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# Optimization of an indoor arena for behavioural studies of target detection in hoverflies

Johan Jakobsson

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Biology Education Centre and Department of Neuroscience, Uppsala University

Supervisor: Karin Nordström

## Abstract

Despite its relatively simple visual system hoverflies (Syrphidae) are capable of complex visually guided behaviour. Recent models suggest that one of the factors used by flies to detect targets is the difference in contrast between the target and the background. An arena was constructed for behavioural experiments aimed at investigating if targets providing different contrasts to the same background have different probabilities of generating a behavioural response. The arena for the experiments consisted of an aquarium modified to encourage natural behaviour of flies of the genera *Helophilus*, *Eristalis* and *Episyrphus*. By making the arena illumination homogeneous and by covering the arena walls with grass, a more natural behaviour was achieved by the reduction of phototaxis and a more normal visitation time on the food sources. Several panorama pictures were also taken at hovering sites for *Helophilus* and *Episyrphus*. These pictures were analysed for common features and were to be used as natural backgrounds in the behaviour experiments. The common features were that the flies were hovering close to food sources, facing an open area and were covered by vegetation from at least one direction. A majority of the pictures also contained visual features producing a sinusoidal like curve with its valley in front of the fly and its peak near the rear of the fly. These common features can be used when designing future behavioural arenas. These arenas could in addition to homogenous light and good wall coverage also incorporate backgrounds of open areas and cover near food plants.

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## Introduction

Despite carrying low resolution compound eyes with less than ten thousand pixels and a small brain with less than a million neurons, hoverflies (Syrphidae) are capable of detecting and interacting with conspecifics in a visually complex environment (Collett and Land 1975). Understanding the mechanisms that enables the fly's simple visual and nervous system to produce robust target detection, might not only provide insights into how our own visual system is designed, but the discovered principles can also provide hints of how to design cheap, simple and reliable systems to provide visual guidance for autonomous machines.

One of the models for target detection in flies is based on studies of the figure detection (FD) neurons of the blowfly. The FD1 neuron in the blowfly gain their selectivity for small targets by inhibition from GABAergic centrifugal horizontal (CH) cells (Warzecha, Egelhaaf et al. 1993). The CH-cells are triggered by the widefield stimuli generated by the animals ego-motion and inhibit the response of the FD1 cells (Warzecha, Egelhaaf et al. 1993) thus tuning the response to small figures. Models for target neurons based upon this mechanisms predict that a response to small targets should only be produced when the speed and direction of the movement of the background do not match that of the target (Higgins and Pant 2004). However, electrophysiological studies have proven that the target neurons in hoverflies, the small target motion detection neurons (STMD), are capable of producing a robust response to small targets even when the movement of the background matches that of the target (Nordström, Barnett et al. 2006). This indicates that additional mechanisms are responsible for distinguishing the target from the background. Recent models suggest the visual systems of flies might also make use of the contrast between the target and the background (Wiederman, Shoemaker et al. 2008).

If hoverflies are using contrasts to detect their targets, this should be possible to verify by behavioural experiments that test for the limitation in the ability of hoverflies to detect targets that produce different contrasts against the same background. We intended to study this with a behavioural study of hoverflies of the genera *Helophilus*, *Episyrphus* and *Eristalis*. Hoverflies were chosen for the behavioural experiment since when males perform their territorial hovering behaviour, they have been reported to be highly prone to display behavioural responses to targets that can be interpreted as conspecific competitors or prospective mates (Fitzpatrick and Wellington 1983a; Fitzpatrick and Wellington 1983b). Since STMD neurons can also be found in female hoverflies (Nordström and O'Carroll 2006), female hoverflies were also planned to be used in the experiment. The female STMD neurons react to targets of the same size as those that triggers an response in the male STMD neurons (Nordström and O'Carroll 2006), indicating that they may have evolved to detect the same type of targets as the male STMDs, possibly conspecifics. This might provide females with the option to take an active role in the mating behaviour of hoverflies. If the STMDs in the female hoverflies

have evolved to detect possible mates, a stimulus that have proven to be capable of producing a behavioural response in male hoverflies should also produce a behavioural response in females.

The targets to be used in the experiments were glass beads, painted to provide varying contrast to the background. These glass beads were moved by the use of a counter weight system of our own design. To provide that flies with natural backgrounds to the stimuli, several 360° panorama images were taken at hovering sites for hoverflies. These images were also analysed for common features to gain additional insights to the territorial behaviour of hoverflies.

A majority of the taken panorama images showed common features that had previously been noted to be important for hovering sites, such as the presence of food plants, coverage by vegetation and that the flies were facing an open area. In addition, several of the images contained visual features that together create a wave like form with its valley in the part of the image that represented the direction the hovering fly was facing, and its peaks near the parts of the image that the back of the fly were facing.

Before the behavioural test could be performed the test arena for the experiments had to be optimized to encourage natural behaviour and thereby also a response to the stimulus. The largest alteration made to the arena during the optimization was the addition of lamps to make the light more homogenous, and an improved wall coverage in the form of grass. While the alterations to the arena were made, the arena was filmed daily to evaluate if the alterations resulted in a more natural behaviour.

Even if these improvements did not led to observed reactions to the stimuli. The optimization of the arena led to the flies behaving more naturally, by spending less of their flight time trying to fly through the walls and ceilings, and that they visited all of the available food sources for intervals corresponding more to that which have previously been reported in field studies for hoverflies (Nuttman and Willmer 2008). The insight from the experiment that homogeneous light and good covering of the walls are needed for normal behaviour can be used to construct future arenas for studies of target detection in hoverflies.

## Methods

### Panorama pictures

The photos for the production of the panorama pictures were taken at observed hovering locations in the Botanical Garden, at Stadsparken and at Stabby Backe in Uppsala. All of the photos were taken during sunny days with low wind speeds in September 2011. For each site, the direction that the fly had been facing when it was hovering was noted, and the illuminance of the site from the sky was measured. The illuminance was measured with a lux meter placed in the position that had been occupied by the fly, directed upwards towards the sky.

The photos were taken with a Panasonic LX3 camera mounted on a tripod with a panorama head. The nodal point of the camera was placed at the same height and position as the observed hoverfly had previously occupied. At each site 18 pictures were taken at 20° intervals. These pictures were assembled with the use of Adobe Photoshop CS5 and ArcSoft Panorama maker 5 pro, resulting in panorama images covering 360° of the hover site. The produced panorama images had a width between 16 200 and 18 100 pixels and a height between 2 800 and 3 500 pixels, corresponding to a vertical coverage of 126° to 176° of the flies visual field.

### Animals

For the optimisation of the arena hoverflies of three different genera was used: *Helophilus*, *Episyrphus* and *Eristalis*. These flies had been collected from the Botanical Garden in Uppsala and the meadow in front of the Biomedical Centre of Uppsala University. Between their capture and their release into the arena all of these flies except the *Eristalis sp* were kept in 6°C cold storage, with access to food in the form of honey and pollen on a moist paper. The *Eristalis sp* had been caught the week before the optimisation steps that were analysed, and had been kept in the aquarium since their capture.

Due to the low survivability of *Episyrphus sp* and *Helophilus sp*, flies from these genera were added at the beginning of the day to replace flies that had died during the night. The *Eristalis sp* never needed to be replaced. At the end of the first week no additional flies were added (Table 1 contains a summary of which genera were used on which recorded day, together with a notation of the time between capture and recorded day)

**Table 1:** The genera and amount of flies for each day of the recorded optimization step, together with weeks since capture within brackets.

| Week of experiment | Day | Referred to as  | <i>Episyrphus</i> sp<br>(weeks since capture) | <i>Eristalis</i> sp<br>(weeks since capture) | <i>Helophilus</i> sp<br>(weeks since capture) |
|--------------------|-----|-----------------|---|--|---|
| 2                  | 1   | Week 1          | 3 (2)   | 1 (1)  |   |
|                    | 2   | Week 1          | 1 (2)   | 1 (1)  |   |
|                    | 3   | Week 1          |   | 1 (1)  |   |
| 3                  | 1   | Start of week 2 | 2 (8)   | 1 (2)  |   |
|                    | 2   | Start of week 2 | 1 (8)   | 1 (2)  | 4 (5)   |
|                    | 3   | End of week 2   |   | 1 (2)  | 5(5)  |

## Arena

A 50 cm high, 49 cm wide and 1 m long aquarium was used as the arena. The top of the aquarium was covered by two glass plates. The glass beads that were planned to be used as targets in the behavioural experiment were attached to and moved by a counterweight system of our own design. The counterweight system consisted of strings and a rubber belt connected to pulleys (Figure 1). The strings and the belt were made to move at various speeds by using varying amount of counterweights. By suspending the glass bead in a thin metal string and attaching this string to different parts of the rubber belt and strings of the counterweight system, the target could be made to move either vertically and horizontally. This counterweight system was mounted on one of the short ends of the arena.

To provide the flies with a natural environment, the floor of the aquarium was covered with dried grass and fresh vegetation. The flies were given access to the same food that they had been fed on in cold storage: honey and pollen placed on a moist paper in a Petri dish. The flies were given additional access to water. All of these sources of sustenance were placed close to the counterweight system to encourage territorial behaviour close to the stimuli (see Figure 2 for the arrangement). The aquarium also contained more natural food sources in form of fresh plants that Syrphid flies had been observed to feed on in the field (personal observation). These consisted of mixed flower bouquets of *Dasiphora fruticosa* (Shrubby Cinquefoil) collected from one of the gardens of the Biomedical Centre of Uppsala University, *Achillea millefolium* (yarrow) that had been collected from the same meadow, and four pots of planted *Calluna vulgaris* (heather, Figure 2). To calculate the flight speed a ruler was attached to the wall opposite to the camera and a measuring tape were attached to the glass plate.

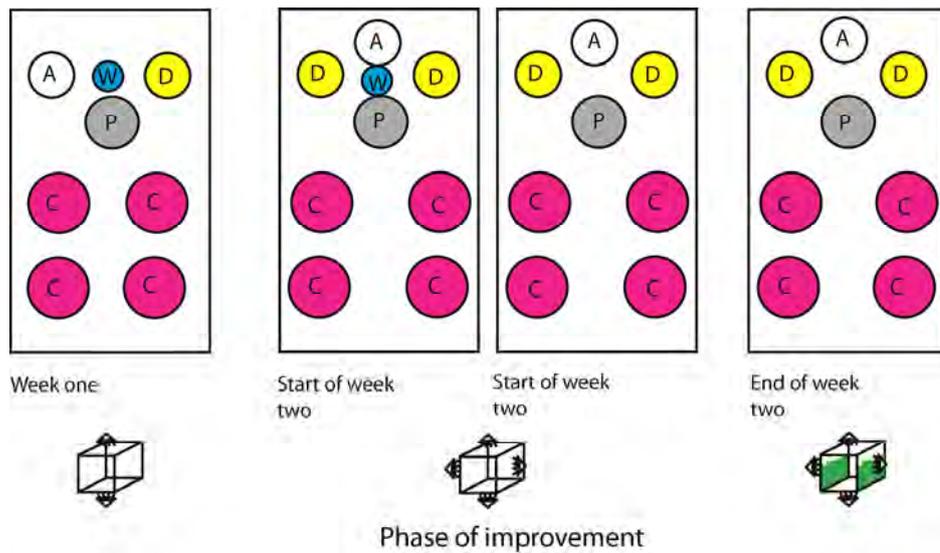
Initially two 12 Watt fluorescent lamps were placed on top of the aquarium and two 0.27 watt cold lamps were placed below. At the second week of the experiment two of the 28 watt fluorescent ceiling lamps were taken down and mounted on the long sides of the aquarium, in order to make the illumination homogenous and to reduce the positive phototaxis. The intensity of the light inciding and reflected from the walls and the ceiling of the aquarium was registered by a lux meter in the middle of the aquarium. The hourly

temperature change inside the aquarium and in the surrounding room was measured for the two different illumination conditions.

All of the walls except the one facing the camera were covered with a single sheet of 0.125 mm polyester film. The walls were emphasised by the addition of a pattern drawn on the polyester film that covered the long walls and the ceiling, and at the end of week two by the addition of dried grass to the long walls (Table 2 contains a list of all the alterations that were made to the aquarium and when they were made).



**Figure 1:** The frame containing the counter weight system that generates the horizontal and vertical motion of the suspended bead.



**Figure 2:** Illustration of the arrangement of the vegetation and the corresponding illumination in the aquarium during the different phases. The aquarium was filmed from the short side (lower part of the illustrations). The letters in the figures represent D=*Dasiphora fruticosa*, A=*Achillea millefolium*, C=*Calluna vulgaris*, W= Water sours, P= the Petri dish with honey and pollen on a moist paper. The pictograms below represent the different conditions of illumination and if the long walls were cover by grass.

**Table 2:** Total length of recorded sequences analysed from each day, the alterations to the aquarium and when they were done.

| Week of the experiment | Day | Referred to as  | Number of analysed minutes | Changes made to the aquarium   |
|------------------------|-----|-----------------|----------------------------|--|
| 2                      | 1   | Week 1          | 40                         |  |
|                        | 2   | Week 1          | 60                         |  |
|                        | 3   | Week 1          | 60                         |  |
| 3                      | 1   | Start of week 2 | 60                         | One fluorescent lamp of 28 watt was added to each of the long sides of the aquarium. An additional bouquet of <i>D. fruticosa</i> was added to the aquarium in a position that allowed the speed of the flies to be easier to calculate. The <i>C. vulgaris</i> were separated and moved towards the long side to make it easier to see if flies were feeding on the Petri dish. |
|                        | 2   | Start of week 2 | 60                         | Additional water source was removed after an <i>Episyrrhus sp</i> were found floating in it before the onset of the filming of the aquarium.   |
|                        | 3   | End of week 2   | 60                         | Grass was added to the long sides of the aquarium. Three flowers from <i>D. fruticosa</i> were placed on the Petri dish to encourage the flies to land.  |

## Filming

The arena was filmed each day to enable evaluating the effect of the improvements made to the aquarium during the following two weeks. The arena was also filmed when the counterweight system was used to evaluate the speeds that it was able to generate.

The aquarium was filmed with of a Sony HDR-XR155E camera filming 1920 x 1080 pixels at 50 fps interlacing. The camera was filming through the short end opposite to the stimuli. The first day the flies were filmed during for 20 minutes at 9 am, 12 noon and 3 pm. After this first day the aquarium was filmed from 9 am to 5 pm with two exceptions in the second week: Due to technical difficulties during the first and last day of the second week the onset of the experiment were postponed until 10 am.

## Data analysis

From the resulting movies 20 minutes from the onset of the experiment, 20 minutes beginning from 12 noon and 20 minutes beginning from 3 pm were selected for analysis.

Due to non optimal camera angle that was not corrected until before the filming at 12 noon during the first day, the morning segment from this day was not analysed (Table 2 contains a summary of the amount of footage that was analysed from each day).

These selected segments were analysed frame by frame by the use of VideoLAN VLC media player and Adobe Premier Pro CS5. During the analysis notes were made regarding interactions, the amount of impacts that flies made against the walls and the ceiling, time spent on the different food sources, and flight time. The impacts were measured as the amount of impacts per second of flight time for each individual fly on each separate flight occasion. The time spent on the food sources for each fly during each individual visit was measured and its mean was compared between each group. These measurements were also summarised as the total time spent on the food sources per recorded hour per fly, to see if the total time spent on the food sources also varied between the groups. The flight time was recorded as the length of each individual flight time per flight occasion, the mean of this time was compared between each group. Just like the measured time on the food sources, the flight time was summarized as the total time per fly per recorded hour to see if the alterations affected the total flight time.

## Statistics

The data from the films was divided into three groups based upon the large changes that were made to the aquarium. The groups are referred to as: Week one, start of week two, and end of week two.

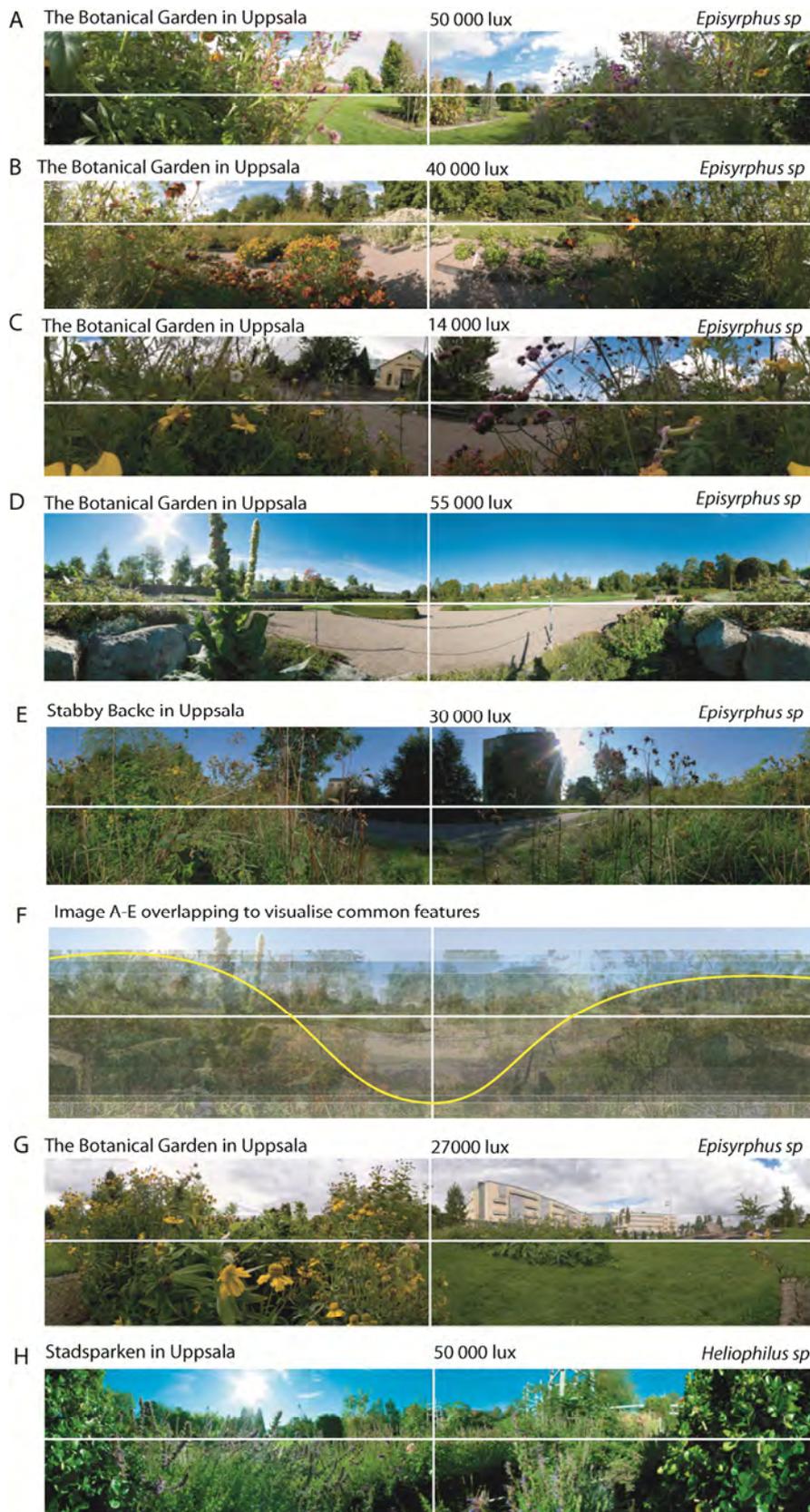
The resulting data were analysed and plotted in IBM SPSS Statistics 19, using the Mann Whitney u test with a Bonferroni correction for multiple comparisons between the three different recorded conditions, with significances allocated to  $p < 0.05$ .

## Results

### Panorama images

To analyse the common factors of hover sites for hoverflies and to provide a natural background for the stimuli, seven panorama images were produced. Six of the panorama images were taken at hover sites for *Episyrphus sp* and one for *Helophilus sp*. For each panorama image the height and the direction of the hoverfly were noted and marked with a cross.

The common factors for the hovering positions in the panorama images (Figure 3), were that in all the flies were hovering close to vegetation containing flowering flowers, that they were covered from at least one side by physical barrier in the form of vegetation or features with vegetation on them, and that they were facing a large open area (with the picture of Figure 3H). In all of the panorama pictures except one (Figure 3G, the back of the fly was covered by a physical barrier consisting of, or containing vegetation Figure 3A-E and H). Five of the panorama images had additional features in common (Figure 3A-E): The visual features create a wave like form with its top close to the rear of the fly and its bottom in front of the fly (Figure 3F).



**Figure 3:** (A to E and G to H): The panorama images of hover sites with location, genus and measured incident light at the hovering position of the fly. The direction that the fly was facing and the height of the hovering fly relative to the surrounding features is marked with a cross overlapping each image. (F) Images A to E superimposed to visualise common visual features. The noted wave like form that can be found in pictures A to E is marked in picture F by a yellow line.

## Response to the stimuli and interactions

To see if the counterweight system was able to generate speeds similar to those reported for hoverflies in field studies (Collett and Land 1978), the movement of the beads attached to the counterweight system were filmed when the counterweight system were driven by different amounts of weights. The analyses of these films showed that the counterweight system was able to move the bead vertically or horizontally in 0.4 m/s to 1.0 m/s (Figure 4), a speed lower than the 10 m/s which has been reported for hoverflies observed in the field (Collett and Land 1978).

One of the main goals of the alterations made to the aquarium was to encourage reactions to the beads. However, during the analysis of the films the flies never displayed any reaction to the moving beads. Neither did they display any interest to react or interact with other flies in the arena. Of the 340 minutes that were analysed in total only three incidents were noted that can be interpreted as interactions. In these three incidents the flies either visually inspected another fly or flew into it, causing the second fly to either flee or in one case beginning to chase the first fly.

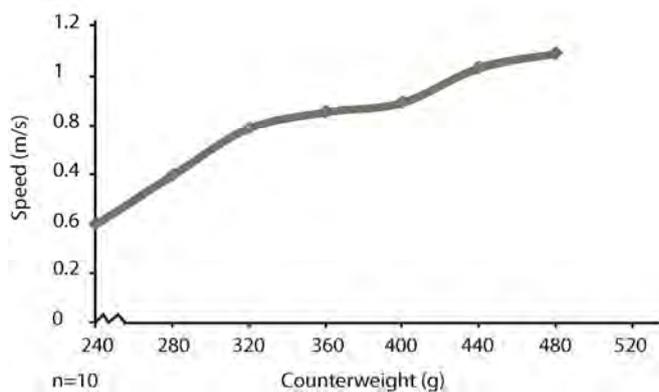


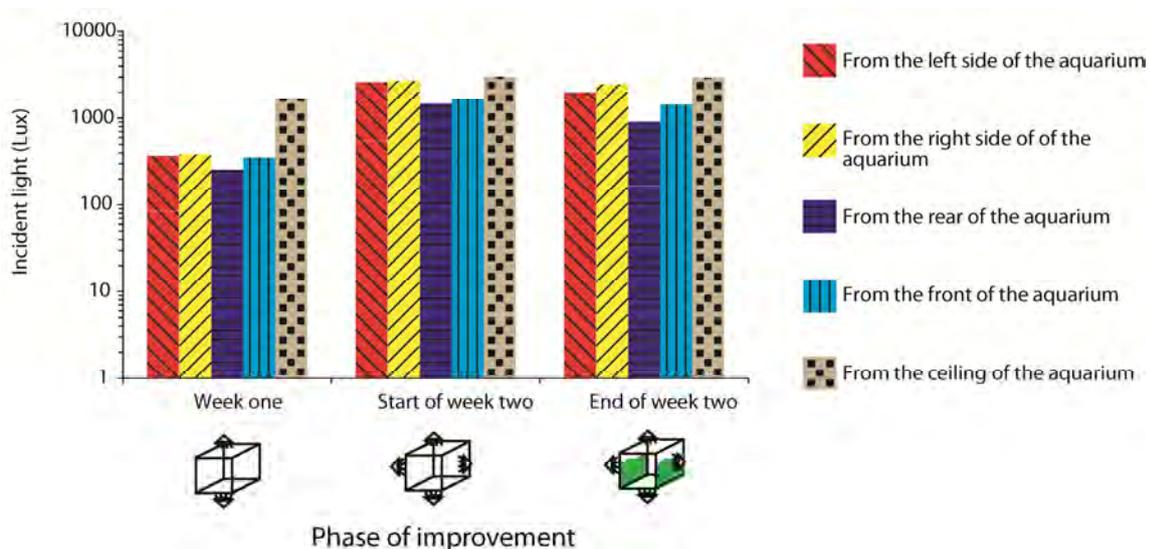
Figure 4: The minimal and maximal possible speed that the counterweight system is capable of generating.

## Light and temperature

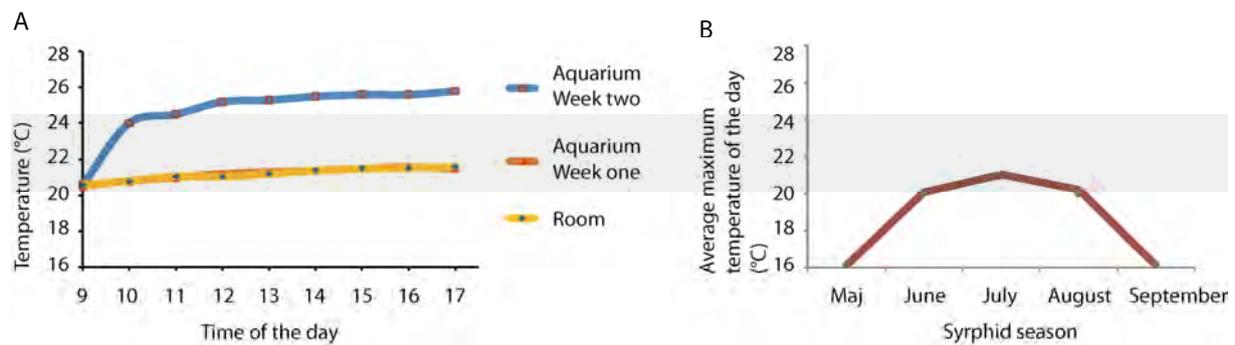
To improve the illumination in the aquarium additional lamps were added during the second week. This extra illumination was added to the sides to make the light more homogeneous.

The addition of the lateral lamps at the start of week two resulted in a stronger and more homogenous light intensity (Figure 5). When grass was added to the walls on the last day of week two, the light intensity of the aquarium was reduced, but not to the levels that were measured before the mounting of the lateral lamps (Figure 5).

The addition of extra lamps on the sides resulted in an increase in the temperature of the aquarium (Figure 6A). The temperatures of the aquarium before and after the addition of the extra lamps were compared to the average maximum temperature in Uppland during the hoverfly season (Figure 6B). This comparison showed that the temperatures in the aquarium were higher than the normal average maximum temperature of the hoverfly season. However, the normal average maximum temperature of the day of these months does not correspond to the maximum temperatures that can occur during these months. Therefore the temperature of the aquarium was also compared to those that had been reported in field studies of hoverflies (Nielsen 1966; Fitzpatrick and Wellington 1983a).



**Figure 5:** The incident light of the three different phases of improvements that were made to the aquarium. The notations of the different sides of the aquarium are relative to the position of the camera. The rear of the aquarium refers to the short side that the camera is placed against, the front of the aquarium refers to the short side that the camera is facing, and the left and right sides the long sides to the left and right to the camera. The pictograms below represent the different conditions of illumination and if the long walls were cover by grass.



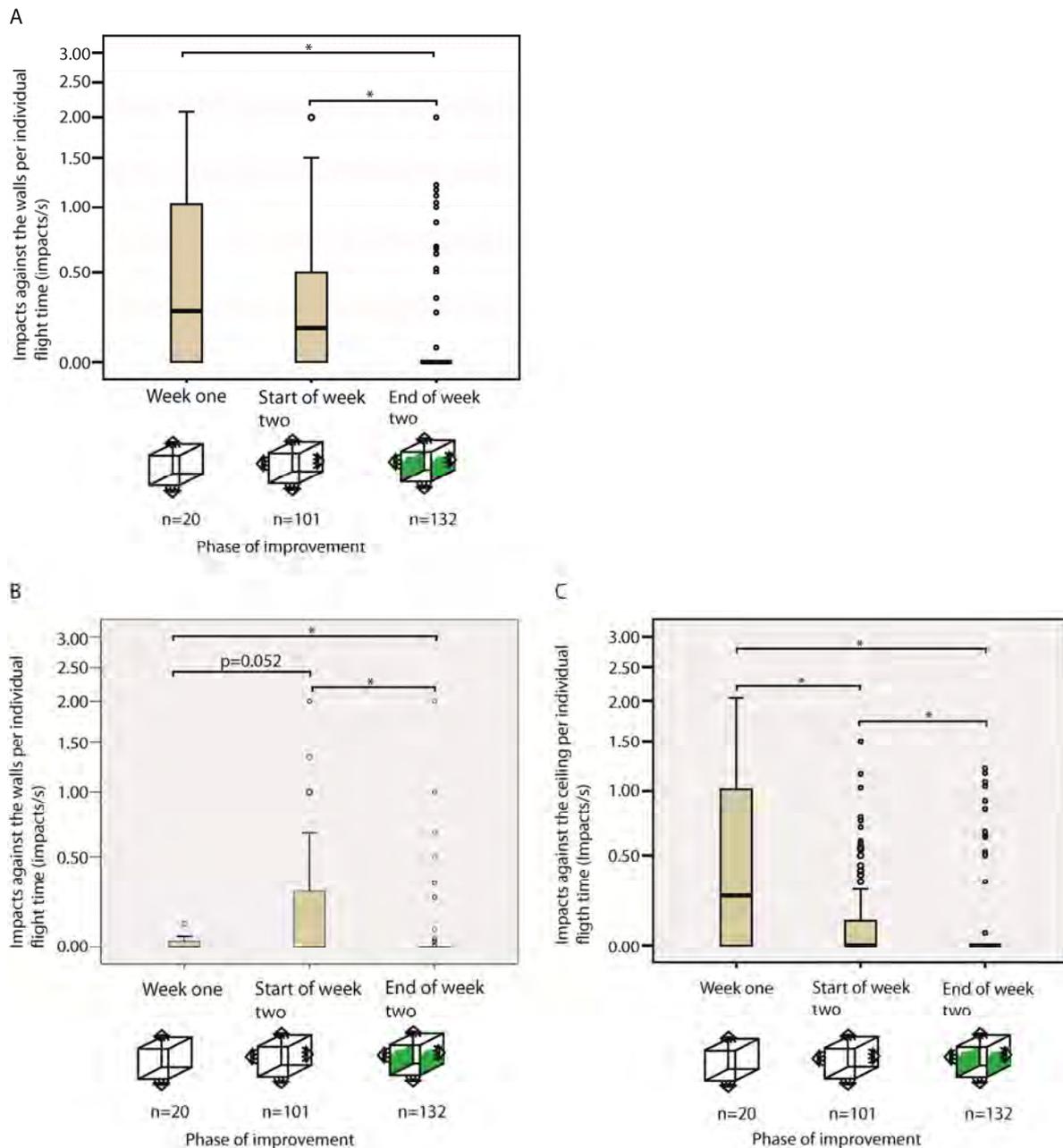
**Figure 6:** (A) The measured temperature of the aquarium and the room. The measurements were performed hourly on two different occasions, the first corresponding to week one overlapping the temperature of the room, and the second on to week two. (B) Average maximum temperature during the summer in Uppland, based on data from SMHI (SMHI 2011).

### Impacts against the walls and the ceiling

The homogenisation of the light and the addition of cover on the walls were expected to reduce positive phototaxis. During analysis of the films from the beginning of week two it was noted that some of the flights that contained impacts against the walls often ended with the fly flying from one wall to the other picking up enough speed to knock itself out against the wall and crashing on the floor of the aquarium.

The data was first analysed for the total amount of impacts per second. In the comparison of the total amount of impacts per second between the first week and the beginning of the second week, no significant difference could be found ( $U=921$ , ns, Figure 7A). At the end of week two the total amount of impacts per second was significantly reduced compared to week one ( $U=3792$ ,  $p<0.025$ ) and the start of week two ( $U=606.00$ ,  $p<0.025$ , Figure 7A).

When the impacts were separated into impacts per second against the walls, and against the ceiling, further differences became apparent. The impacts per second against the ceiling was significantly lower in the beginning of week two compared to week one ( $U=590.50$ ,  $p<0.025$ , Figure 7B), but the impacts per second against the walls showed a trend of increasing at the beginning of week two ( $U=1390.50$ ,  $p=0.052$ , Figure 7C). At the end of week two the impacts per second against the ceiling was significantly lower compared to week one ( $U=541.50$ ,  $p<0.025$ ) and the beginning of week two ( $U=5525.50$ ,  $p<0.025$ , Figure 7B). These reductions were also significantly lower for the amount of impacts against the walls for both week one ( $U=1009.00$ ,  $p<0.025$ ) and the beginning of week two ( $U=4079.50$ ,  $p<0.025$ , Figure 7C).



**Figure 7:** (A) Box plots representing total amount of impacts per second of flight for each individual flight occasion. (B) The total amount of impacts against the walls per second of flight for each individual flight occasion. (C) The total amount of impacts against the ceiling per second of flight for each individual flight occasion. The figures below the x-axis of each plot represent the different conditions of illumination in the aquarium at the time of measurement and if the long walls were covered by grass. The quartiles represent the upper and lower 25<sup>th</sup> percentiles, the whiskers represent values within 1.5 times that of the interquartile range. Values outside the whiskers are treated as outliers and marked in the plot by independent circles. Significant differences ( $p < 0.05$ ) are marked with an asterisk in the figures. Trends are marked by their p-value.

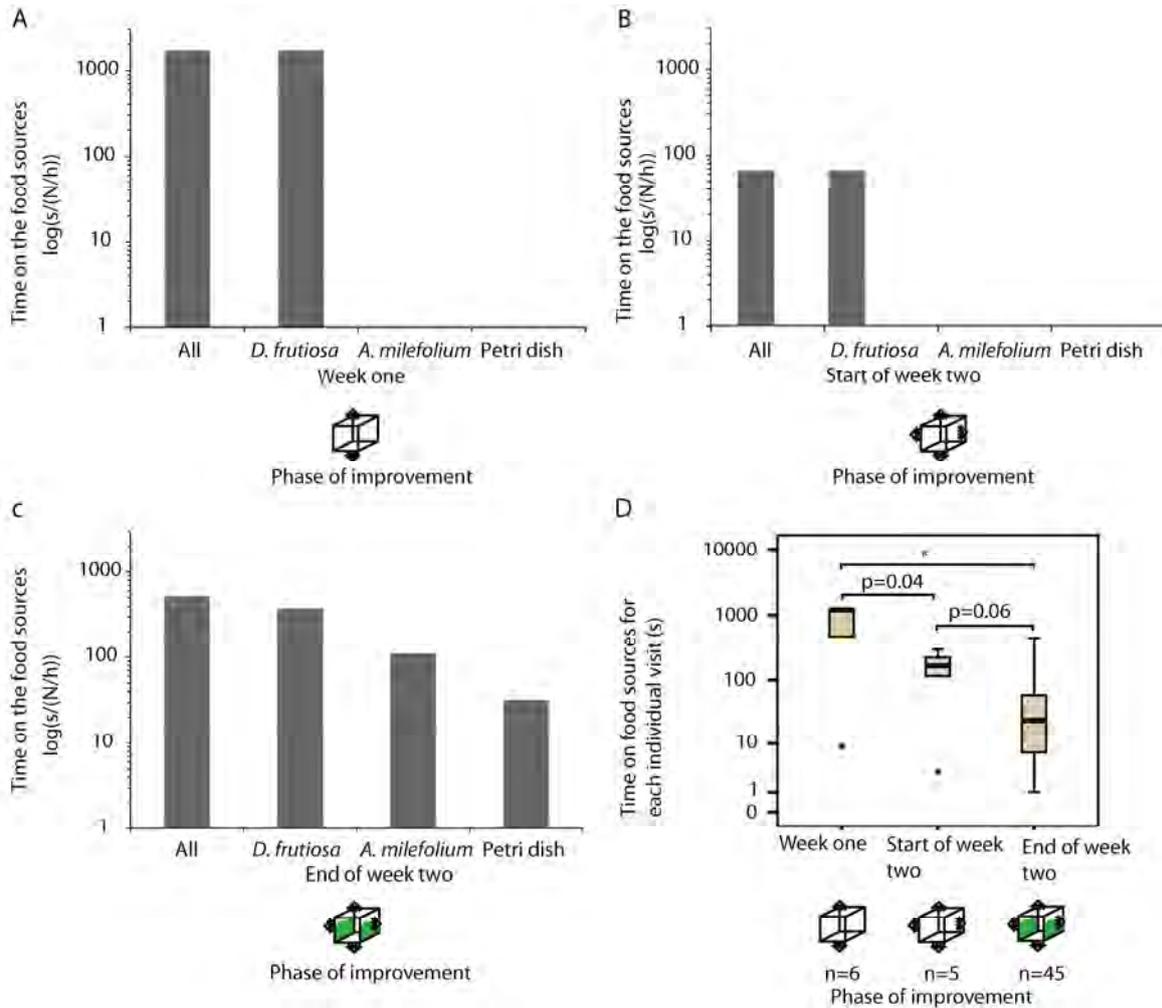
## Food sources

The flies in the aquarium were given access to natural food sources in the form of three different flowers that the flies had been observed to use in the field (personal observation), complemented with non natural food sources that they had fed from when they had been kept in cold storage. The total time and the mean time on the food sources were analysed to determine the food preferences of the flies and as a measurement of their activity. Due to the fact that *C. vulgaris* never started to bloom the

*C. vulgaris* were never treated as a food source during the analysis. Neither were the flies seen using the additional water source. The flies showed a strong preference for the *D. fruticosa* and no observations were made when the flies landed or feed on the *A. millefolium* or the Petri dish before the last day of week two.

The longest time spent on the food sources were recorded during the first week (Figure 8A). The shortest time spent on the food sources were recorded at the beginning of the second week (Figure 8B). The time spent on the food sources at the end of the second week were longer than that of the start of week two, but not as long as that of the week one (Figure 8C).

The mean time spent on the food sources showed a trend of being lower (Mann-Whitney U,  $U=4.00$ ,  $p=0.04$ ) at the start of week two (mdn=165 s) compared to week one (mdn=12000 s, Figure 8C). The mean time spent on the food sources at the end of week two (mdn=24 s) showed a trend of being lower those registered at the start of week two ( $U=55.00$   $p=0.06$ ) and was significantly lower than week at one ( $U=29.50$ ,  $P<0.025$ , Figure 8D).



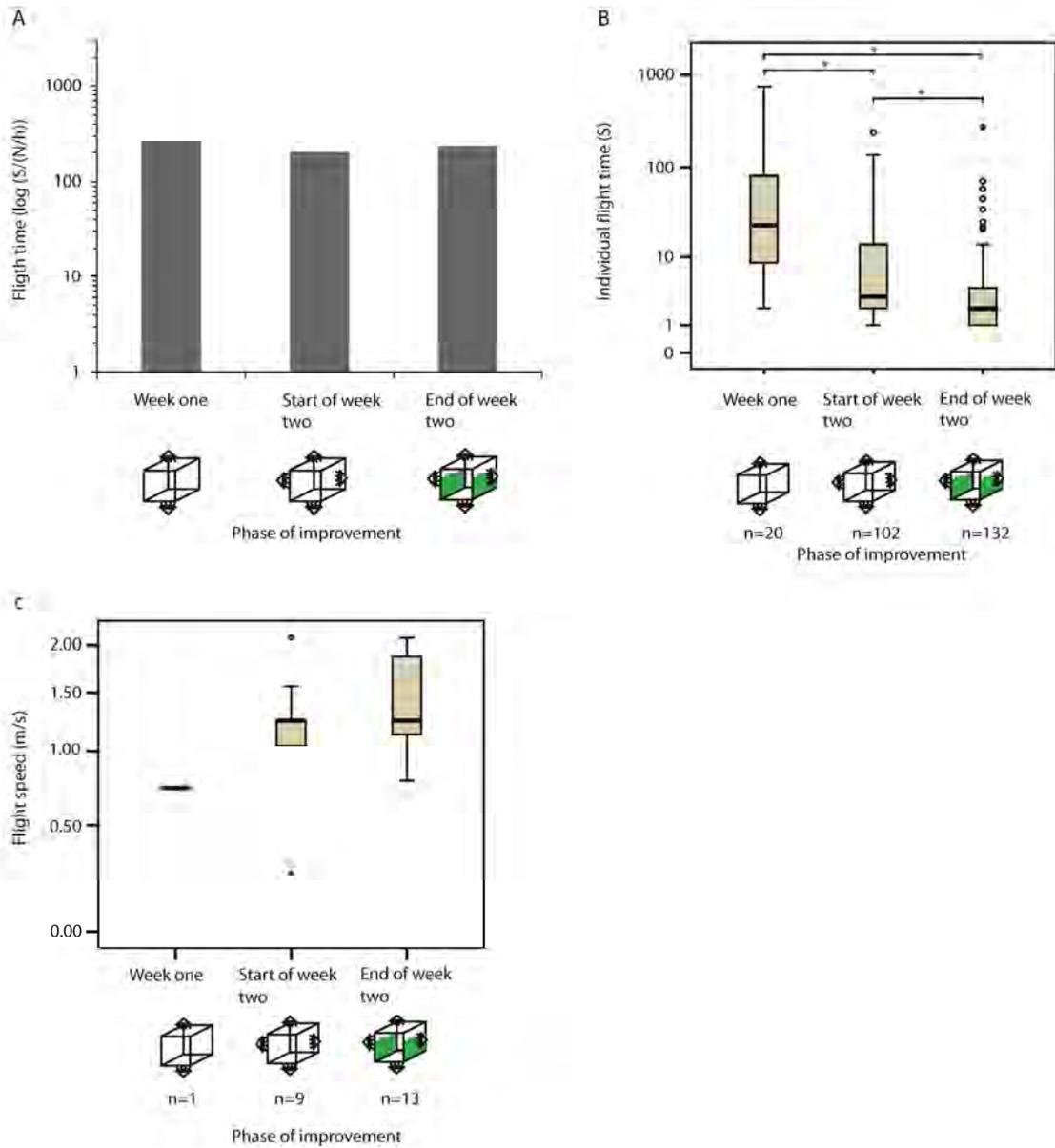
**Figure 8:** (A, B and C): Total amount of time per fly per recorded hour on the different food sources for the three different aquarium conditions. The time has been normalized for the amount of flies in the aquarium during each observation and the amount of hours that each condition was observed. (D) Time for each individual visit on the food sources. The figures below the x-axis of each plot represent the different conditions of illumination in the aquarium at the time of measurement and if the long walls were covered by grass. The quartiles represent the upper and lower 25<sup>th</sup> percentiles, the whiskers represent values within 1.5 times that of the interquartile range. Values outside the whiskers are treated as outliers and marked in the plot by independent circles. Significant differences ( $p < 0.05$ ) are marked with an asterisk in the figure and trends are marked with their p-value.

## Flight time and flight speed

The normalized total amount of flight time and the mean flight time was compared between the three different groups as a measurement of activity. The longest normalized total flight times were found at week one and at the end of week two, which was calculated to four minutes per fly, per recorded hour (Figure 9A). This was slightly longer than the beginning of week two, where the normalized total flight time was calculated to 3.5 minutes per fly per recorded hour (Figure 9A). The mean individual flight time was continually reduced between the groups. The mean individual flight time recorded in week one (mdn= 23.00 s) was significantly higher than both that of the start of week two (mdn= 3.00 s,  $U=473.50$ ,  $p < 0.025$ ) and that which had been recorded at the end of week two (mdn= 2.00 s,  $U=295.50$ ,  $p < 0.025$ , Figure 9B). The mean flight time was also

significantly lower at the end of week two (mdn=2.00 s) compared to the start of week two (mdn=3.00 s,  $U=4858.50$ ,  $p<0.025$ , Figure 9B).

Before the addition of the ruler and the measuring tape, flight speed could only be determined when a fly flew the known distance from one wall to the opposite wall. Due to the rarity of these observations during the first week of the experiment the flight speed was only calculated for one flight vector, thus making it impossible to statistically analyse changes. After the addition of the ruler and the measuring tape, enough flight vectors were observed to allow statistically evaluation. The flight speeds observed at the start and the end of week two were not found to be significantly different ( $U=74.5$ , ns), with a maximum flight speed of 2 m/s recorded in both conditions (Figure 9C).



**Figure 9:** (A) Total amount flight time per hour normalized for the amount of flies in the aquarium during each observation and the amount of hours that each condition was observed. (B) Box plots of the flight time of each flight occasion performed by the flies during the three different conditions that were analysed. (C) Flight speeds for individual flights observed during the different conditions. The figures below the x-axis of each plot represent the different conditions of illumination in the aquarium at the time of measurement and if the long walls were covered by grass. The quartiles represent the upper and lower 25<sup>th</sup> percentiles, the whiskers represent values within 1.5 times that of the interquartile range. Values outside the whiskers are treated as outliers and marked in the plot by independent circles. Significant differences ( $p < 0.05$ ) are marked with an asterisk in the figures.

## Discussion

### Panorama images

In all of the panorama images the flies had been hovering close to flowering food plants and at least one of their sides had been covered by vegetation. In all of the pictures except the hovering site of a *Helophilus sp.*, the flies were also facing a large open area. The presence of food plants, the cover of vegetation and open space that the flies are facing when it was guarding its territory, have previously been documented as common factors for a typical hovering site for Syrphid flies (Fitzpatrick and Wellington 1983a; Fitzpatrick and Wellington 1983b). A possible benefit that male flies might gain by facing the open area, is that it allows their small target motion detectors in their frontally directed acute field (Gronenberg and Strausfeld 1991) to detect females flying from the open area towards the food sources. That the flies were covered from at least one side and in all picture except one (Figure 3G) also from their rear, might have the benefit of making them harder to detect by predators by reducing the angles from which predators can detect them.

In five of the panorama images (Figure 3A-E) the visual features were found to create a wave like form with its top close to the back of the hovering fly and with its bottom in front of the fly. This sine wave pattern has not been previously reported from observations of hovering sites for hovering flies. That the highest features of the physical barriers occurs after or coincide with the top of the wave like form might be a coincident, since the front of the fly was facing the opening of the vegetation and thereby also the valley of the wave like form. To verify that the wave like form is not an artefact caused by a feature found in most openings of vegetation at ground level, further pictures would need to be taken of openings in ground vegetation at non hovering sites. It could also be falsified by taking additional panorama pictures of hovering sites for *Episyrphus* to see if more sites as the one in Figure 3G can be found or if the hovering behaviour of this picture is merely a rare exception to the pattern that is indicated by Figure 3A-E. If these verifications prove that the presence of visual features in the hovering sites that form a wave like form is not a coincident, this might indicate a simplification in the flies behaviour for the finding of hover sites, possible genetically encoded. This simplification would allow the flies to find good hovering sites facing an open area with good cover by simply seeking for visual features that form the described wave like form.

To find additional common properties between the panorama images, they could be further analysed with a Fourier analysis, to see if the part of the panorama images that the flies was facing contain features that would provide a better contrast than those that are behind and on the sides the fly. Furthermore these images could be analysed by one of the current models for STMD neurons. One of these models have been developed by Wiederman et al (2008) to be capable of responding to false positives. It is possible that the open areas that the flies chose to face in the taken pictures contain a lesser amount of false positives than the rear and lateral visual features.

## Response to the stimuli and interactions

Only a few interactions were observed during the analyses of the recorded films, nor did the flies show any interest to the glass beads. The counterweight system's maximum speed was calculated to 1 m/s, a speed lower than a hoverfly's maximum speed of 10 m/s (Collett and Land 1978). But since hoverflies are known to be curious and highly aggressive toward all objects that might be interpreted as a possible mate or competitor (Fitzpatrick and Wellington 1983a; Fitzpatrick and Wellington 1983b), the low speed of the counterweight system should be sufficient to produce a response.

The most important factors for interaction between hoverflies are sufficient light intensities (Maier and Waldbauer 1979; Fitzpatrick and Wellington 1983a) and temperatures between from 17°C to 27°C (Nielsen 1966; Fitzpatrick and Wellington 1983a). Thus the most likely explanation to the lack of interaction and the response to the stimuli is that one or both of these variables were outside the required levels that were needed to produce a response. The temperatures of the aquarium ranged from 20.6°C to 25.8°C. These temperatures were found to be higher than the average maximum temperature of the day during the hoverfly season (Figure 6A and B) but they are within the critical values for behavior (Nielsen 1966; Fitzpatrick and Wellington 1983a). It is more likely that the intensity of the light was too low to encourage territorial behaviour. The strongest illuminance that were in the aquarium of 3000 lux is far from the lowest illuminance value of 14 000 lux registered at the observed hovering site with the lowest luminance. Even if the maximum light levels in the aquarium was lower than at the hover sites, the light levels should still be sufficient to allow natural vision in the flies, since the light levels in the aquarium are far greater than the 150-300 lux that have been successfully used during electrophysiological experiments on hoverfly vision (Nordström, Barnett et al. 2006).

## Impacts against the walls and the ceiling

When the additional lamps were mounted on the sides of the aquarium the total amount of impacts were not reduced (Figure 7A), merely shifted from the ceiling to the sides of the aquarium (Figure 7B and C). Both the number of impacts against the walls and the ceiling were significantly reduced during the last day of week two. Previous behavioural studies of flies, have given the walls texture by covering them with cloth (Wehrhahn, Poggio et al. 1982) or randomly generated cloud patterns (Geurten, Kern et al. 2010). It is possible that the pattern drawn on the polyester film was not dense enough to be interpreted as a barrier for the flies, thus only resulting in the flies trying to fly between the lines that the pattern consisted of. That the amounts of impacts against the walls of the aquarium were reduced during the last day might be because the grass presented a texture dense enough for the flies to detect as a barrier, thus discouraging them from flying towards the lights on the sides. That the amount of impacts against the ceiling were also reduced on the last day, may be because the natural detectable barrier on

the sides of the aquarium provided a less stressful environment, resulting in the flies shifting their focus from trying to escape the arena to instead investigate its features. Previous studies of flies in behavioural arenas have noted that flies more often approaches the walls of small arenas (Geurten, Kern et al. 2010). But since many previous behavioural studies have successfully used arenas smaller than ours (Wehrhahn, Poggio et al. 1982; Boeddeker, Roland et al. 2003; Geurten, Kern et al. 2010), the size of the arena was probably not the cause of the initially observed impacts against the walls.

### Food sources

The mean and total amount of time per fly per hour spent on the food sources was continually reduced. In addition, the flies were only observed to visit the *A. millefolium* and the Petri dish during the end of week two (Figure 8C). The normal visitation time of a hoverfly to a food source is 1 to 54 seconds (Nuttman and Willmer 2008). The mean observed visitation time that corresponds closest to this value (24 seconds) was noted at the end of week two (Figure 8D). That the flies visited the Petri dish during the last day could also be an result of the addition of the *D. fruticosa* flower on the Petri dish, since hoverflies are known to prefer food sources with yellow details (Nuttman and Willmer 2008), but this would still not explain why they did not visit the *A. millefolium* earlier since it also contains yellow details. It is more likely that the more normal time on the food sources and the recorded interest of all food sources were a consequence of the improvement of the illumination of the aquarium and the added grass cover on the walls. Since the resolution of the camera was low compared to its distance to the food sources to see the proboscis of the fly, it was not possible to determine if the flies were actually feeding on the plants. It is therefore possible that the long periods that the flies spent on the food sources, during the earlier stages of the experiment do not represent active feeding, but rather represent inactivity and that it was a mere coincident that the fly happened to spend its inactive time by sitting on one of the possible landing sites that we registered visitations to.

## Flight time and flight speed

The total flight time per fly per hour stayed approximately the same for each of the groups (Figure 9A). But the individual flight path times were continuously reduced (Figure 9B). That the individual flight time of the flies was continuously reduced between the different stages of improvement could be explained by the fact that the flies had spent a longer time in cold storage, but that would also have resulted in the total flight time being reduced. Since this did not happen, a more likely explanation is that many of the flights that were performed when the lights were mounted on the sides of the aquarium ended with the fly violently crashing against the walls, thus possibly ending the flights prematurely. That the individual flight time was reduced during the last day could be a result of that the fly could more easily detect the physical barrier when the grass was mounted on the walls, preventing them to try to find a way to fly through the walls toward the fluorescent lights and instead shifted their focus thus performing short flights between the different food sources.

The maximal calculated flight speed of the flies 2 m/s second was lower than the 10 m/s which have been reported for hoverflies observed in the field (Collett and Land 1978) but higher than flights speed observed in studies using smaller arenas (Geurten, Kern et al. 2010). It is possible that our arena is too small to allow the flies to reach their maximum speed before they run into a physical barrier and that an even bigger arena might result in higher flight speeds.

## Conclusion

The behavioural study indicates that it should be possible to perform behavioural experiments on syrphid flies. But for these experiments to be successful they should be performed in a larger arena to allow higher flight speeds. The walls of the flight arena should be covered by natural vegetation and be illuminated by light sources that are capable of producing high homogeneous light intensities, without raising the temperature above 27°C. The common features of the hovering sites could also be incorporated in future arenas, in the form of good cover placed close to the food sources and by pictures of open areas as the background displayed in front of the food sources. If these conditions were to be satisfied, future experiments could be successfully performed in indoor flight arenas, to study the target detection of hoverflies.

## References

- Boeddeker, N., K. Roland, et al. (2003). "Chasing a dummy target: smooth pursuit and velocity control in male blowflies." Proceedings - Royal Society of London. Biological sciences **270**: 393-399.
- Collett, T. S. and M. F. Land (1975). "Visual Control of Flight Behavior in Hoverfly, *Syritta pipiens* L." Journal of Comparative Physiology **99**(1): 1-66.
- Collett, T. S. and M. F. Land (1978). "How Hoverflies Compute Interception Courses." Journal of Comparative Physiology **125**(3): 191-204.
- Fitzpatrick, S. M. and W. G. Wellington (1983a). "Contrasts in the Territorial Behavior of 3 Species of Hover Flies (*Diptera, Syrphidae*)." Canadian Entomologist **115**(5): 559-566.
- Fitzpatrick, S. M. and W. G. Wellington (1983b). "Insect Territoriality." Canadian Journal of Zoology-Revue Canadienne De Zoologie **61**(3): 471-486.
- Geurten, B. R. H., R. Kern, et al. (2010). "A syntax of hoverfly flight prototypes." Journal of Experimental Biology **213**(14): 2461-2475.
- Grönenberg, W. and N. J. Strausfeld (1991). "Descending pathways connecting the male-specific visual system of flies to the neck and flight motor." Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology **169**(4): 413-426.
- Higgins, C. M. and V. Pant (2004). "An elaborated model of fly small-target tracking." Biological Cybernetics **91**(6): 417-428.
- Maier, C. T. and G. P. Waldbauer (1979). "Dual Mate-Seeking Strategies in Male Syrphid Flies (*Diptera Syrphidae*)." Annals of the Entomological Society of America **72**(1): 54-61.
- Nielsen, T. R. (1966). "Species of the genus *Helophilus* (*Dipt., Syrphidae*), found on Jæren, Rogaland." Norsk Entomologisk Tidsskrift **13**: 427-439.
- Nordström, K., P. D. Barnett, et al. (2006). "Insect Detection of Small Targets Moving in Visual Clutter." PLoS Biol **4**(3): 378-386.
- Nordström, K. and D. C. O'Carroll (2006). "Small object detection neurons in female hoverflies." Proceedings of the Royal Society B-Biological Sciences **273**(1591): 1211-1216.
- Nuttman, C. and P. Willmer (2008). "Hoverfly visitation in relation to floral colour change (*Diptera : Syrphidae*)." Entomologia Generalis **31**(1): 33-47.
- SMHI (2011). "Temperatur." Retrieved 2011-01-18, from <http://www.smhi.se/klimatdata/meteorologi/temperatur>.

Warzecha, A. K., M. Egelhaaf, et al. (1993). "Neural circuit tuning fly visual interneurons to motion of small objects. I. Dissection of the circuit by pharmacological and photoinactivation techniques." Journal of Neurophysiol **69**(2): 329-339.

Wehrhahn, C., T. Poggio, et al. (1982). "Tracking and chasing in houseflies (*Musca*) An analysis of 3-D flight trajectories." Biological Cybernetics **45**(2): 123-130.

Wiederman, S. D., P. A. Shoemaker, et al. (2008). "A model for the detection of moving targets in visual clutter inspired by insect physiology." PloS One **3**(7): e2784.