Box Jellyfish Use Terrestrial Visual Cues for Navigation

Report

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Summary

Box jellyfish have an impressive set of 24 eyes of four different types, including eyes structurally similar to those of vertebrates and cephalopods [1, 2]. However, the known visual responses are restricted to simple phototaxis, shadow responses, and object avoidance responses [3–8], and it has been a puzzle why they need such a complex set of eyes. Here we report that medusae of the box jellyfish _Tripedalia cystophora_ are capable of visually guided navigation in mangrove swamps using terrestrial structures seen through the water surface. They detect the mangrove canopy by an eye type that is specialized to peer up through the water surface and that is suspended such that it is constantly looking straight up, irrespective of the orientation of the jellyfish. The visual information is used to navigate to the preferred habitat at the edge of mangrove lagoons.

Results and Discussion

In an attempt to understand the reason for the many eyes in box jellyfish, we investigated one of the two types of lens eye (the upper lens eye) and found that it is highly specialized for looking up through the water surface. Two striking specializations were found: (1) the eye is suspended such that it passively orients the visual field straight upward at all times, irrespective of the orientation of the jellyfish body, and (2) the size of the visual field agrees closely with the angle of 97° (Snell's window), within which the full 180° terrestrial field is compressed by refraction through the water surface. These surprising features strongly suggest that the upper lens eyes are involved in behaviors that exploit either celestial or terrestrial visual cues. We had observed that medusae of our study species, _Tripedalia cystophora_, rapidly swim back to the preferred habitat at the edge of mangrove lagoons after they are transferred away from the edge, and we went on to show that this behavior is driven by visual detection of the mangrove canopy. We further observed that this navigation breaks down at distances predicted by the resolution of the upper lens eyes and when the canopy was obscured from sight.

Background

For well over 100 years it has been known that cubomedusae, or box jellyfish, possess a unique visual system [9, 10]. They have four identical sensory structures, called rhopalia, each carrying six eyes of four morphological types: the upper and lower lens eyes, the pit eyes, and the slit eyes [1, 11–15]. Two of the eye types, the upper and lower lens eyes, have image-forming optics and resemble vertebrate and cephalopod eyes [2, 16, 17]. The role of vision in box jellyfish is known to involve phototaxis, obstacle avoidance, and control of swim-pulse rate [4–6, 18], but more advanced visually guided behaviors have not been discovered prior to this report.

Most known species of box jellyfish are found in shallow water habitats where obstacles are abundant [19]. Medusae of the study species, _T. cystophora_, live between the prop roots in Caribbean mangrove swamps [8, 20]. Here they stay close to the surface [8] to catch their prey, a phototactic copepod that gathers in high densities in the light shafts formed by openings in the mangrove canopy. The medusae are not found in the open lagoons, where they risk starvation [5]. As a result, their habitat is a restricted zone under the mangrove canopy, typically less than 2 m wide. Here we investigate whether the medusae use vision to find their preferred habitat at the edge of the mangrove lagoons and to remain within it.

Vision through the Upper Lens Eyes

From earlier studies [2], we were intrigued by the upper lens eyes, of which there is one on each of the four rhopalia. These eyes point upward in vertically oriented medusae, but because of the heavy crystal (statolith) and the flexible stalk of the rhopalium, it seemed possible, as speculated earlier [7], that they maintain this orientation even when the medusae swim with a horizontally oriented body axis. To test this, we made close-up video recordings of freely swimming _T. cystophora_ medusae and monitored the rhopalial orientation at different body orientations (Figures 1A and 1B). This demonstrated that the upper lens eyes and the pit eyes always point straight upward, with no observable deviation, whereas the lower lens eyes and the slit eyes constantly point obliquely downward. Observations of tethered medusae confirmed that the rhopalia maintain a strictly vertical orientation, irrespective of the orientation of the bell, even when the animal is completely upside down (see Figure S1 available online). The muscles present in the stalk [21] seemed to have no influence on the vertical orientation of the rhopalium, and in the close-up video recordings the rhopalia were never observed to actively move.

The constantly upward-pointing upper lens eye has a retinal geometry indicating a much smaller visual field than that of the downward-pointing large lens eye. Using a previously described optical model of the upper eye [2], we determined the visual field to be close to circular, with a width of 95°–100° (Figure 1C; see Experimental Procedures for details). The precise orientation of naturally suspended rhopalia (Figures 1A and 1B), together with the position of the upper lens eye within the rhopalium [2], allowed us to determine that the circular visual field is centered on the vertical (with an estimated accuracy of ±5°). This vertically centered visual field, of just below 100°, closely matches Snell's window (the 97° circular window through which an underwater observer can see the entire 180° of the terrestrial world compressed by refraction as the light passes through the water surface; Figures 1C and 1D). This, along with the preference of medusae for the top 10 cm of the water column, is a strong indication that the upper
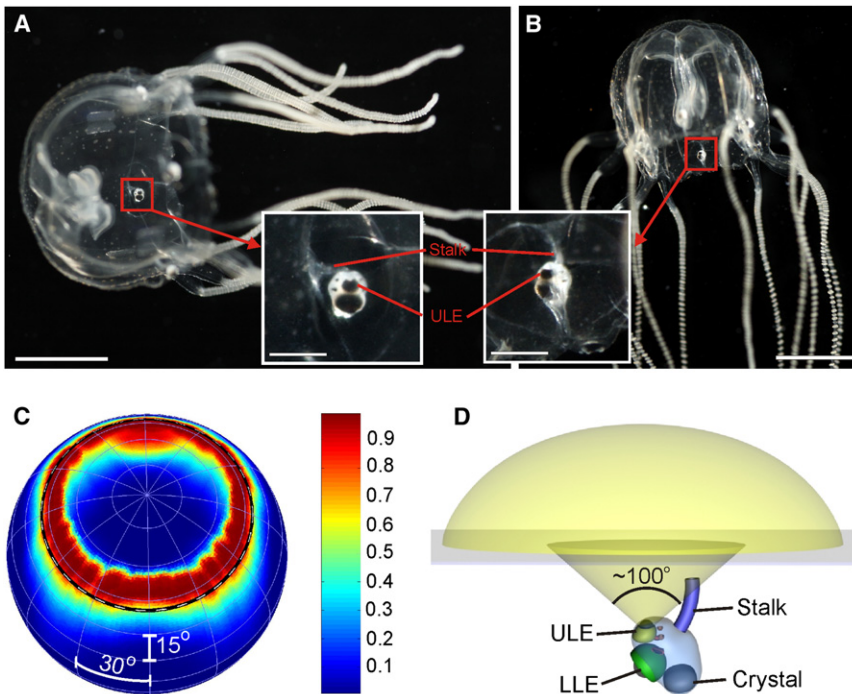
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Figure 1. Rhopalial Orientation and Visual Field of the Upper Lens Eye

(A and B) In freely swimming medusae, the rhopalia maintain a constant vertical orientation. When the medusa changes its body orientation, the heavy crystal (statolith) in the distal end of the rhopalium causes the rhopalial stalk to bend such that the rhopalium remains vertically oriented. Thus, the upper lens eye (ULE) points straight upward at all times, irrespective of body orientation. The rhopalia in focus are situated on the far side of the medusa and have the eyes directed to the center of the animal.

(C) Modeling the receptive fields of the most peripheral photoreceptors in the ULE (the relative angular sensitivity of all peripheral rim photoreceptors are superimposed and normalized according to the color template). The demarcated field of view reveals a near-perfect match to the size and orientation of Snell's window (dashed line).

(D) The visual field of the ULE, of just below 100°, implies that it monitors the full 180° terrestrial scene, refracted through Snell's window. LLE denotes lower lens eye. Scale bars represent 5 mm in (A) and (B) and 500 μm in insets.

lens eye is indeed specialized for looking up through the water surface to exploit terrestrial or celestial visual cues.

With this result, it is tempting to speculate that the upper lens eye is used to detect the mangrove canopy through Snell's window, such that the approximately 1 cm large animals can find their habitat between the mangrove prop roots and remain there even in the presence of tidal or storm-water currents. To evaluate the possibility that the upper lens eye detects the position of the mangrove canopy through Snell's window, we made still pictures using a wide-angle lens looking up through Snell's window in the natural habitat. The pictures were taken from just under the surface to make Snell's window cover the same area of the surface as seen by the medusae. In the pictures, it was easy to follow the mangrove canopy, which shifted from covering most of Snell's window to covering just the edge of Snell's window when the camera was slowly moved outward to about 20 m away from the lagoon edge (Figure 2).

To determine what medusae of *T. cystophora* would see with their upper lens eyes, we used the optical model [2] of the eye to calculate the point-spread function of the optics at different retinal locations. Applying these point-spread functions to still images of Snell's window in the mangrove swamp, we were able to simulate the retinal image formed in the upper lens eyes as a jellyfish moves about in the mangrove lagoon. The results (Figure 2) confirm that despite the severely underfocused eyes and blurred image [2], the approximately 5 m tall mangrove canopy can be readily detected at a distance of 4 m from the lagoon edge and, with some difficulty, can be detected even at a distance of 8 m (detection depends on the amount of surface ripple and the height of the mangrove trees). These results thus predict that if *T. cystophora* medusae use their upper lens eyes to guide them to the correct habitat at the lagoon edge, then they would swim toward this edge if they are closer than about 8 m away from it. Also, if they are farther out in the lagoon, surface ripple and their poor visual

resolution will prevent detection of the mangrove canopy, and the animals would not be able to determine the direction to the closest lagoon edge.

Behavioral Assessment of Visual Navigation

Experiments were conducted on wild populations of *T. cystophora* medusae in the mangrove lagoons near La Parguera, Puerto Rico. Preliminary tests demonstrated that if jellyfish were displaced about 5 m from their habitat at the lagoon edge, they rapidly swam back to the nearest edge, independent of compass orientation. To make controlled experiments, we introduced a clear experimental tank consisting of a cylindrical wall and a flat bottom, open upward, to the natural habitat under the mangrove canopy. When the tank was filled with water, it was lightly buoyant such that the walls extended 1–2 cm above the external water surface, effectively sealing off the water around the animals but without affecting the visual surroundings. A group of medusae was released in the tank, and as long as the tank remained under the canopy, the medusae showed no directional preference but occasionally bumped into the tank wall. The tank, with the trapped water and medusae, was then slowly towed out into the lagoon from the original position under the mangrove canopy. In steps of 2–4 m, starting at the canopy edge, the positions of the medusae within the tank were recorded by a video camera suspended under the tank. At all positions, from the canopy edge and outward, the medusae ceased feeding and swam along the edges of the tank, constantly bumping into it, suggesting that they responded to the displacement (Figure 3). Most importantly, their mean swimming direction differed significantly from random and coincided with the direction toward the nearest mangrove trees (Table S1). This behavior was indicated already at the canopy edge but was strongest when the tank was placed 2 or 4 m into the lagoon (Figure 3). At 8 m from the canopy edge, the medusae could still detect
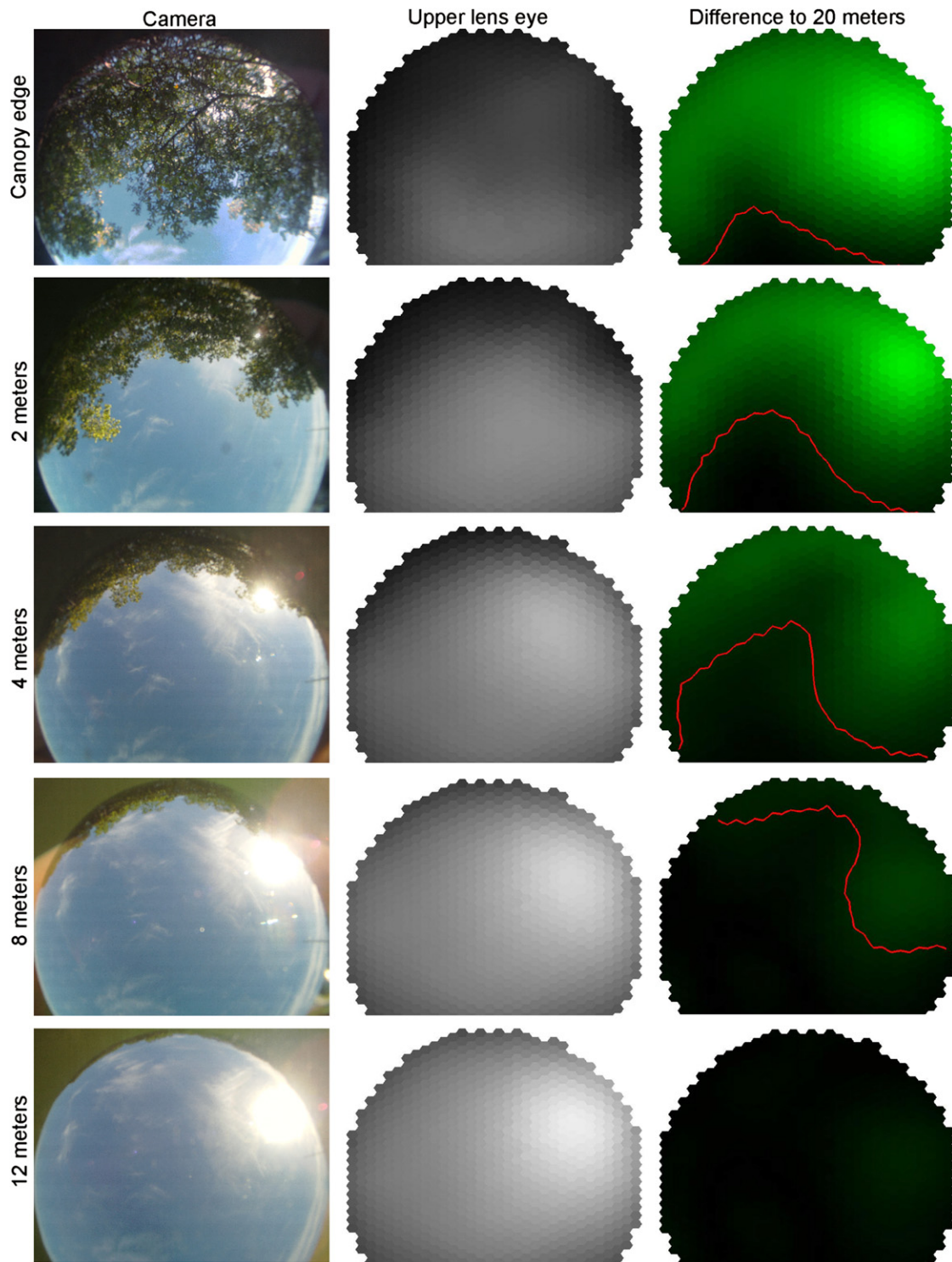
Figure 2. Snell's Window Seen through the Jellyfish Eyes

The first column shows wide-angle still pictures of Snell's window, captured just below the surface at 0, 2, 4, 8, and 12 m from the lagoon edge. The missing part of Snell's window opposite the canopy was removed because it contained the photographer. The second column shows the same images processed by the optical model to mimic the view seen through the upper lens eyes of *T. cystophora* medusae (the gray level represents the calculated relative photon catch in the receptor cells of the retina). The third column shows the difference between images from the experimental distances and a 20 m image. Green indicates higher intensities at 20 m. Red line indicates 12% contrast. Modeling of the image in the upper lens eyes indicates that, despite the poor resolution, jellyfish vision is good enough to detect the mangrove canopy through Snell's window at 8 m, but not at 12 m.
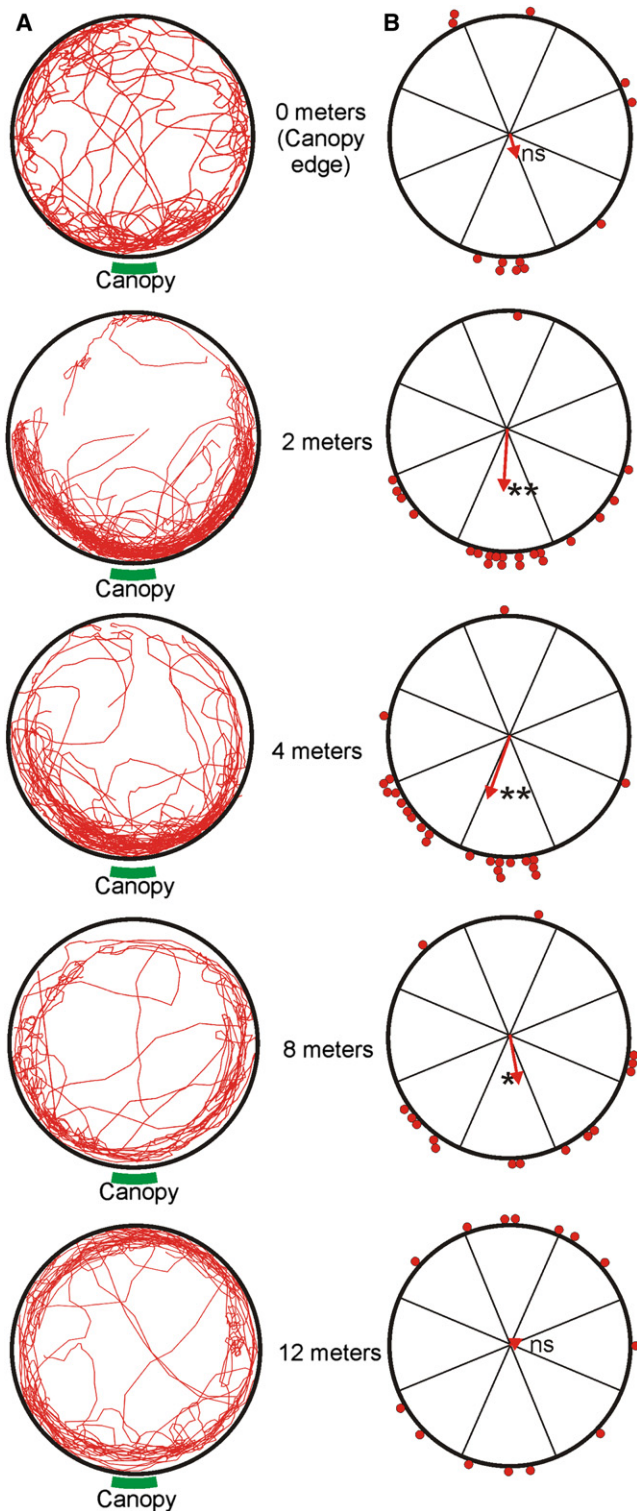
Figure 3. Navigation Behavior of Medusae of *T. cystophora* When Displaced from Their Habitat

(A) Swim trajectories within the circular tank, at the five experimentally tested distances (the green bar indicates the direction to the nearest canopy). At all five distances, the medusae swam along the edge of the tank, demonstrating that the medusae respond to the displacement from their habitat between the mangrove roots.

(B) Statistical analysis of the swim pattern. The red arrow indicates the mean vector of the preferred swimming direction for all medusae at the given distance to the mangrove canopy (a longer arrow indicates a more uniform
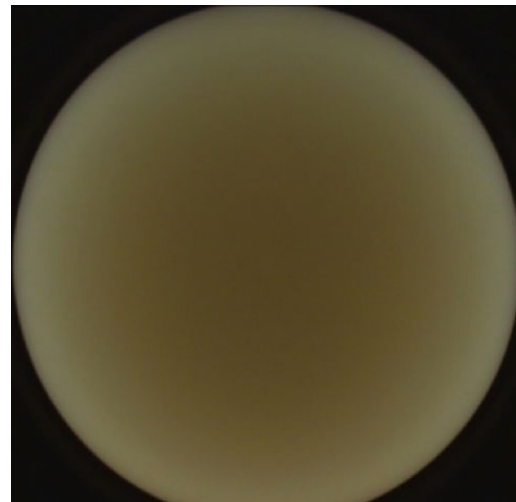

Figure 4. The Complete 180° Underwater Visual Scene in the Mangrove Lagoon

The picture is an average of five pictures taken straight downward just under the surface at a distance of 4 m from the canopies, where the depth is approximately 2.5 m. No visually detectable objects are present, and the light distribution is close to symmetrical, suggesting that navigation by underwater visual cues is not possible in the mangrove lagoon.

the direction to the nearest canopy, but at 12 m they swam randomly along the edge of the tank (Figure 3).

Conclusions

Visual detection of the mangrove canopy by the upper lens eye is the only plausible explanation for the behavioral results. Chemical or mechanical cues cannot have guided the medusae because of the enclosed experimental tank. Further, because their navigational ability depends on the distance to the mangrove trees and is not compromised by the sun being at zenith, we can rule out celestial or other compass cues. The poor visibility in the turbid mangrove lagoon (<1 m) precludes visual navigation by underwater cues. Video recordings demonstrated that no underwater structures or reliable intensity gradients could be seen beyond the canopy edge (Figure 4). The distribution of polarized light underwater could give directional cues, but from the random orientation of the photosensitive membranes in the eyes of *T. cystophora* medusae, polarized vision can be ruled out [2, 11, 12]. Finally, when the visual detection of the mangrove canopy was obscured above water by a bright white sheet (Figure 5), and with the underwater visual scene intact, none of the tested medusae were able to navigate toward the nearest canopy edge. The behavioral experiments thus confirm our hypothesis that medusae of *T. cystophora* detect terrestrial visual structures (the mangrove canopy) through Snell's window and use them as navigational cues. Modeling of visual resolution in the upper lens eye (Figure 2) offers further support by the good match between behavioral data and the predicted distance at which the upper lens eye retina of *T. cystophora* medusae can visually detect the canopy.

swimming direction). The red dots mark the preferred direction of the individual medusae. At 2, 4, and 8 m, the mean vector differs significantly from random and coincides with the direction to the nearest canopy. NS denotes not significant; *p < 0.05, **p < 0.001.
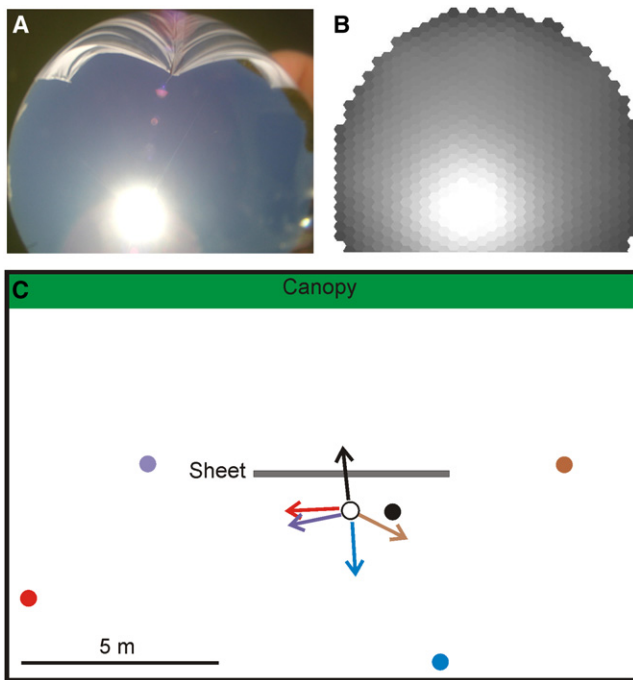
Figure 5. Mangrove Canopy Obscured by a White Sheet

(A) The visual scene in Snell's window when a 2 × 5 m white sheet is put up about 4 m in front of the canopy. The sheet completely blocks sight of the canopy, and this disrupts the ability of the medusae to navigate.

(B) The picture from (A) when processed by the optical model of the upper lens eye. The canopy signature is close to cancelled by the white sheet.

(C) Results from the experiments with obscured canopy. The open circle is the release point of the five meduse; the colored arrows indicate the approximate initial heading. The colored circles indicate the approximate end point of each medusa after 2 min.

It is surprising to find such a navigational system in an animal as basal as a jellyfish. The central nervous system of *T. cystophora* medusae consists of ganglia in the rhopalia and a ring nerve connecting the rhopalia [21–24]. It is not an entirely trivial task to detect the position and orientation of the border between the dark mangrove canopy and the bright sky in Snell's window, but we must assume that the necessary neural circuitry is present in the rhopalial ganglia. Even though these ganglia are unusually large for a cnidarian, each only contains about 1000 neurons [22], and it subserves five eyes in addition to the upper lens eye. It remains to be investigated whether navigational processing in these ganglia resembles that of bilaterian brains.

From an evolutionary viewpoint, the use of terrestrial cues does not seem to be the most straightforward source of information for a marine organism, especially not for a jellyfish. A possible explanation for this peculiarity is that canopy navigation has evolved by modification of a sun compass. Other species of box jellyfish that do not live at the edge of mangrove lagoons have somewhat different upper lens eyes that have been implicated as specializations for detecting the solar position [25]. Scyphozoan medusae of the genus *Mastigas*, along with the common moon jelly *Aurelia aurita*, has also been shown to migrate using the solar position [26, 27]. Here it is suggested that the migration helps the medusae aggregate for reproduction (*Aurelia*) or helps them stay in their saline lakes (*Mastigas*).

Our work demonstrates that despite the lack of a conventional brain, box jellyfish are able to perform seemingly sophisticated behaviors such as navigation by terrestrial visual cues. So far, this navigation is the only known purpose of the upper lens eye, and the lower lens eye seems to have an equally restricted use in repulsion and attraction to nearby underwater structures [3, 4]. Eyes supporting a single visual behavior presumably represent an early stage in the evolution of visual systems. Different eyes for different behaviors probably require less neural processing than if information for different behaviors has to pass through the same eye. The box jellyfish solution may thus be linked to the absence of a central brain, but it defeats the idea that a central brain is a prerequisite for advanced behavior.

Animals

All medusae used for the behavioral experiments were adult males and females of *T. cystophora* (bell height of 8–10 mm). They were experimentally manipulated in their native mangrove swamp in La Parguera, Puerto Rico. For video recordings, animals were collected and filmed within 2 days.

Measurements of Eye Orientation

Close-up video recordings were made to monitor the eye orientation on five nonmanipulated medusae swimming freely in a small tank (10 cm height × 20 cm width × 2 cm depth). A Sony PowerHAD video camera, equipped with a Micro Nikkor 105 mm lens and set to a fixed shutter time of 0.002 s, was used for the recordings. A total of 25 min of video was analyzed. Still pictures were taken with a Nikon D200 camera equipped with the same lens as the video camera. Rhopalial orientation was also observed on tethered medusae, where the bell was rotated to different orientations (Figure S1).

Optical Modeling

A Matlab (2007a, Mathworks) application was made to convert input images into retinal images in the upper eye of *T. cystophora* medusae. The application has a hexagonal pixel array corresponding to the approximately 400 photoreceptors of the retina. Each pixel samples across a unique point-spread function corresponding to angular sensitivities derived from a geometrical-optical model of the eye [2]. Diffraction can be safely ignored because of the much more severe blurring due to underfocusing [2]. The application was fed still images taken in the natural habitat by a high-definition video camera equipped with a wide-angle lens (∼120° × 80° underwater, GoPro Hero, Woodmans Lab).

Behavioral Experiments

In the preliminary trials, three sets of five medusae were tested. Five medusae were collected and tested on the northern shore on the lagoon, five were collected and tested on the southern shore, and five were collected on the northern shore but tested on the southern shore. The medusae were tested one at a time. In each of the tank experiments, 6–8 medusae were tested simultaneously in a round tank (diameter = 45 cm, height = 10 cm) floating with the walls extending 1–2 cm above the surface, effectively separating the tank water from the surrounding water. The tanks were filled with water from under the canopy, ensuring that the chemical composition was that of the habitat. A standard video camera, equipped with a fish eye objective, was mounted under the tank and recorded the behavior of the medusae. The position of the tank was secured by an anchor at successively increasing distances from the canopy edge: 0 m (directly under the canopy edge), 2 m, 4 m, 8 m, and 12 m. Both anchor and camera were attached to the tank with 1 mm thick wire. Every time the tank was moved, the medusae were manually dispersed in the tank, and their behavior was then recorded for 2.5 min. Because of the manipulation, only the last 2 min were used in the analyses. During these 2 min, the experimenter rested low in the water at least 4 m farther into the lagoon, beyond the visual range of the medusae. The experimental series, including the five distances, were repeated three times each with a new group of medusae and with the nearest canopy in different compass bearings. The videos were analyzed in a custom-made program for Matlab, which returned swim trajectories and time spent in predefined tank segments, with a temporal resolution of 1 s. Only medusae that could be followed the entire 2 min were used in the analysis. All experiments were performed in August 2007 on sunny days with the sun position close to zenith (∼82°–88°). A final

set of experiments was conducted to test above-water visual cues versus underwater cues. Here, five medusae were released one at a time 5 m from the canopy, but the canopy was visually obscured by a 2 × 5 m bright white sheet placed approximately 4 m from the canopy. The underwater visual scene was left intact. The approximate initial heading of the medusae was noted, and so was the end point after 2 min of swimming.

Statistics

For statistical analysis of the behavior, the experimental tank was divided into eight segments of 45°, one of which was aligned with the direction to the nearest mangrove trees. The number of times a medusa was observed in each area was then used to create a vector corresponding to the preferred swimming direction of the individual medusae. For each distance to the canopy, a mean vector was calculated and tested against a random distribution with circular statistics in a custom-made program (H_0: direction does not differ from random, n = 12, 21, 22, 15, and 14 at 0, 2, 4, 8, and 12 m, respectively). If the mean vector differed significantly from random, it was tested against the predicted direction toward the nearest canopy (H_0: direction differs from prediction). For details on the statistics, see [28]. Table S1 summarizes the results of the tests.

Supplemental Information

Supplemental Information includes one table and one figure and can be found with this article online at doi:10.1016/j.cub.2011.03.054.

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