Pupillary response to light in three species of Cubozoa (box jellyfish)

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Abstract: Pupillary response under varying conditions of bright light and darkness was compared in three species of Cubozoa with differing ecologies. Maximal and minimal pupil area in relation to total eye area was measured and the rate of change recorded. In Carukia barnesi, the rate of pupil constriction was faster and final constriction greater than in Chironex fleckeri, which itself showed faster and greater constriction than in Chiropsella bronzie. We suggest this allows for differing degrees of visual acuity between the species. We propose that these differences are correlated with variations in the environment which each of these species inhabit, with Ca. barnesi found fishing for larval fish in and around waters of structurally complex coral reefs, Ch. fleckeri regularly found acquiring fish in similarly complex mangrove habitats, while Ch. bronzie spends the majority of its time in the comparably less complex but more turbid environments of shallow sandy beaches where their food source of small shrimps is highly aggregated and less mobile.

Key words: box jellyfish, cubozoan, pupillary mobility, vision

Introduction

The primary function of pupil constriction and dilation in complex eyes is to enable light regulation on the retina which serves to increase sensitivity and acuity (Erichsen et al. 2000, Douglas et al. 2005). Comparative studies on visual systems in vertebrate fauna have shown that differences in this basic pupillary movement can dictate the visual awareness capabilities required to cope with the lifestyle demands of a particular species within the ecological niche in which they operate, for example vertebrates operating in complex environments and variable light intensity show more rapid and greater pupil constriction resulting in better control over visual acuity than those with restricted pupil responses (Arrese 2002, Litherland et al. 2009).

Within the invertebrate fauna, complex eyes are relatively uncommon and are currently known from only two phyla: Mollusca and Cnidaria. Research into the visual systems of these invertebrates has mainly focused on the structure and optics and particularly in regards to their evolutionary development (O’Connor et al. 2009). Although limited studies have investigated pupillary movement of invertebrates (Douglas et al. 2005, O’Connor et al. 2009), none exist directly comparing pupillary movement between species in relation to their habitat and lifestyle.

Members of the Phylum Cnidaria have developed a variety of visual systems ranging from simple eye spots to pigment cup ocelli (Yamasu & Yoshida 1973, Yamasu & Yoshida 1976, Singla 1974, Blumer et al. 1995, Nordström et al. 2003) to advanced complex eyes similar to those found in vertebrates and potentially derived by convergent evolution (Nilsson et al. 2005). These complex camera-type eyes belong to the medusa stage of the cubozoans (box jellyfish) (Berger 1898, Pearse & Pearse 1978, Martin 2002, Nilsson et al. 2005). Research into cubozoan visual systems has noted that the medusa of all species studied possess four sensory clubs or ‘rhopalia,’ each housing a pair of simple light sensitive pigment cups, a pair of light sensitive pigment slits, and a pair of complex eyes (O’Connor et al. 2010, Garm et al. 2011). Both complex eyes have a cornea, a lens, a pigment layer, an iris and a retina (Piatigorsky et al. 1989, Hamner et al. 1995, Martin 2002, Nilsson et al. 2005, O’Connor et al. 2009) with the lower of the two complex eyes displaying pupil movement (Nilsson et al. 2005, O’Connor et al. 2009). However, studies have demonstrated the inability of these complex visual structures to focus sharply questioning what level and type of image these
animals are able to produce (Nilsson et al. 2005, O’Connor et al. 2009).

To examine whether invertebrate fauna possessing complex eyes demonstrate a similar relationship between changes in pupil size and habitat as observed in vertebrate fauna, we investigated differences in the rate and extent of pupil movement in the lateral complex eye of three species of cubozoan found in Tropical North Queensland, Chiropsella bronzie Gershwin, 2006, Carukia barnesi Southcott, 1967, and Chironex fleckeri Southcott, 1956. These three species show a distinct difference in their feeding ecologies. Mature medusae of Ch. fleckeri actively hunt (Kinsey 1986) and feed on fish (Kinsey 1986, Carrette et al. 2002), Ch. bronzie passively captures its prey, the small marine shrimp Acetes sibogae australis Colefax, 1940, by swimming through bait balls (Carrette et al. 2002), and Ca. barnesi actively lures and feeds on larval planktonic fish (Underwood & Seymour 2007, Courtney et al. 2015). The environment in which each of these species are commonly found also varies; Ch. bronzie inhabits the shallow waters of sandy foreshores (Barnes 1961, Gordon et al. 2004) while their prey is located in generally slightly turbid waters (Kinsey 1986); Ch. fleckeri is more often found in the complex environments of the mangroves roots along river banks (Kinsey 1986, Hartwick 1991, Gordon & Seymour 2012); Ca. barnesi, a reportedly offshore medusae (Southcott 1967, Kinsey 1986, Williamson et al. 1996, Kingsford et al. 2012), is regularly found in coral-lined bays of offshore islands (Kingsford et al. 2012). If light regulation in complex eyes of invertebrate species operates according to environmental niche requirements as has been shown for vertebrates, the contrasts in habitat and feeding ecologies displayed by these three species of box jellyfish should be reflected by differences in the functional capacities of their pupils. This study elaborates on the previous research documenting pupil constriction in the cubozoans, Tripedalia cystophora Conant, 1897 (Nilsson et al. 2005) and Ch. bronzie (O’Connor et al. 2009) and presents these findings in relation to the ecological niche in which each species operates.

Materials and Methods

Live medusae were caught at Mission Beach, 17.8694° S, 146.1069° E (Chironex fleckeri), Port Douglas, 16.4836° S, 145.4653° E (Chiropsella bronzie) and Double Island near Cairns, 16.7259° S, 145.6839° E (Carukia barnesi). Animals were transported directly to an aquarium facility and eyes used in this experiment were excised within 12 hours of capture. Entire rhopalia were detached from live animals at the top of the rhopalial stalk as per O’Connor et al. (2009). No more than two rhopalia were used from any individual animal with a single excised rhopodium used only once in any experiment. Each rhopodium was embedded in a wax cup containing seawater (at the same salinity and temperature at which the animals were collected) on a mounting slide with the lateral compound eye facing upwards. The slide was then placed under a dissecting microscope (SZ-ST, Olympus, Japan) and covered in darkness for 30 minutes to ensure maximum pupil dilation and adjustment. The lateral compound eyes of C. bronzie were subjected to three different light intensities. The highest (2000 µmol photons s⁻¹ m⁻²) represented a high light intensity the organism would normally not encounter. The lowest (600 µmol photons s⁻¹ m⁻²) represented the light intensity at 1 m depth in the field. These intensities were achieved by shining a variable cold light source (halogen globe, 3200°K) directly onto the eye. The medium intensity (1400 µmol photons s⁻¹ m⁻²) which is full sunlight, was obtained by placing the excised rhopalia in direct sunlight.

Light intensities were measured by placing the photoreceptive end of a light meter (LuxFc, N19Q1367, Dicksmith Electronics, Australia) at the same distance from the cold light source or direct sunlight as the excised rhopalia. Using a series of digital photographs taken at 60 second intervals for the first 10 minutes and then every 5 minutes thereafter for a total of 20 minutes, the total surface area of the eye was measured followed by the total pupil area (represented by a dark area in the centre of the eye) (Fig. 1). These measurements were converted to a percentage of the maximum pupil opening (100%) as determined from the photograph taken immediately after the initial 30 minutes of dark adaptation for each trial.

For experiments carried out on all three species in direct sunlight (1400 µmol photons s⁻¹ m⁻²), a total of seven eyes from four mature Ch. fleckeri medusae, five eyes from

Fig. 1. Single rhopalium of Chironex fleckeri showing 4 types of visual structures. UCE, upper compound eye (with fixed pupil); CO, cup-shaped ocelli; SO, slit-shaped ocelli; LCE, lateral compound eye (with moveable pupil); ST, statolith; P, pupil opening; I, iris. Scale bar represents 0.5 mm
three mature *Ch. bronzie* medusae, and eight eyes from four mature *Ca. barnesi* medusae were tested. Pupil dilation was measured in the same manner as outlined above.

Data analysis was performed using SPSS version 14. Differences between the pupil dilation of the three species and time in direct sunlight where investigated using general linear model analysis.

**Results**

The pupils in the lateral compound eye of *Chiropsella bronzie* responded differently under the three alternate light intensities to which these eyes were exposed. Time to maximal pupil contraction was fastest at 15 minutes in 2000 µmol photons s⁻¹ m⁻² with a maximum pupil contraction to 48% of maximum dilation (Fig. 2).

When the eyes of all three species where exposed to direct sunlight, the pupils of *Carukia barnesi* contracted more rapidly and to a greater extent than did *Chironex fleckeri* which likewise contracted more rapidly and to greater extent than *Ch. bronzie* pupils (*F* = 3.51, 22*¹*136 d.f, *p* <0.001) (Fig. 3). Maximal pupil contraction in all three species occurred at approximately 10 minutes. Under sustained exposure to each light intensity, the pupils of all three species were not able to maintain the fully contracted state presumably due to muscle fatigue.

**Discussion**

As with vertebrates, pupillary movement in invertebrate cubozoans appears to vary with the ecological niche occupied by different species. *Carukia barnesi* has a faster pupil reaction time and greater constriction capabilities than *Chironex fleckeri*, which has faster and greater pupillary movement than *Chiropsella bronzie*. We believe these differences in eye function are related to the particular ecological niche exploited by these three cubozoan species. For *Ch. bronzie*, a medusae regularly found on calm shallow beach fronts (Kinsey 1986, Gordon et al. 2004) in low visibility water, visual acuity would not be of prime importance and would therefore not confer any major visual advantage. The diet of these medusae consists solely of *Acetes sibogae australis* (Carrette et al. 2002), which are commonly found in aggregations of thousands m² in the waters of sandy foreshores (Omundsen et al. 2000). These marine shrimp prefer water with low visibility resulting from the presence of their main food source of organic material in the water column (Omundsen et al. 2000). *Ch. bronzie* appears to randomly swim through these dense prey schools catching the shrimp on their tentacles (Kinsey 1986) and as such, a tightly contracting pupil affords no real advantage. Poor vision has similarly been linked to feeding ecology in the nocturnal box jellyfish *Copula sivickisi* Stiasny, 1926, which possesses underfocused eyes with very low temporal resolution (Garm et al. 2016). The bioluminescence of their dinoflagellate prey can be detected with low intensity vision, however individual prey items cannot be observed due to low spatial resolution. Although, a peak spectral sensitivity of 460 nm enhances the contrast of the coral structures needed for shelter at dawn, demonstrating a compromise between prey capture and habitat detection.

The comparative lack of obstacles in the environment where *Ch. bronzie* are found, compared to those of *Ch. fleckeri* and *Ca. barnesi*, reduces the need for increased visual acuity. In the cases of *Ca. barnesi* and *Ch. fleckeri*, medusae of these species are known to inhabit the more structurally complex three dimensional environments of mangroves (*Ch. fleckeri*, Gordon & Seymour 2012) and coral reefs (*Ca. barnesi*, Kingsford et al. 2012). These medusae have to contend not only with numerous physical structures such as tree roots or coral branches, but also low level fluctuations in light as well as fast moving vertebrate prey. Under laboratory conditions, *Ch. fleckeri* have been shown to use vision in obstacle avoidance (Hamner et al. 1995), whereas *Ch. bronzie* displayed very poor obstacle avoidance capabilities in a similar experiment (Garm et al. 2007b). As a result, the visual acuity of *Ch. fleckeri* compared to that seen in *Ch. bronzie* would presumably have a
positive selection pressure. Similarly, increased visual acuity brought about by a greater depth of field as a result of the smaller pupil openings in *Ch. fleckeri* and *Ca. barnesi*, would assist these animals in better negotiating their environment.

These results support and elaborate on a study (O’Connor et al. 2009) that linked variation in eye structure and optics of *Ch. bronze* and *Tripedalia cystophora*, with distinct ecological habitats of each species. O’Connor et al. (2009) noted a ten-fold faster reaction time to maximum pupil dilation in *T. cystophora* (1 minute to max. dilation) under high intensity light compared to the maximum contraction times recorded for the three species of this study. However, this may also be a factor of differences in the habitat and behaviour of *T. cystophora* which swims in and out of light shafts in short periods of time (Garm et al. 2007a) and uses vision for prey capture (Buskey 2003) and orientation to specific habitat features in their mangrove habitats (Garm et al. 2011). *Tripedalia cystophora* and *Ch. fleckeri* both inhabit mangrove roots at lagoon edges. Nilsson et al. (2005) determined that although the compound eyes of *T. cystophora* have the potential for sharp image focus, the ray paths in *T. cystophora* result in the eyes being severely underfocused. This was deduced to remove high spatial frequencies allowing the animal to visualise the large mangrove structures in its environment, but not small floating particles. However, if the animals’ prey source is considered, and compared with the visual sensitivity now demonstrated in *Ch. fleckeri*, this could perhaps be interpreted as a trade-off between prey detection and obstacle avoidance. Both *T. cystophora* and *Ch. fleckeri* must be able to visualise the large mangrove structures dominating their environment, however whilst *Ch. fleckeri* also requires sharp image focus to locate fast agile prey fish, *T. cystophora* feeds on dense copepod swarms (Buskey 2003) similar to *Ca. bronze*, negating the need of both species to visualise fine details. This demonstrates an important relationship between visual acuity and feeding ecology. Studies on marine vertebrates have also shown vast differences in the operational speed of pupil movement which they suggest may be correlated to the ecological niche each species operates in (Douglas et al. 1998, Douglas et al. 2002).

Cubozoans are an appropriate taxon for further studies of visual ecology in invertebrates due to the similarities in morphology of their visual systems but differences in ecological niches. To further this hypothesis of the relationship between pupil movement and environmental requirements, additional studies are needed before any constructive conclusions can be made regarding the advantages (or disadvantages) offered to cubozoan medusae by varying rates of contraction. These findings should be linked with studies on how, if at all, these medusae are able to process images collected by their relatively complex visual system.

Acknowledgements

We would like to thank Damain Rigg and Avril Underwood for some of the initial work and comments on the manuscript.

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