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Pattern and contrast dependent visual response in the box jellyfish *Tripedalia cystophora*

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Abstract

Cubomedusae possess a total of 24 eyes of which some are structurally similar to vertebrate eyes. Accordingly, the medusae also display a range of light guided behaviours including obstacle avoidance, diurnal activity patterns, and navigation. Navigation is supported by spatial resolution and image formation in the so-called upper lens eye. Further, there are indications that the obstacle avoidance requires image information from the lower lens eye. Here we use a behavioural assay to examine the obstacle avoidance behaviour of the Caribbean cubomedusa *Tripedalia cystophora* and test whether it requires spatial resolution. The possible influence of the contrast and orientation of the obstacles is also examined. We show that the medusae can only perform the behaviour when spatial information is present, and fail to avoid a uniformly dark wall, directly proving the use of spatial vision. We also show that the medusae respond stronger to high contrast lines than to low contrast lines in a graded fashion and propose that the medusae use the contrast as a semi reliable measure of distance to the obstacle.

Introduction

Eyes come in a great variety of shapes and sizes and are used for an impressive range of light guided behaviours from simple light detection to highly advanced intraspecific communication (Land and Nilsson, 2012). A normal assumption is that the degree of complexity found in the eyes and the behaviour they support is closely correlated with the brainpower of the organism. It has been considered an enigma, therefore, that cubomedusae, or box jellyfish, possess very elaborate visual equipment (Wehner, 2005). All known species of box jellyfish have the same overall arrangement of the eyes. They have four sensory structures called rhopalia situated in rhopalial niches along the lower part of the bell and each of them carries six eyes of four distinct morphological types (Hertwig and Hertwig, 1878; Claus, 1878; Berger, 1898; Werner, 1975; Yamasu and Yoshida, 1976; Martin, 2004). There is an upper and a lower lens eye, a pair of pit and a pair of slit eyes. The lens eyes are structurally similar to vertebrate and cephalopod eyes in that they have a spherical lens with a graded refractive index, an upright hemisphere shaped retina, a cornea, a vitreous space and in the case of the lower lens eye, a movable iris (Nilsson et al., 2005).

Box jellyfish also display a more diverse behavioural repertoire than any other known cnidarian and several of them are light guided (Garm and Ekström, 2010). Here it should be kept in mind that almost all the behavioural data stem from a single species of box jellyfish, *Tripedalia cystophora* from the Caribbean, and thus many more behaviours are bound to surface when more species are examined. *T. cystophora* is found between the prop roots of mangrove trees where they prey on a single species of copepod gathering in light shafts between the roots (Stewart, 1996). The medusae use their eyes to seek out the light shafts where they passively hunt (Buskey, 2003).

They rest on the bottom of the mangrove lagoon at night (Garm et al., 2012). Every morning they have to navigate back to their habitat between the roots and this they do by visually detecting the mangrove canopy through the water surface (Garm et al., 2011). The medusae are also able to detect and avoid obstacles in their surroundings (like the prop roots) and for this they probably use their lower lens eye (Garm et al., 2007b).

The morphological and optical data indicate that both lens eyes are image forming with a spatial resolution in the range of 10-20 degrees (Nilsson et al., 2005). In the case of the upper lens eye the use of spatial information is confirmed by the navigation behaviour, where they see the direction to the canopy (Garm et al., 2011). The obstacle avoidance behaviour also indicated the use of spatial information and true image formation, in that its onset was correlated with the size of the obstacle on the retina (Garm et al., 2007b). Still, since the obstacles were dark on a bright background the behaviour could also be triggered by a directional drop in light intensity when approaching the obstacles and thus be part of a simpler positive phototaxis.

Here we have examined the obstacle avoidance behaviour of *T. cystophora* and tested whether an image of the obstacle in needed to accomplish this behaviour or whether it is a case of positive phototaxis. In a behavioural assay the medusae were presented with a visual scene of either alternating dark and bright stripes in different orientations or an uniformly grey wall. We hypothesized that the medusae would only be able to avoid the striped walls and not the uniform walls without contrast. Further, we hypothesized that vertical stripes with high contrast would evoke the strongest response, since they would be the most visible and have the most resemblance with the natural obstacles, the prop roots.

Material and Methods

Animals

We used adult sized animals (7-9 mm in bell diameter) from our cultures at the University of Copenhagen. The animals were cultured in 250 l tanks with recycled sea water at 28^{o}C and psu = 30. In the culture tanks the light:dark cycle was 8:16 hours and the medusae reached adult size in about 2 month. A total of 36 medusae were used and each medusa was only used in one assay.

Behavioural arena

The experiments were conducted in a round 3 liter tank with a diameter of 16 cm. The tank was filled with water from the culture tank to minimize stress, which can be induced by moving the animals to water with changes in salinity and/or chemical composition. The water depth was approximately 12 cm and the water was kept at 28^{o}C by placing the tank on a heating plate. The wall of the tank had a changeable visual scene of either 2 cm wide grey and white stripes or uniformly grey. Undiffused light came from a 11W fluorescent blub (OSRAM longlife, OSRAM GmbH, Augsburg, Germany) situated approx. 50 cm above the center of the tank thereby creating an even illumination of the behavioural arena. The light intensity measured at the surface straight under the lamp was 76 W/m^2.

In the case of the stripes (contrast and orientation experiments) three different orientations were used: vertical, 45^{o} oblique and horizontal. All three orientations were tested with five different grey tones resulting in contrasts of 0.93, 0.71, 0.39, 0.27, and 0.17. The contrast was calculated as $(I_w-I_g)/(I_w+I_g)$ where I_g is the intensity of reflected light from the grey strips and I_w the intensity reflected from the white stripes both corrected by the absorption spectrum of the 500 nm opsin (Govardovskii et al., 2000) present in the lower lens eye (Coates et al., 2006; Garm et al., 2007a). The intensities were measured from 350 nm to 700 nm using a radiospectrophotometer (ILT900W, International Light Technologies Inc., Peabody, MA) with the sensor held perpendicular to the wall at a distance of 1 cm and 1 cm below the surface.

With the uniformly dark walls (intensity experiments) five different grey tones were used one at a time. The grey tones were chosen to match the mean intensities of the white stripe and the five different grey stripes respectively (with an accuracy of +/- 5%), such that the intensity of the reflected light of the darkest uniformly grey wall matched the mean of reflected light of the white and the darkest grey stripe. Light intensities were measured as for the contrast experiments.

<u>Behavioural protocol</u>

At the onset of each behavioural assay a medusa was placed in the center of the arena and left to adjust for 5 min, after which they had re-extended their tentacles and swam with normal pulse rate. After this acclimation each medusa was tested with either five striped walls with the same orientation but varying contrast (contrast and orientation experiments) or the five uniformly dark walls (intensity experiments). The visual scene was changed every 4 min and the order of contrast/darkness was randomized. The experimental series (acclimation plus 5 tests) lasted 25 min and was repeated eight times using eight different experimental animals in the case of vertical stripes and uniform grey tones. The experiments with oblique and horizontal stripes were repeated ten times using ten medusae. The swim pattern during the last 2.5 min with each visual scene was recorded from above using a video camera (Sony handycam DCR-HC40, Sony Corp., Tokyo, Japan). The timing equals earlier experiments (Garm et al., 2007b).

Data analysis

The video recordings were turned into swim trajectories with a temporal resolution of 1 s using a custom made program for Matlab 2011a (MathWorks Inc., Natick, MA). From the trajectories the average distance to the wall was calculated. A temporal resolution of 0.5 s was also tested for three recordings to make sure the sampling rate was sufficient to resolve the swim pattern and no difference was found. The number of avoidance responses for each individual and each visual scene was counted manually and the distance to the wall at the behavioural onset was determined for each avoidance response. An avoidance response is defined as the medusa swimming towards the wall and then turning a minimum of 120^{o} in 2-3 swim contractions with an increased pulse rate (see supplementary materials, video 1, for example). Finally, in the contrast experiments the distance of the avoidance response (when the medusa started turning) was turned into visual angle of the stripes following the equation $\tan \frac{1}{2}\alpha = a/b$, where α is the visual angle, a the width of the stripe (2 cm), and b the distance of avoidance. All statistical tests were performed in Biostat 2008 Professional (version 5.4.0.0, AnalystSoft, Vancouver, Canada) and were one-way ANOVAs followed by Tukey-Kramer post hoc test unless otherwise stated. Fishers LSD post hoc test was used in cases of uneven variances (distance when avoidance and visual angle when avoidance).

Contrasts in the habitat

As it was not possible to make light intensity measurements from small confined areas in the mangrove habitat of the medusae, we used an indirect approach. Pictures of the mangrove habitat in Puerto Rico including several prop roots and medusae were taken at noon with a standard underwater camera. A representative of these RGB pictures showing the typical habitat was chosen. The red channel was removed from the picture using the program Corel PhotoPaint (version X3, Corel Corporation, Canada) to better match the spectral sensitivity of the medusae and it was turned into 8 bit grey scale. The average pixel value (0 = black, 255 = white) was then determined from a rectangular area (500 pixels) of a prop root and from the neighboring area in the water. These pixel values were used as relative estimates of the light intensity and the contrast between the root and water was calculated in the following way: $(PV_w – PV_r)/(PV_w + PV_r)$, were PV_w = pixel value from the water and PV_r.= pixel value from the root. The procedure was repeated for 4 roots at different distances to the camera. The absolute distances were not measured but the relative distance was determined by where in the picture the root intersected the water surface.

Results

Contrast experiments

In the experiments using grey and white stripes the medusae performed many clear obstacle avoidances. With increasing contrast (c) from 0.17 to 0.93 the medusae responded with a stronger obstacle avoidance response for all three orientations of the stripes. In the swim trajectories it is seen that medusae made only few turns and came close to the wall when contrast was low (figs. 1-3). At c = 0.93 the medusae frequently turned and stayed centered in the tank (figs. 1-3). This is in contrast to the results from the intensity experiments with the uniform grey walls (fig. 4).

The behavioural change with contrast is confirmed when the average distance to the wall is calculated (fig. 5a). With the vertical stripes and the lowest contrast the medusae had an average distance of 2.5 cm to the wall whereas when c = 0.93 the average distance was 5.7 cm (all behavioural data are summarized in table 1). These differences are significant between a given contrast level and all other except the neighboring levels (one-way ANOVA, $F_{4, 35}$=15.1, p<0.0001, followed by Tukey-Kramer post hoc, 0.0001<p<0.0071). The results are the same for the oblique stripes except here there is also a significant difference between c=0.39 and c=0.71 (one-way ANOVA, $F_{4, 45}$=49.2, 0.001<p<0.016). With the horizontal stripes there were no differences in the average distance to the wall between the four lowest contrasts but they stayed significantly farther away from the darkest stripes than the four others (one-way ANOVA, $F_{4, 45}$=12.6, 0.0001<p<0.0003).

The medusae not only stayed farther away from the wall of the tank they also performed more obstacle avoidances per min with higher contrast (fig. 5b, table 1). The highest rate, 3.3 min-1, was obtained with the vertical stripes and c=0.93. This was significantly higher than the rates with vertical stripes at c=0.17, 0.27 and 0.39 (one-way ANOVA, $F_{4, 35}$=11.7, 0.0001<p<0.0002) and c=0.71 also resulted in significantly more avoidances than c=0.17 (p=0.011). With the oblique stripes there was also an increase with contrast (fig. 5b) and here all differences were significant (one-way ANOVA, $F_{4, 44}$=61.3, 0.0001<p<0.044) except between c=0.17 and c=0.25 (p=1). In the experiments with horizontal stripes only c=0.93 produced significantly more avoidances than the four other contrasts (one-way ANOVA, $F_{4, 45}$=16.2, 0.0001<p<0.0002).

The increasing number of avoidances with higher contrast stripes was also performed at a longer distance from the stripes (fig. 6a, table 1). On average the obstacle avoidance responses were performed 2.2 cm from the wall with vertical stripes at c = 0.17 but 4.6 cm from the wall at c = 0.93. The differences are significant between c=0.93 and the four other contrasts (one-way ANOVA, $F_{4, 134}=8.7$, $p<0.0001$, followed by Fisher LSD post hoc, $0.0001<p<0.012$) and between c=0.27 and c=0.71 (p=0.015). In the case of the oblique stripes only the three highest contrasts could be tested, since n=1 for c=0.17 and 0.27. Still, the avoidances were performed significantly farther away from the wall at c=0.93 than at c=0.39 and 0.71 (one-way ANOVA, $F_{2, 125}=26.8$, $p<0.0001$, followed by Fisher LSD post hoc, $p<0.001$). With the horizontal stripes and c=0.93 the avoidances were performed 4.6 cm from the wall and this was farther away than with the four other contrasts (one-way ANOVA, $F_{4, 81}=8.5$, $p<0.0001$, followed by Fisher LSD post hoc, $0.0004<p<0.023$). The width of the stripes at the distance of avoidance was turned into visual angle on the retina and the average of these angles varied from 52^o (horizontal, c=0.17) to 25^o (oblique, c = 0.93) (fig. 6b, table 1). This transformation of the data had no significant effect on the statistics. The smallest visual angle provoking an avoidance response was 15^o.

Intensity experiments

When presenting the medusae with uniformly grey tank walls the obstacle avoidance behaviour was almost completely abolished even with the darkest grey tone matching the mean intensity of the white and the darkest (black) stripe. For all five grey tones they swam with few turns and stayed most of the time in the periphery of the tank often touching the wall (fig. 4). This resulted in them having the same average distance to the tank wall, 2.5 – 2.9 cm (one-way ANOVA, $F_{4, 35}=0.63$, p=0.64) (fig.

5a). Further, at the three highest intensities (matching c = 0.17, 0.27 and 0.39) no avoidances were seen and only very few with the two darker walls (0.05 avoidances per min in both cases). This slight increase with darker walls was not significant (one-way ANOVA, $F_{4, 35}$=0.75, p=0.57).

Orientation experiments

When comparing the response to stripes with the same contrast but different orientation and the corresponding grey tone interesting differences are seen. With the two lowest contrasts all four different experimental conditions resulted in the same general distance to the wall (fig. 5a) (one-way ANOVA, p=0.052 and 0.36 respectively). At c=0.39 only the vertical stripes kept the medusae farther away than the corresponding grey tone (one-way ANOVA, $F_{3,32}$=4.03, p=0.014). When taking one step further up in contrast the vertical and oblique stripes gave similar results and both significantly higher than the horizontal stripes and grey tone (one-way ANOVA, 0.00044<p<0.0052). With the highest contrast (c=0.93) the three different stripes caused the medusae to keep the same distance to the wall, 5.7 – 5.9 cm, (0.75<p<0.99), which in all cases were significantly farther away than the corresponding grey tone (one-way ANOVA, $F_{3, 32}$=37.4, p<0.0001, p<0.0001).

A similar picture is seen with the rate of avoidances (fig. 5b). Here the vertical stripes produced a stronger response than the three other visual scenes already at c=0.27 (one-way ANOVA, $F_{3, 32}$=5.5, 0.005<p<0.03). At c=0.71 both the vertical and oblique stripes caused more avoidances than the grey tone (one-way ANOVA, $F_{3, 32}$=9.5, 0.0007< p<0.001) and the vertical more avoidances than the horizontal stripes (p=0.038). With the highest contrast all stripes gave similar responses all significantly higher than the corresponding grey tone (one-way ANOVA, $F_{3, 32}=16.8$, $0.0001<p<0.0004$) (fig. 5b).

When considering the average distance of the avoidances and the average visual angles there were no significant differences between the four experimental conditions at any of the contrasts (fig. 6).

<u>Contrast in the natural habitat</u>

A relative measure of contrast between the prop roots and the surrounding water as a function of distance was obtained from an underwater photo (fig. 7a). Four roots in the picture were analyzed with root 1 being the closest and root 4 the furthest away. In the picture adjusted to the spectral sensitivity of *T. cystophora* (fig. 7b) there was a correlation between relative distance and relative contrast. Root 1 had a contrast of 0.39, for root 2 and 3 it was 0.24 and the most distant root 4 had a relative contrast of 0.15.

Discussion

The results presented here clearly demonstrate that the visually guided obstacle avoidance described for cubomedusae (Garm et al., 2007b) is dependant on actual detection of the obstacle using spatial information and not a mere positive phototaxis. When we presented medusae of *Tripedalia cystophora* with a visual scene without spatial information the behaviour disappeared even though the overall brightness of the wall equaled that of a scene with stripes resulting in many avoidances. We also show that for all three orientations of the stripes an increasing contrast made the medusae stay farther away from the wall and perform more avoidances. Finally we found that the orientation of the obstacle influences the strength of the response, with vertical stripes causing the strongest response followed by the oblique with an intermediate effect and lastly the horizontal stripes resulting in the weakest response.

<u>Contrast dependent obstacle avoidance</u>

Our experiments returned a surprising result. We expected the obstacle avoidance response to have a contrast threshold triggering the behaviour. That is, once a certain contrast is present on the retina the medusa would acknowledge the presence of the obstacle and start the response. The results strongly indicate that this is not the case, since there is a gradual change of the response strength (measured as average distance to wall, rate of avoidances and object size on retina) more or less proportional with the change in contrast, at least for the vertical stripes. This could be because a higher contrast means greater certainty that there is an obstacle and thus a greater “willingness” to respond, but there is another possible explanation and the two are not mutually exclusive.

It would be of great advantage for the medusae if they were able to tell the distance to the obstacle and not start the avoidance response until within a certain distance. This would ensure that they do not perform unnecessary responses interfering with their foraging behaviour in the light shafts between the roots (Stewart, 1996; Buskey, 2003). There are several ways to visually determine the distance to an object. The most exact are also the most advanced using parameters such as depth of focus, relative movements and relative size combined with knowledge of absolute size (Land and Nilsson, 2012). These are all mechanisms demanding acute vision and much neural processing, which are resources not available to the jellyfish. But there are also more simple ways to estimate the distance to an object in the visual scene. The medusa can take advantage of the water in the mangrove swamp being turbid with visibilities often down to about a meter (Garm et al., 2011). This means that due to light absorption and scattering the contrast of a given object decreases steeply with distance and that contrast, therefore, can be used as a semi reliable measure of distance. This is supported by the underwater photo of the prop roots, which are the naturally occurring obstacles (fig. 7). Even in this habitat with complex light distribution there is still an overall decrease in contrast between the roots and the surrounding water with distance. This taken together with our behavioural results show that the obstacle avoidance response has a built in mechanism for distance detection probably enabling effective foraging between the roots while still avoiding collisions. To our knowledge these are the first behavioural data pointing to the use of this mechanism for distance evaluation in any aquatic animal.

Pattern dependent obstacle avoidance

Interestingly, the contrast dependency varied with the orientation of the stripes. At the highest contrast, c=0.93, there was no difference between vertical, oblique and horizontal stripes, but differences were seen in the general distance to the wall and the rate of avoidances at lower contrasts. The medusae responded the strongest to the vertical stripes and already at c=0.27 this scene provoked more avoidances than any of the other visual scenes. At c=0.71 both the vertical stripes and the oblique stripes made the medusae stay farther away from the wall than the horizontal stripes and the grey tone. Finally, the response to the horizontal stripes did not differ from the grey tones until we used the highest contrast. This shows that the more vertical an obstacle (or contrast line), the stronger the medusae will react to it and this is in good concordance with the most often encountered obstacles, the prop roots. As seen in figure 7 the roots typically have an orientation varying between 45^{o} oblique and vertical. Almost no horizontal lines are seen in the natural habitat. This is a good example of a matched filter (Wehner, 1987), where the visual system is matched to the natural visual scene.

There are two ways orientation filters can be implemented in the visual system of *T. cystophora*. Either, all contrast lines are detected equally by the eye independent of orientation and then some contrast line orientations are filtered away by the CNS. The other possibility is that the retina is better at detecting some orientations of contrast lines than others. The latter is favored by our behavioural data, since the medusae respond equally strong to all three orientations at the highest contrast, indicating than any high contrast object can trigger the response. The separation of the different orientation could then be accomplished by directional contrast enhancement, such that vertical contrast lines would be enhanced and horizontal not. Support for such enhancement through lateral inhibition is offered by the presence of synapses between neighboring photoreceptors in the lens eyes (Gray et al., 2009). It would require that the synapses specifically inhibit horizontal neighbors, though, and whether this is the case is not known.

<u>Image processing with limited brainpower</u>

The obstacle avoidance behaviour is probably controlled by the lower lens eyes (Garm et al., 2007b). From our earlier morphological and optical modeling of *T. cystophora* we know that both the upper and lower lens eye allow for spatial resolution (Nilsson et al., 2005). The slit eyes might also acquire spatial information but in the vertical plane only (Garm et al., 2008). In the case of the upper lens eyes it was shown that the animals do indeed use the spatial information when they navigate from the mangrove lagoon to their habitat between the prop roots (Garm et al., 2011).

With the present work we have now shown that *T. cystophora* also requires spatial resolution in order to avoid obstacles. The data again point to the lower lens eyes controlling the behaviour. The upper lens eyes and the pit eyes point upwards observing Snell's window (Garm et al., 2011) and do not see the underwater roots. The only other eyes observing the underwater world are the slit eyes but they should preferably detect horizontal lines. Further, the minimum size of the obstacle on the retina able to evoke a response (15^{o}) nicely matches the calculated resolution of the lower lens eye varying between 10^{o} and 20^{o} depending on the area of the retina (Nilsson et al., 2005). The possible image formation in the slit eyes, seeing the world in horizontal bands, is intriguing and still awaits proof from behavioural experiments.

Cnidarians are often accused of being brainless (Wehner, 2005), but there is no doubt that at least hydromedusae and cubomedusae possess a central nervous system (Passano, 1976; Mackie, 2004; Skogh et al., 2006; Garm et al., 2007c). In cubomedusae the CNS is composed of four parallel rhopalial nervous systems (RNS) interconnected by a ring nerve (Satterlie, 2002; Garm et al., 2007c; Satterlie, 2011). From electrophysiological experiments and morphological examinations it is indicated that the visual processing mostly takes place in the RNS (Satterlie and Nolen, 2001; Parkefelt et al., 2005; Garm and Mori, 2009; Parkefelt and Ekström, 2009). In the adult medusa only about 1000 neurons are found here besides the photoreceptors (Skogh et al., 2006). This limited number of neurons has to process spatial information from at least the two lens eyes and possibly also the slit eyes. Considering the amount of neuronal power often dedicated to visual processing (Thorpe et al., 1996; Masland, 2012) this is somewhat surprising. Such a system stresses the need for the above mentioned matched filters, which ensures that irrelevant information is removed and that only the essential information in processed by the CNS. These filters are often applied already in the very periphery at the sensors (Barth, 2000) and the suggested lateral inhibition in the retina enhancing vertical stripes would be a clear example of this. In vision matched filters may result in so-called special purpose eyes (Land and Nilsson, 2006), where the animal has several eye types each specialized in taking up a narrow spectrum of information supporting one or a few behaviours only. The visual system of box jellyfish is a textbook example of special purpose eyes and this is probably one of the explanations for how they support an elaborate behavioural repertoire with their sparse CNS.

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Reference List

Barth, F. G. (2000). How to catch the wind: spider hairs specialized for sensing the movement of air. *Natur Wissenschaften* **87**, 51-58.

Berger, E. W. (1898). The histological structure of the eyes of cubomedusae. *J Comp Neurol* **8**, 223-230.

Buskey, E. J. (2003). Behavioral adaptations of the cubozoan medusa *Tripedalia cystophora* for feeding on copepod (*Dioithona oculata*) swarms. *Mar Biol* **142**, 225-232.

Claus, C. (1878). Ueber *Charybdea marsupialis*. *Arbeiten aus dem zoologischen Institut Universität Wien* **1**, 1-56.

Coates, M. M., Garm, A., Theobald, J. C., Thompson, S. H., and Nilsson, D. E. (2006). The spectral sensitivity in the lens eyes of a box jellyfish, *Tripedalia cystophora*. *J Exp Biol* **209**, 3758-3765.

Garm, A., Anderson, F., and Nilsson, D. E. (2008). Unique structure and optics of the lesser eyes of the box jellyfish *Tripedalia cystophora*. *Vision Res* **48**, 1061-1073.

Garm, A., Bielecki, J., Petie, R., and Nilsson, D. E. (2012). Oposite patterns of diurnal rhytms in the box jellyfish *Tripedalia cystophora* and *Carybdea sivickisi*. *Biol Bull* **222**, 35-45.

Garm, A., Coates, M. M., Seymour, J., Gad, R., and Nilsson, D. E. (2007a). The lens eyes of the box jellyfish *Tripedalia cystophora* and *Chiropsalmus sp.* are slow and color-blind. *J Comp Physiol A* **193**, 547-557.

Garm, A. and Ekström, P. (2010). Evidence for multiple photosystems in jellyfish. *International Review of Cell and Molecular Biology* **280**, 41-78.

Garm, A. and Mori, S. (2009). Multiple photoreceptor systems control the swim pacemaker activity in box jellyfish. *J Exp Biol* **212**, 3951-3960.

Garm, A., O'Connor, M., Parkefelt, L., and Nilsson, D. E. (2007b). Visually guided obstacle avoidance in the box jellyfish *Tripedalia cystophora* and *Chiropsella bronzie*. *J Exp Biol* **210**, 3616-3623.

Garm, A., Oskarsson, M., and Nilsson, D. E. (2011). Box jellyfish use terrestrial visual cues for navigation. *Curr Biol* **21**, 798-803.

Garm, A., Poussart, Y., Parkefelt, L., and Nilsson, D. E. (2007c). The ring nerve of the box jellyfish *Tripedalia cystophora*. *Cell Tissue Res* **329**, 147-157.

Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G., and Donner, K. (2000). In search of the visual pigment template. *Visual Neurosci* **17**, 509-528.

Gray, G. C., Martin, V. J., and Satterlie, R. A. (2009). Ultrastructure of the retinal synapses in cubozoans. *Biol Bull* **217**, 35-49.

Hertwig, O. and Hertwig, R. (1878). Das Nervensystem und die Sinnesorgane der Medusen. Leipzig: Vogel. pp. 1-186

Land, M. F. and Nilsson, D. E. (2006). General-purpose and special-purpose visual systems. In: *Invertebrate vision* (eds. Warrant, E. J. and Nilsson, D. E.), pp. 167-210. Cambridge: Cambridge University Press.

Land, M. F. and Nilsson, D. E. (2012). Animal eyes. Oxford: Oxford University Press. pp. 1-221

Mackie, G. O. (2004). Central neural circuitry in the jellyfish *Aglantha*: A model "simple nervous system". *Neuro-Signals* **13**, 5-19.

Martin, V. J. (2004). Photoreceptors of cubozoan jellyfish. *Hydrobiol* **530/531**, 135-144.

Masland, R. H. (2012). The neuronal organization of the retina. *Neuron* **76**, 266-280.

Nilsson, D. E., Gislén, L., Coates, M. M., Skogh, C., and Garm, A. (2005). Advanced optics in a jellyfish eye. *Nature* **435**, 201-205.

Parkefelt, L. and Ekström, P. (2009). Prominent system of RFamide immunoreactive neurons in the rhopalia of box jellyfish (Cnidaria: Cubozoa). *J Comp Neurol* **516**, 157-165.

Parkefelt, L., Nilsson, D. E., and Ekström, P. (2005). A bilaterally symmetric nervous system in the rhopalia of a radially symmetric cubomedusa. *J Comp Neurol* **492**, 251-262.

Passano, L. M. (1976). Strategies for the study of the coelenterate brain. In: *Coelenterate Ecology and Behavior* (ed. Mackie, G. O.), pp. 639-645. London and New York: Plenum Press.

Satterlie, R. A. (2002). Neural control of swimming in jellyfish: a comparative story. *Can J Zool* **80**, 1654-1669.

Satterlie, R. A. (2011). Do jellyfish have central nervous systems? *J Exp Biol* **214**, 1215-1223.

Satterlie, R. A. and Nolen, T. G. (2001). Why do cubomedusae have only four swim pacemakers? *J Exp Biol* **204**, 1413-1419.

Skogh, C., Garm, A., Nilsson, D. E., and Ekström, P. (2006). The bilateral symmetric rhopalial nervous system of box jellyfish. *J Morphol* **267**, 1391-1405.

Stewart, S. E. (1996). Field behavior of *Tripedalia cystophora* (class Cubozoa). *Mar Freshw Behav Physiol* **27**, 175-188.

Thorpe, S., Fize, D., and Marlot, C. (1996). Speed of processing in human vision. *Nature* **381**, 520-522.

Wehner, R. (1987). Matched filters neural models of the external world. *J Comp Physiol A* **161**, 511-532.

Wehner, R. (2005). Brainless eyes. *Nature* **435**, 157-158.

Werner, B. (1975). Bau und lebensgeschichte des polypen von *Tripedalia cystophora* (Cubozoa, class. nov., Carybdeidae) und seine bedeutung für die evolution der Cnidaria. *Helgoländer Wiss Meeresuntersuch* **27**, 461-504.

Yamasu, T. and Yoshida, M. (1976). Fine structure of complex ocelli of a cubomedusan, *Tamoya bursaria* Haeckel. *Cell Tissue Res* **170**, 325-339.

Figure legends

Figure 1

Examples of swim trajectories from the experiments with vertical stripes. The data are from the same medusa presented with all five different contrast settings. Each trajectory represents 2.5 min with a time resolution of 1 s. At the low contrast end (c = 0.17 and 0.27) there is little response from the medusa, which performs few turns and has several contacts with the wall of the tank. As contrast increases the medusa starts responding stronger and stronger and at c = 0.93 it makes many obstacle avoidances and stays close to the center of the tank during the entire 2.5 min of the experiment. The numbers on the axes indicate the distance to the wall in cm.

Figure 2

Examples of swim trajectories from the experiments with oblique stripes. The data are from the same medusa presented with all five different contrast settings. Each trajectory represents 2.5 min with a time resolution of 1 s. At the low contrast end (c = 0.17 - 0.39) there is little response from the medusa, which performs few turns and often comes close to the wall of the tank. At c = 0.71 and 0.93 it makes many obstacle avoidances and stays close to the center of the tank during the entire 2.5 min of the experiment. The numbers on the axes indicate the distance to the wall in cm.

Figure 3

Examples of swim trajectories from the experiments with horizontal stripes. The data are from the same medusa presented with all five different contrast settings. Each trajectory represents 2.5 min with a time resolution of 1 s. The medusa does not seem to respond to the stripes until presented with the highest contrast, c=0.93 (compare with figure 1). The numbers on the axes indicate the distance to the wall in cm.

Figure 4

Examples of swim trajectories from the intensity experiments. The data are from the same medusa presented with all five different intensity settings. Each trajectory represents 2.5 min with a time resolution of 1 s. When the wall is uniformly grey the medusa fails to respond to the increasing darkness and makes almost no obstacle avoidances even when presented with the darkest wall matching in light intensity the average between the white and the darkest stripes (compare with figure 1). The numbers on the axes indicate the distance to the wall in cm.

Figure 5

Spatial information and contrast triggers obstacle avoidance. The bars indicate the average and the error bars the standard error of mean (n=8 for vertical stripes and grey tones, n=10 for oblique and horizontal stripes). **(A)** In the contrast experiments with the striped wall the medusae respond to darker stripes by keeping a longer distance to the wall. **(B)** With the stripes the medusae also respond to increasing contrast with an increasing number of avoidances. In the intensity experiments with the grey tones almost no avoidances were seen. The pattern of the bars follows the orientation of the stripes in the experiments. Lines above bars indicate significant differences at the 0.05 (*) or 0.01 (**) level, see Results section for statistics.

Figure 6

Distance when avoiding obstacles. The bars indicate the average and the error bars the standard error of mean (n is indicated by number in bars). **(A)** The more avoidances produced by increasing contrast are also performed farther away from the wall. There was no significant difference between the three different orientations of the stripes when tested with the same contrast. **(B)** When turning the distance of avoidance into angular size of the stripes it is seen that, independent of orientation, at the highest contrast they take up about 25 degrees on the retina. The pattern of the bars follows the orientation of the stripes in the experiments. See Results section for statistics.

Figure 7

Relative contrasts in the natural habitat. **(A)** Photo from the natural habitat of *T. cystophora* showing the natural obstacles, the prop roots. The relative distance from the camera to the root was determined for four roots by their intersection with the surface (coloured lines). The higher up in the picture the intersection the closer the root is to the camera. (B) The RBG photo has the red channel removed and is turned into grayscale to match the spectral sensitivity of the lens eyes of *T. cystophora*. The relative contrast (coloured number) was calculated from the pixel values in two boxes of 500 pixel each (coloured boxes). One box of pixels from the edge of the root and one box from the water just next to it. The farther away the root the lower the contrast (picture courtesy Dan-E Nilsson).

Figure 1

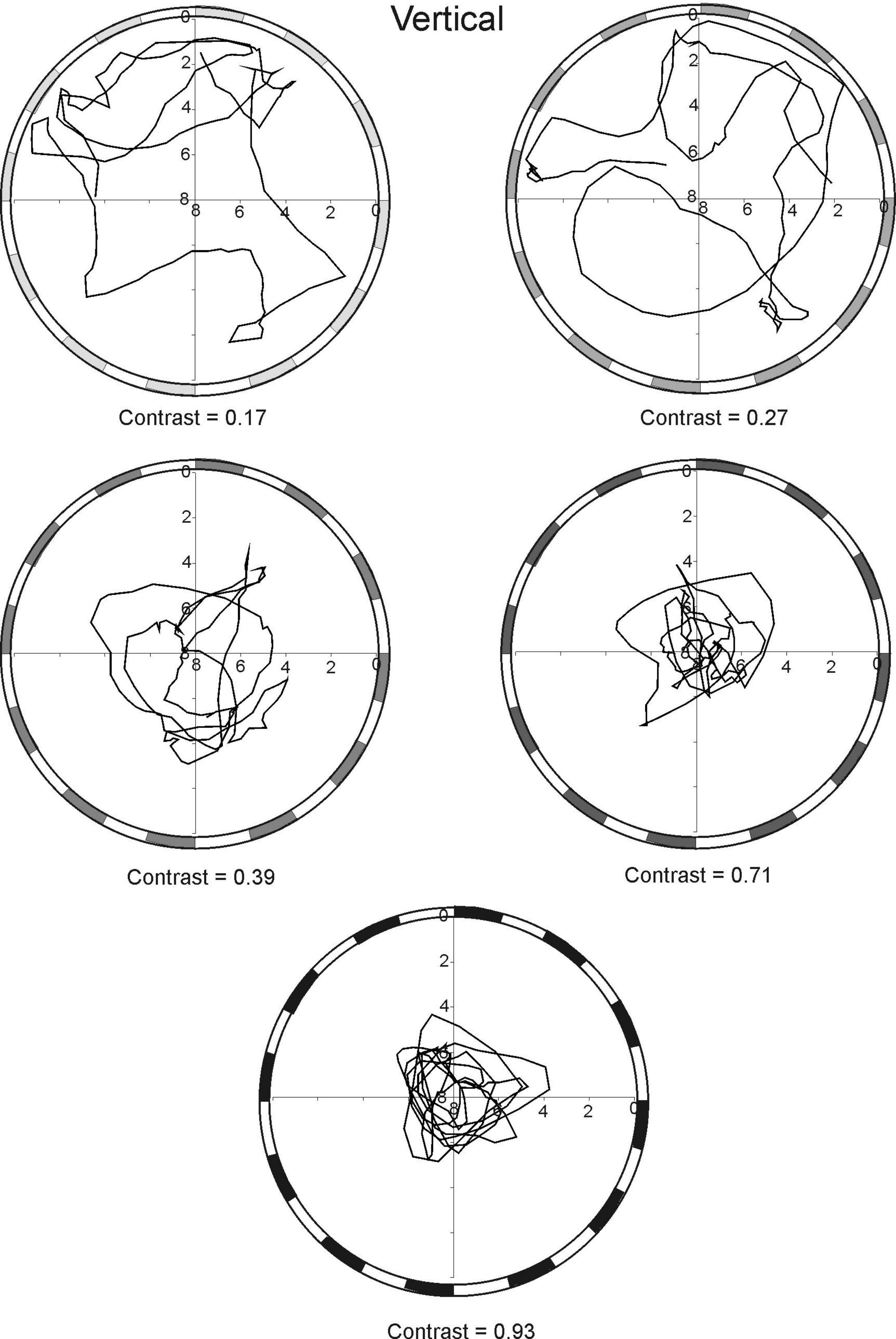

Vertical
Contrast = 0.17
Contrast = 0.27
Contrast = 0.39
Contrast = 0.71
Contrast = 0.93

Figure 2

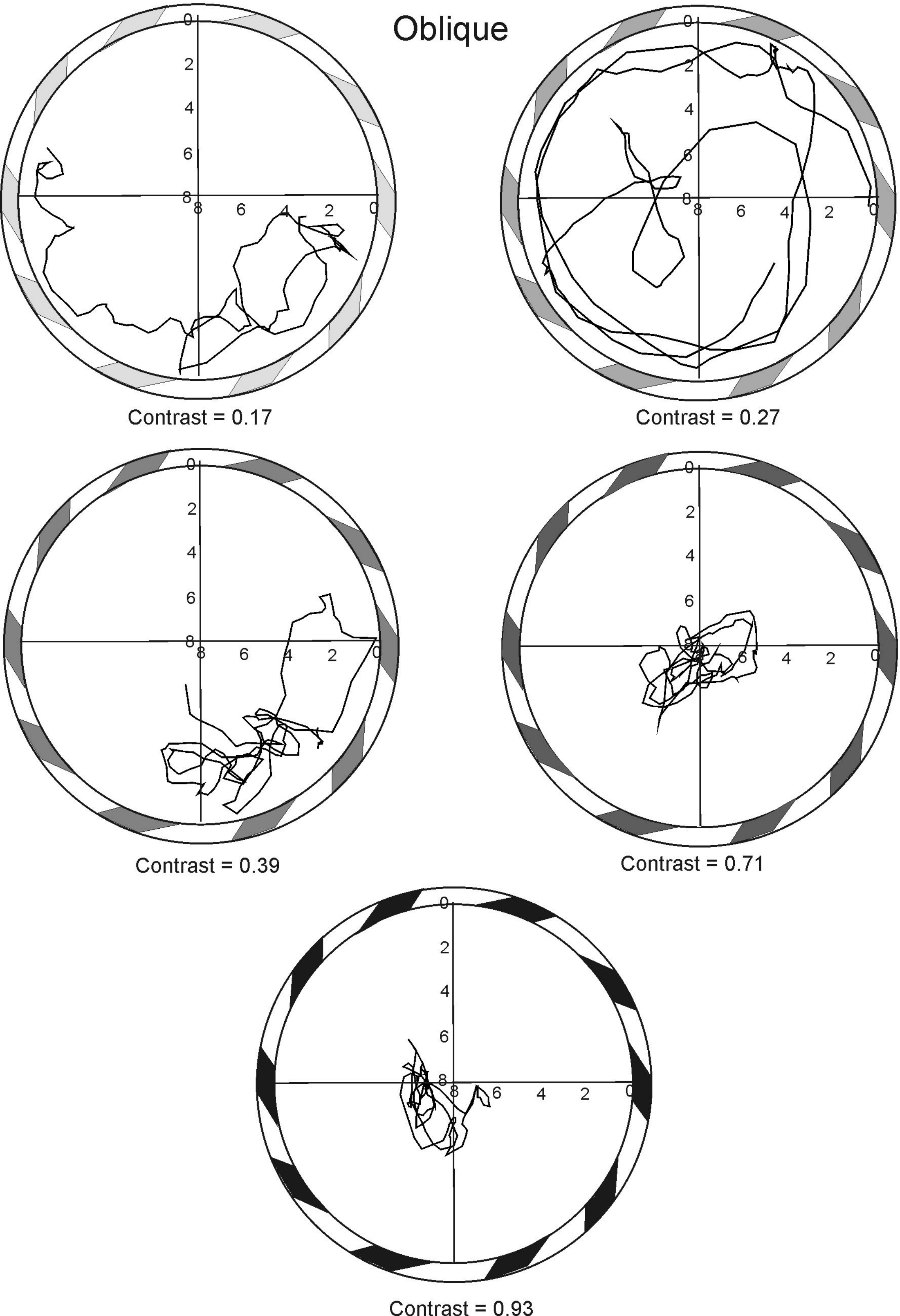
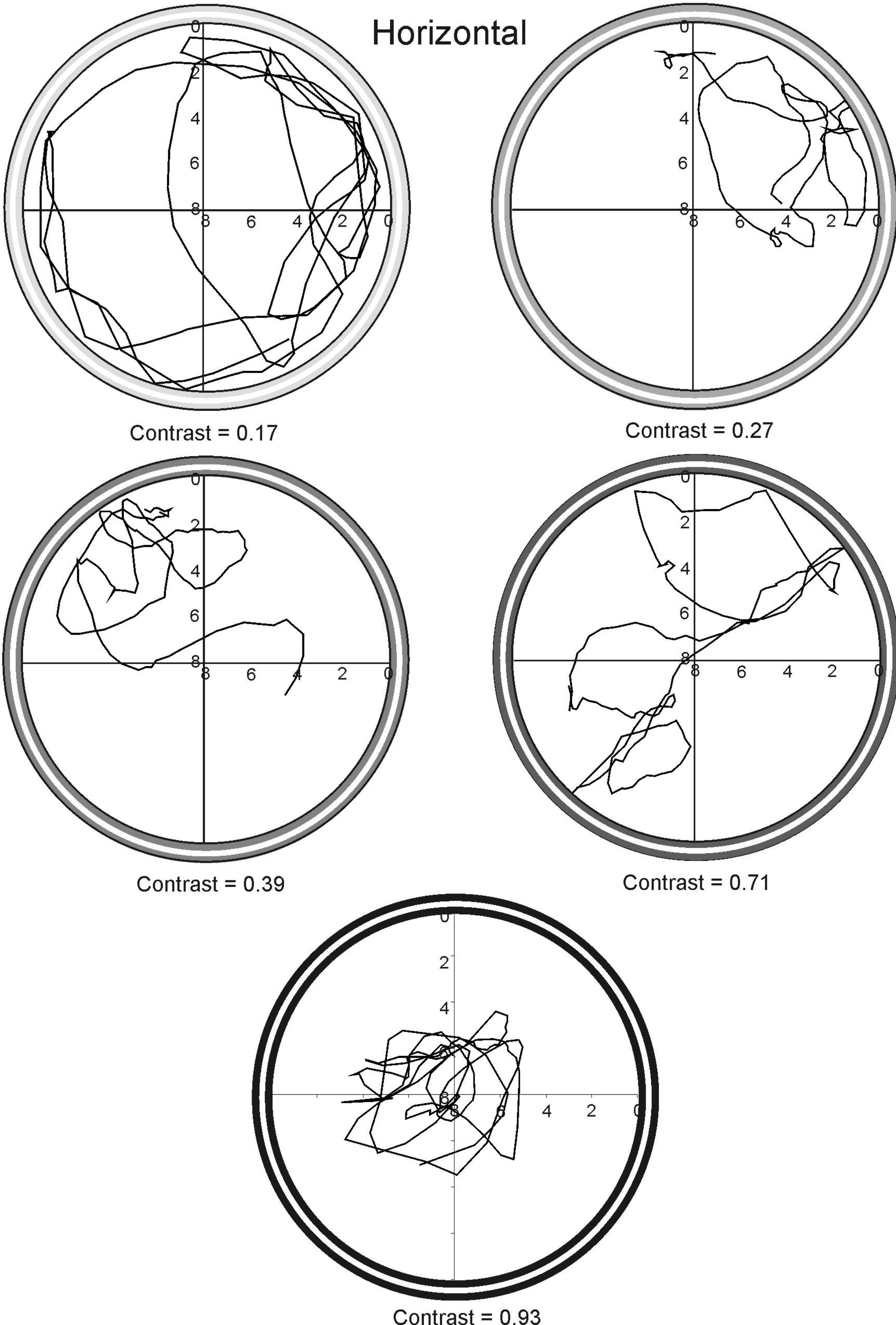

Oblique
Contrast = 0.17
Contrast = 0.27
Contrast = 0.39
Contrast = 0.71
Contrast = 0.93

Figure 3



Horizontal
Contrast = 0.17
Contrast = 0.27
Contrast = 0.39
Contrast = 0.71
Contrast = 0.93

Figure 4

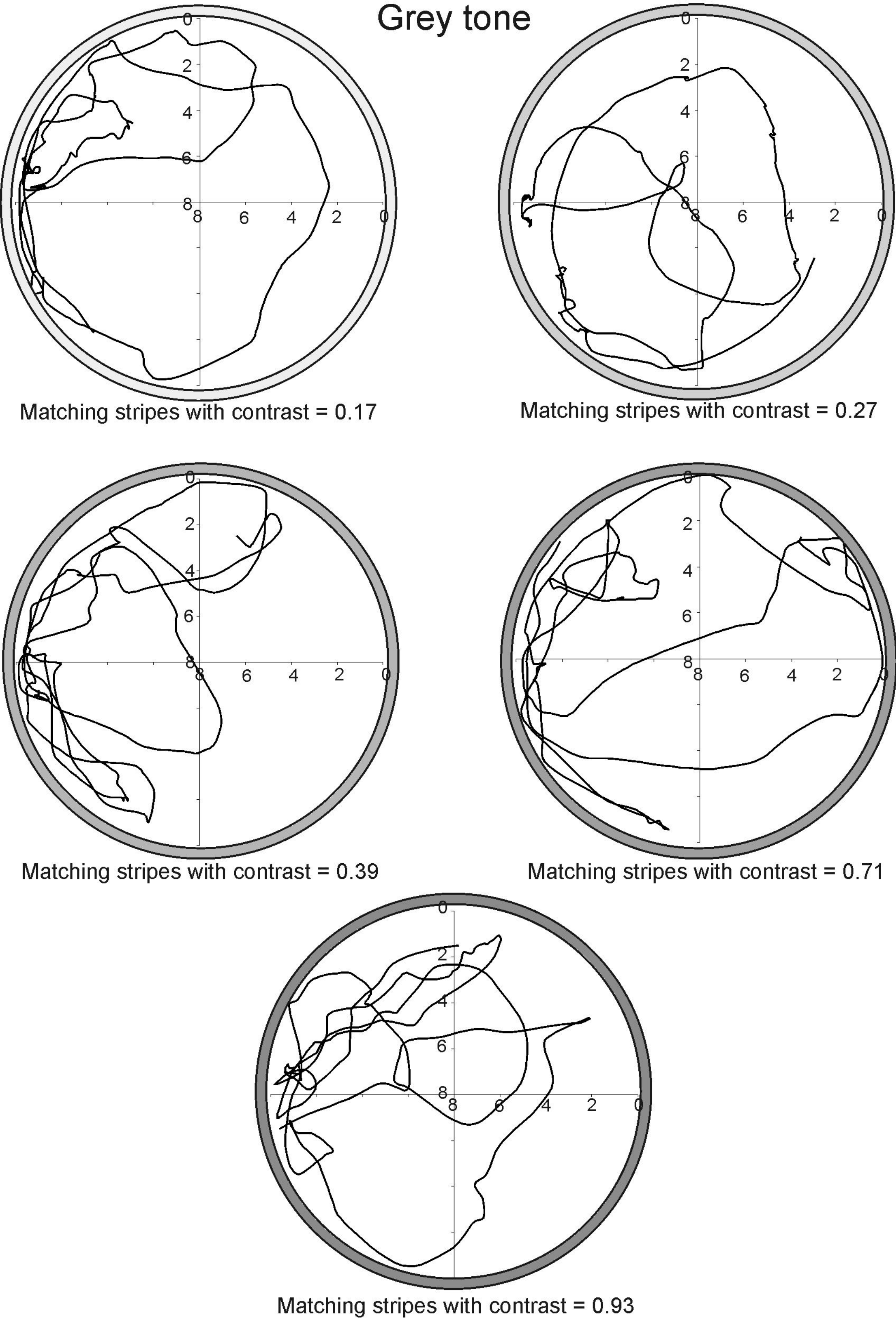

Grey tone
Matching stripes with contrast = 0.17
Matching stripes with contrast = 0.27
Matching stripes with contrast = 0.39
Matching stripes with contrast = 0.71
Matching stripes with contrast = 0.93

Figure 5

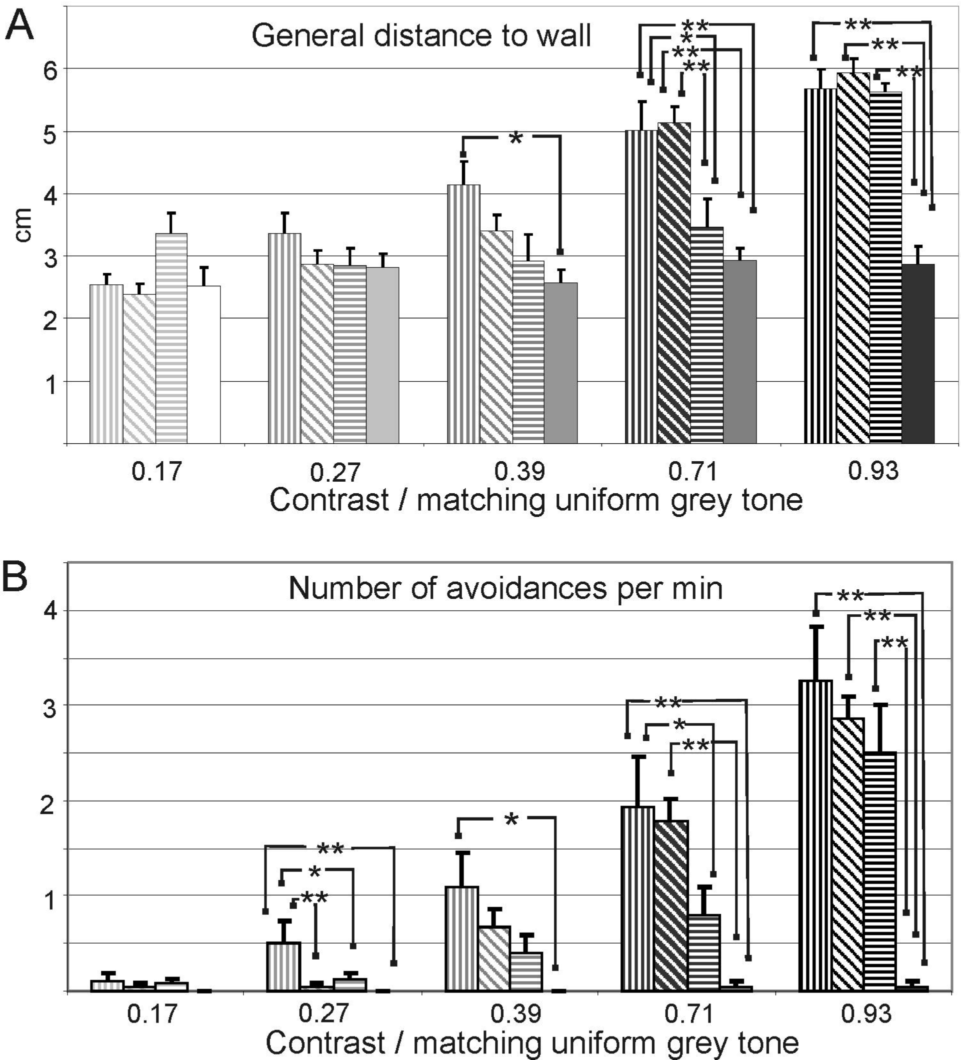

A
General distance to wall
cm
6
5
4
3
2
1
0.17
0.27
0.39
0.71
0.93
Contrast / matching uniform grey tone
B
Number of avoidances per min
4
3
2
1
0.17
0.27
0.39
0.71
0.93
Contrast / matching uniform grey tone

Figure 6

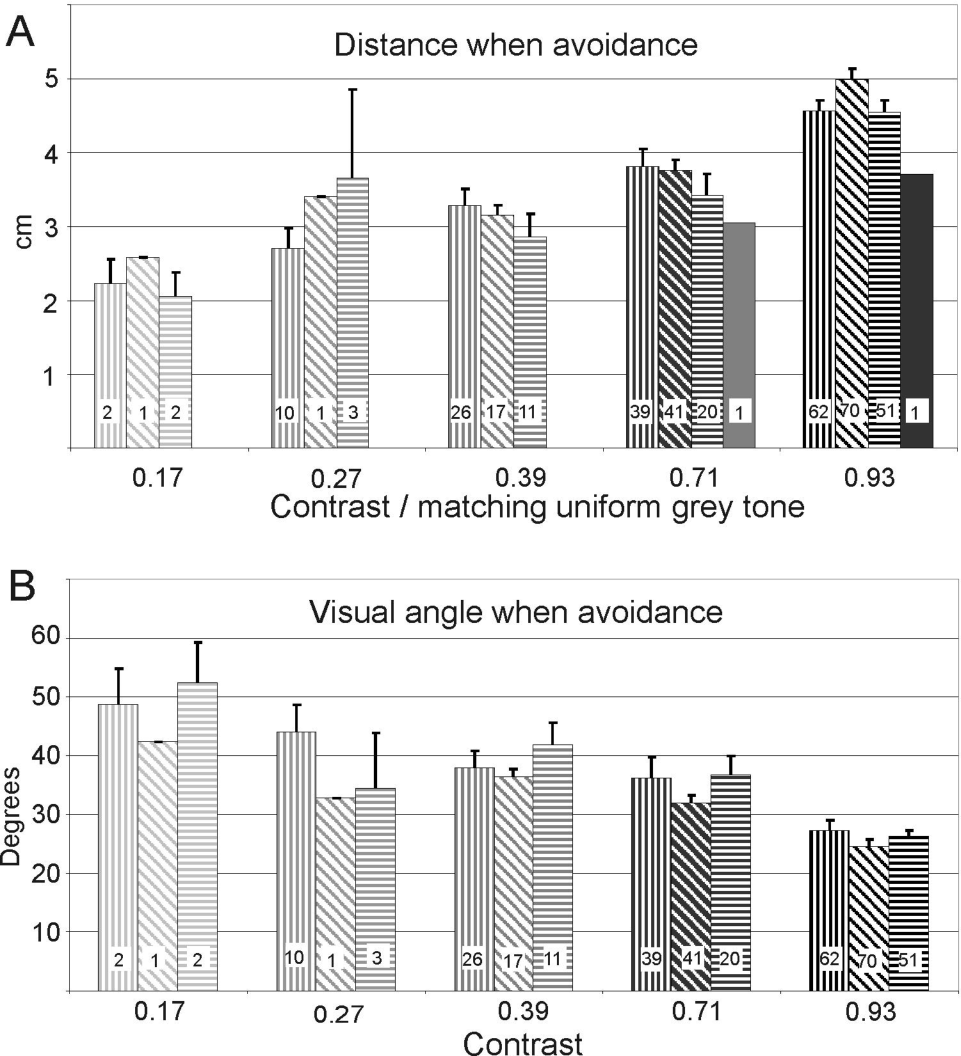

A
Distance when avoidance
cm
5
4
3
2
1
2 1 2
10 1 3
26 17 11
39 41 20 1
62 70 51 1
0.17
0.27
0.39
0.71
0.93
Contrast / matching uniform grey tone
B
Visual angle when avoidance
Degrees
60
50
40
30
20
10
2 1 2
10 1 3
26 17 11
39 41 20
62 70 51
0.17
0.27
0.39
0.71
0.93
Contrast

Figure 7

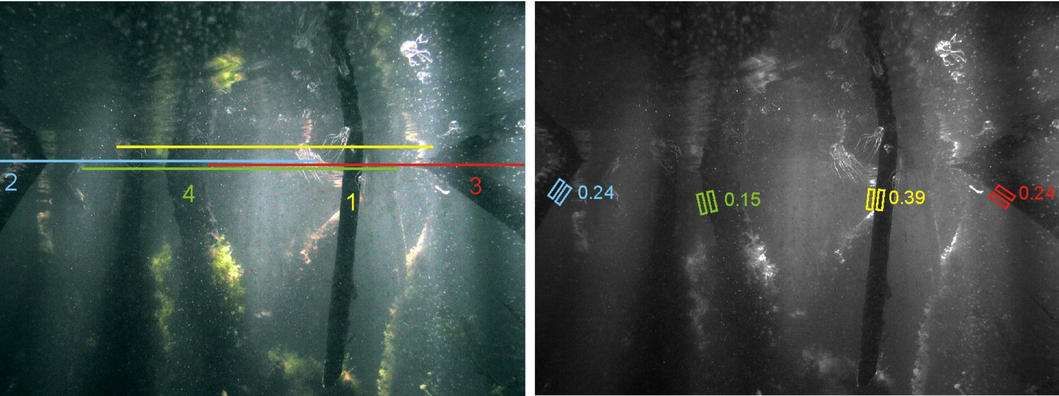


Table 1, summery of behavioural data

	Contrast	General distance to wall in cm	Number of avoids per min	Distance to wall when avoid in cm	Visual angle of stripes when avoid
Vertical	c=0.17	2.5 ± 0.5	0.1 ± 0.1	2.2 ± 0.3	49 ± 6
	c=0.27	3.4 ± 0.9	0.5 ± 0.2	2.7 ± 0.3	44 ± 5
	c=0.39	4.1 ± 1.0	1.1 ± 0.4	3.3 ± 0.2	38 ± 3
	c=0.71	5.0 ± 1.2	1.9 ± 0.5	3.8 ± 0.2	36 ± 4
	c=0.93	5.7 ± 0.8	3.3 ± 0.6	4.6 ± 0. 2	27 ± 2
Oblique	c=0.17	2.4 ± 0.2	0.04 ± 0.04	2.6	42
	c=0.27	2.9 ± 0.2	0.04 ± 0.04	3.4	33
	c=0.39	3.4 ± 0.3	0.7 ± 0.2	3.1 ± 0.1	36 ± 1
	c=0.71	5.1 ± 0.3	1.8 ± 0.2	3.8 ± 0.2	32 ± 1
	c=0.93	5.9 ± 0.2	2.9 ± 0.2	5.0 ± 0.2	25 ± 1
Horizontal	c=0.17	2.3 ± 0.2	0.08 ± 0.06	2.1 ± 0.3	52 ± 7
	c=0.27	3.4 ± 0.3	0.1 ± 0.06	3.6 ± 1.2	34 ± 9
	c=0.39	4.1 ± 0.4	0.4 ± 0.2	2.9 ± 0.3	42 ± 4
	c=0.71	5.0 ± 0.5	0.8 ± 0.3	3.4 ± 0.3	37 ± 3
	c=0.93	5.7 ± 0.3	2.5 ± 0.5	4.6 ± 0.2	26 ± 1
Grey tone	matching c=0.17	2.5 ± 0.3	0	n.a.	n.a.
	matching c=0.27	2.8 ± 0.2	0	n.a.	n.a.
	matching c=0.39	2.6 ± 0.2	0	n.a.	n.a.
	matching c=0.71	2.9 ± 0.2	0.05 ± 0.05	3.1	n.a.
	matching c=0.93	2.9 ± 0.3	0.05 ± 0.05	3.7	n.a.

All values are mean ± s.e.m.