved away from the light source, exhibiting negative phototaxis.

Kinesis is a behaviour pattern in which the organism changes its rate of movement in relation to the intensity of the stimulus. Flatworms for example respond to chemical

[1] Cratchley, K, Handbook of animal types.

gradients, they move in a straight line until they detect an increase in chemical concentration. Their path then becomes more random, the increasing chemical gradient means that flat worms increase their rate of turning in the area of the meat until they touch it, they may then begin feeding [2]. When animals such as woodlice that are accustomed to inhabiting a damp environment are placed in a dry environment their rate of activity increases. The woodlouse locating a damp environment will speed up again increasing its chances of survival. In kinesis unlike tax is the animal does not go in any particular direction [3].

Maggots have photoreceptors on each side of their heads to gauge the direction from which the light comes. They will move their heads from one side to the other and sense which side has the most intense light, they will move in the direction with the lowest intensity. If they were to increase their rate of movement and or change the direction of movement with light intensity (a kinesis), should they be on the surface, or reach the surface they would make themselves more vulnerable to predators. This kind of behaviour would be too easy for predators to spot, although it stops when the organism is buried deep enough to be an advantage to them. A change in allele frequencies resulting from their vulnerability in this situation would rather favour the evolution of direct movement away from the light (taxis). The idea would be if the movement was away from the light source, and slowed down as the maggot reached the centre of its abode, where the light intensity was at its least, so that it stayed there. This is a negative phototactic response. As the U.V rays will harm the maggot, moving away from the light will increase the maggot's chance of survival. This would be why most of the maggots moved away from the light, because the UV rays from the light will be harmful to them and it is usually where they are more susceptibly to predators. There pattern of behaviour will make them move away from the light and so in turn away from the most danger increasing their chances of survival.

The light in the investigation was coming from only one direction, if the maggots had some innate behaviour of negative or positive phototaxis they would have moved in a particular direction, either away or towards the light that was present. The results from my investigation illustrate that the maggots showed negative phototactic behaviour as all but one of the maggots moved away from the light. If there is equal intensity of light on both sides the maggot's head where the receptors are, the maggot will move in a straight line because there isn't a direction they can move into that has a lower intensity of light, this could cause the maggot to move towards the light. This could be the cause of the one anomaly that has been observed, maggot number 18 if the maggot was placed facing the light, the intensity of light on both sides of the head would have the same and so the maggot would have moved straight forward into the light.

The maggot exhibits phototaxis behaviour rather than photokinesis behaviour, this could be because phototaxis behaviour is more efficient and in order to sustain the pupal period maggots have evolved to conserve energy. Efficiency conserves energy. A kinesis type movement could attract the attention of predators as it could involve if the maggot was on the surface of the decaying matter for a longer period of time. The longer the exposure on the surface the more vulnerable the maggot is to predators and harmful UV radiation. A change in allele frequencies resulting from their vulnerability in this situation would rather favour the evolution of the direct movement away from light (negative phototaxis).

---

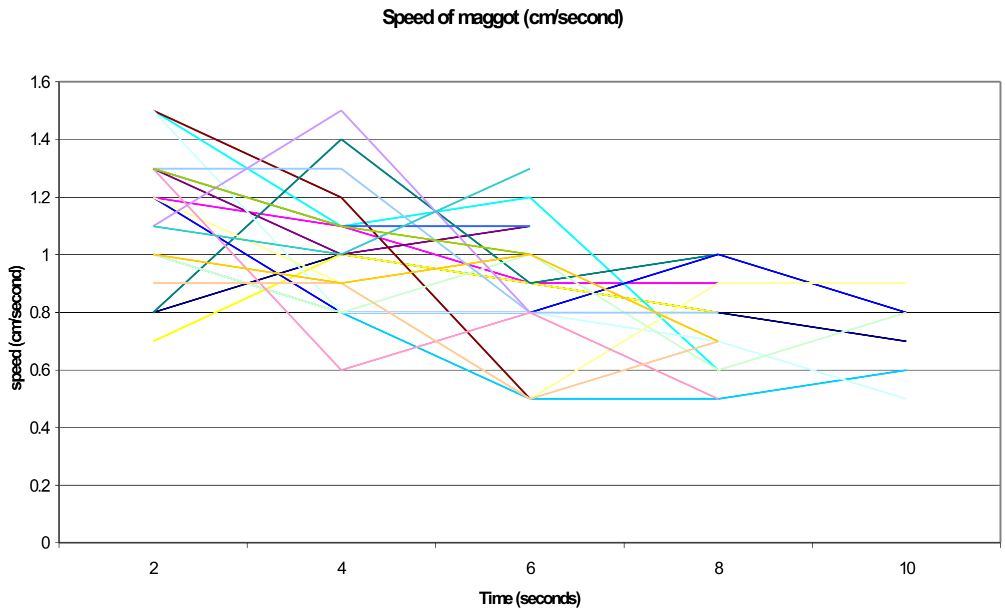
[2] Murray, P and Owens, N Behaviour and populations Harper Collins Publishers 2001 pg.9
[3] Murray, P and Owens, N Behaviour and populations Harper Collins Publishers 2001 pg.8

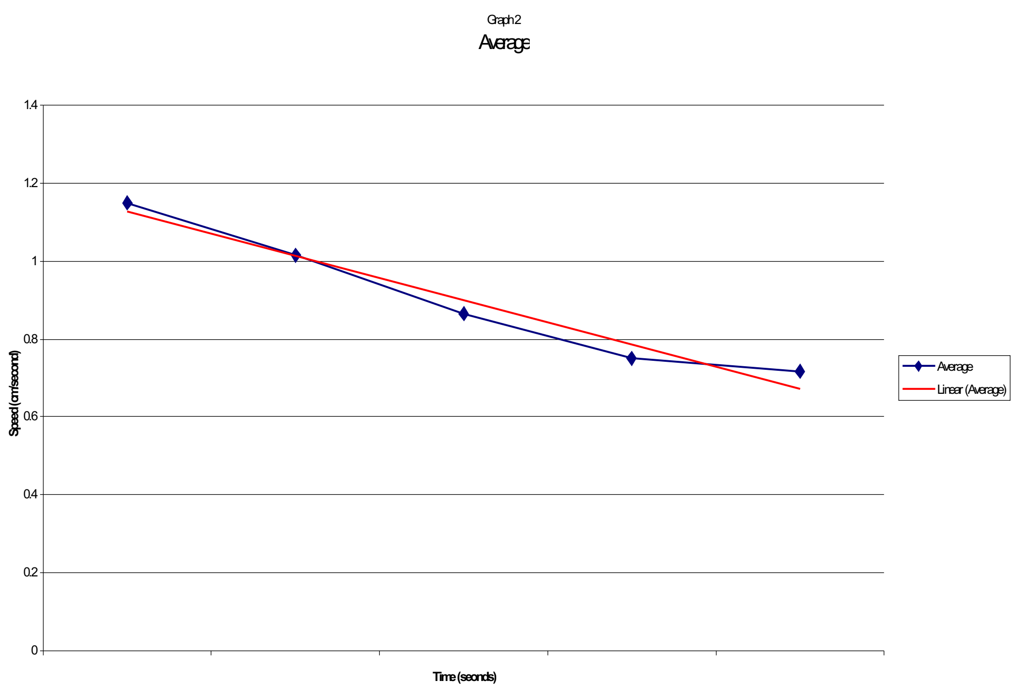
A directional response is also an advantage to the housefly in the later stages of its development. The pupae need to be on the surface so that the hatched flies can leave the area. A directional movement towards the light (positive phototaxis) would be an advantage to the housefly as it again conserves vital energy and the random movement of kinesis would hinder the maggot. In that if the maggot were hatched into a large carcass, it could take a longer period of time to reach the surface using kinesis, expending large amounts of energy in the process. The imago also needs to exhibit positive phototaxis behaviour so that it can quickly and efficiently reach light. The adult fly locates the light in order to warm up, the fly needs to warm up so that it can increase its respiratory rate and produce more energy. More energy increases the fly's chance of survival by enabling it to seek out food and avoid predators. The age of the maggot would therefore affect its response to light. This means that the anomalous result when the maggot moved towards the light could have been caused because it was older and closer to the pupating stage where positive phototaxis behaviour is exhibited.

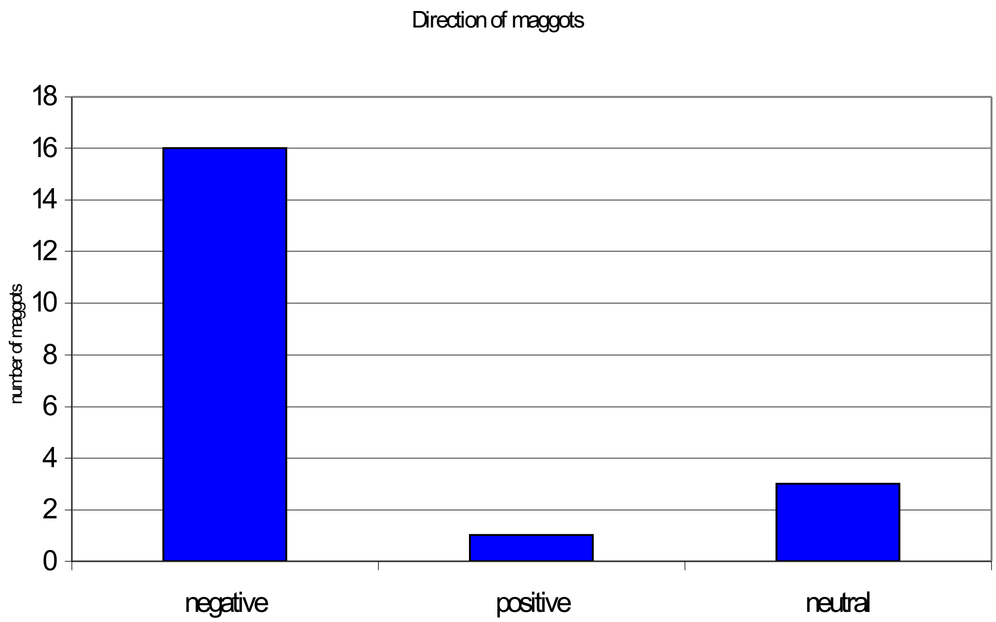
References

- Cratchey, K, Handbook of animal types
- Burton, G, Holman, J, Lazenby, J, Pilling, G, Waddington, O, Salter's Advanced Chemistry, Chemical Ideas, Heinemann (2nd edition) 2000
- www.emrory.edu
- www.iinet.net.edu
- www.ply.com.uk
- www.ups.edu
- Murray, P and Owens, N Behaviour and population Harper Collins Publishers 2001

Appendix


Speed of maggot (cm/second)
speed (cm/second)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
Time (seconds)


Graph2
Average
Speed (cm/second)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Time (seconds)
Average
Linear (Average)



Chi squared test
from other research

Direction	Observed	Expected	O-E	(O-E)2	(O-E)2 /E
Positive	1	6.67	-5.67	32.14	4.81
Negative	15	6.67	8.33	69.38	10.40
Neutral	4	6.67	-2.67	7.12	1.67
				Total	16.25

Distance moved by maggots

	2	4	6	8	10	position
1	20.6	22.6	24.4	25.5	26.4	negative
2	21.6	23.5	25	25.2		negative
3	20.6	23.5	24.3	25.9		negative
4	22.8	23.5	22	23		neutral
5	21.7	23.7	25.8			negative
6	22	24.4	25.4			neutral
7	20.9	23.7	25.2	26.1		negative
8	22	23.5	23	23	23.5	negative
9	21.5	23	24	25.3	25.9	negative
10	22.2	23.7	24	24.5	24.8	negative
11	21.4	22.9	24.9	25.9	26.5	negative
12	21.7	23.5	24.5	26.2	26	negative
13	21.9	24.4	25.6	25.8		negative
14	21.8	23	24.6	25.6		negative
15	21.5	24.5	26.1			negative
16	20.9	22.7	23.7	25.1		negative
17	21.7	23.9	26.1			negative
18	15.5	14	12			positive
19	21.5	30.5	21			neutral
20	21.3	23.1	25.1	26.5		negative

position
negative
negative
negative
neutral
negative
neutral
negative
negative
negative
negative
negative
negative
negative
negative
negative
negative
negative
positive
neutral
negative

position	
negative	16
positive	1
neutral	3

Speed of maggot

maggot	2	4	6	8	10
1	0.80	1.00	0.90	0.80	0.70
2	1.20	1.10	0.90	0.90	
3	0.70	1.00	0.90	0.80	
4	1.50	1.10	1.20	0.60	
5	1.30	1.00	1.10		
6	1.50	1.20	0.50		
7	0.80	1.40	0.90	1.00	
8	1.20	0.80	0.80	1.00	0.80
9	1.00	0.80	0.50	0.50	0.60
10	1.50	0.80	0.80	0.70	0.50
11	1.00	0.80	1.00	0.60	0.80
12	1.20	0.90	0.50	0.90	0.90
13	1.30	1.30	0.80	0.80	
14	1.30	0.60	0.80	0.50	
15	1.10	1.50	0.80		
16	0.90	0.90	0.50	0.70	
17	1.30	1.10	1.10		
18	1.10	1.00	1.30		
19	1.30	1.10	1.00		
20	1.00	0.90	1.00	0.70	
	2	4	6	8	10
Average	**1.15**	**1.02**	**0.87**	**0.75**	**0.72**

The distance travelled at each 2 second interval