A 22219

Volume 193 • Number 7 • July 2007

Journal of Comparative Physiology A

Neuroethology, Sensory, Neural, and Behavioral Physiology

Springer

J Comp Physiol A (2007) 193:547–557
DOI 10.1007/s00359-007-0211-4

ORIGINAL PAPER

The lens eyes of the box jellyfish *Tripedalia cystophora* and *Chiropsalmus sp.* are slow and color-blind

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Received: 19 June 2006 / Revised: 15 January 2007 / Accepted: 18 January 2007 / Published online: 16 February 2007

Abstract Box jellyfish, or cubomedusae, possess an impressive total of 24 eyes of four morphologically different types. Compared to other cnidarians they also have an elaborate behavioral repertoire, which for a large part seems to be visually guided. Two of the four types of cubomedusean eyes, called the upper and the lower lens eye, are camera type eyes with spherical fish-like lenses. Here we explore the electroretinograms of the lens eyes of the Caribbean species, *Tripedalia cystophora*, and the Australian species, *Chiropsalmus sp.* using suction electrodes. We show that the photoreceptors of the lens eyes of both species have dynamic ranges of about 3 log units and slow responses. The spectral sensitivity curves for all eyes peak in the blue-green region, but the lower lens eye of *T. cystophora* has a small additional peak in the near UV range. All spectral sensitivity curves agree well with the theoretical absorbance curve of a single opsin, strongly suggesting color-blind vision in box jellyfish with a single receptor type. A single opsin is supported by selective adaptation experiments.

Keywords Box Jellyfish · Vision · Spectral sensitivity · Response speed · Dynamic range

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Introduction

Box jellyfish, or cubomedusae, are unusual amongst jellyfish in several ways. When observed in their natural habitat it is striking how their behavior resembles that of fish. They are fast swimmers often performing strong directional swimming (Shorten et al. 2005) but also capable of rapid 180° turns. Many species of cubomedusae are found in habitats such as between mangrove roots and in kelp forests (Coates 2003), habitats which are dangerous for most other jellyfish. They are able to navigate between the obstacles in these habitats and under laboratory conditions they have been shown to move away from dark objects and to be attracted to white (Hartwick 1991; Hamner et al. 1995; Matsumoto 1995). It seems intuitive that most if not all of these behaviors are visually guided, and cubomedusae do have eyes.

Eyes are not uncommon among jellyfish and simple eyes are found in many species of both scyphomedusae and hydromedusae (Yamasu and Yoshida 1973; Singla 1974; Toh et al. 1979; Yamamoto and Yoshida 1980; Singla and Weber 1982; Takasu and Yoshida 1984) but the visual system of cubomedusae is much more elaborate (Nilsson et al. 2005). They have four sensory clubs, called rhopalia, each equipped with six eyes (Fig. 1) of four distinct morphological types (Claus 1878; Hertwig and Hertwig 1878; Berger 1898; Yamasu and Yoshida 1976; Laska and Hündgen 1982), suggesting a division of labor between special purpose eyes. Two of the eye-types, the upper and lower lens eyes, are morphologically very similar to the camera-type eyes found in vertebrates and cephalopods and have a spherical lens with graded refractive index, an everse hemispherical retina, a cornea, and in the lower lens eye also a mobile

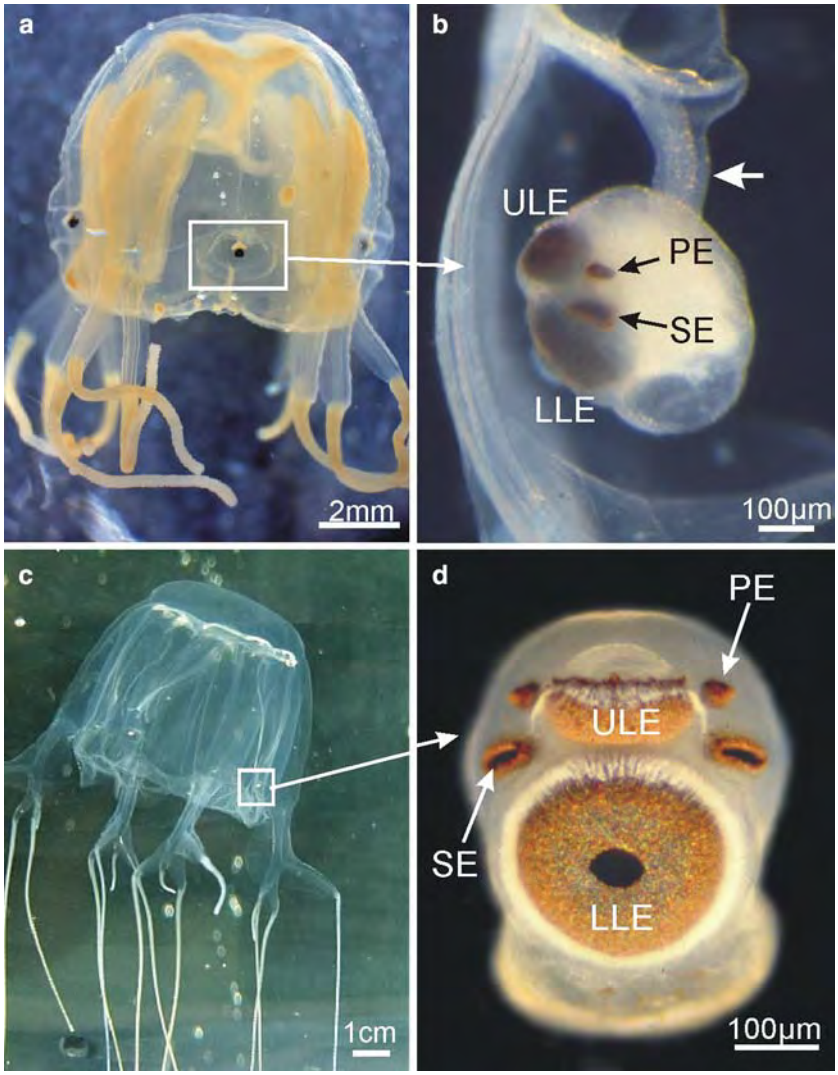

Fig. 1 The species of box jellyfish used in this study were *Tripedalia cystophora* from the mangroves of La Parguera, Puerto Rico (**a**) and *Chiropsalmus sp.* living off sandy beaches in northern Queensland, Australia (**c**). All box jellyfish have four elaborate sensory clubs, the rhopalia (**b**, **d**), each carrying six eyes of four morphologically distinct types: upper lens eye (*ULE*), lower lens eye (*LLE*), a pair of slit eyes (*SE*), and a pair of pit eyes (*PE*). The rhopalia are attached to the rest of the animal through a flexible stalk **(b,** *arrow***)**

pupil (Nilsson et al. 2005). The remaining four eyes are arranged in two pairs, the slit eyes and the pit eyes (Fig. 1) and in the literature they are referred to as simple ocelli (Berger 1898; Pearse and Pearse 1978; Laska and Hündgen 1982).

There has been much speculation for the presence of eyes in cubomedusae. It has been suggested that they play a role in the choice of mate (Lewis and Long 2005) but most often the eyes are believed to be used in active hunting (Larson 1976; Pearse and Pearse 1978; Kinsey 1986; Matsumoto 1995; Raffaele 2005). The only species where the method of prey capture is well studied is the Caribbean *Tripedalia cystophora*, and they are passive hunters (Stewart 1996; Buskey 2003). They cruise back and forth through light shafts between mangrove roots and accidentally catch phototactic copepods. Preliminary observations on *Carybdea rastonii* and *Chironex fleckeri* suggest that these species are also passive hunters (Kinsey 1986; Matsumoto 1995). Therefore it seems unlikely that the eyes are involved in prey capture

The purpose of cubozoan eyes can be elucidated through their visual capacities. In an earlier study we examined the special resolution of the lens eyes of our model species *T. cystophora* and found rather poor resolution (Nilsson et al. 2005). Interestingly, we also found that the shape of the visual fields of individual receptors varied across the retina some being highly asymmetric. This indicates very specific filtering already at the retinal level. Next we examined the spectral sensitivity of the lens eyes of *T. cystophora* (Coates et al. 2006) and found them to be opsin-based probably with a single population of photoreceptors. Still, some uncertainty remained as to whether additional populations of rare receptors are present. Also, studies on the cubomedusa *Carybdea marsupialis* have indicated the presence of several populations of photoreceptors using antibodies against zebra fish opsins (Martin 2002, 2004).

In the present study the spectral sensitivity of *T. cystophora* lens eyes is further investigated using

selective adaptation. For comparison, we also include data from the Australian species *Chiropsalmus sp.* Additionally, we examine the dynamic range and the time-to-peak of the lens eyes of both species. This is done by extracellular electrophysiological recordings and we show that in both species the eyes are slow and have standard dynamic ranges of about 3 log units. We also show that the observed spectral sensitivity curves all indicate the presence of a single opsin.

Material and methods

Adult male and female (8–12 mm in bell diameter) *T. cystophora* were hand collected in the mangrove swamps near La Parguera, Puerto Rico. They were kept in a round 300 l concrete tank with running seawater at about 28°C and used for the experiments within 2 days of capture.

Adult males and females (4–8 cm in bell diameter) of *Chiropsalmus sp.* were either hand collected or caught with a dragnet at Newall Beach north of Port Douglas in Queensland, Australia. The animals were brought back alive to James Cook University in Cairns where they were kept in a round 500 l tank with circulating seawater at 28°C. They were fed pieces of dead prawn several times a day.

Electrophysiology

A rhopalium was cut off approximately midway along the stalk with a pair of fine scissors and transferred using a pipette to a small Petri dish containing sea water. Under a dissection microscope a glass suction electrode (for electrode details see Derby 1995) was applied to the surface area of the rhopalium where the pigmented photoreceptors of either the lower or the upper lens eye was closest to the surface (Fig. 2). Suction was applied until the first slight pigment migration was seen indicating that the pigmented photoreceptors are attached to the tip of the electrode. The reference electrode was placed in the seawater bath. Once the electrode was attached and a signal obtained a black cloth was put over the setup and the rhopalium was left to dark adapt for 15 min.

A Linos microbench system was used for light stimulation. Light from a 12 V/50 W halogen bulb was focused into a light guide, 500 µm in diameter. The light guide was arranged in the Petri dish to stimulate either the upper or the lower lens eye with 40 ms flashes applied using a mechanical shutter controlled by a function generator (TGP10 from TTI, Fort Worth, TX, USA). To check for effects of the flash duration additional experiments were performed on *T. cystophora*, $n = 3$, with 5, 10, 25, and 100 ms flashes. The dynamics of the shutter were monitored using a photodiode. To examine the dynamic range V–log I curves were obtained. A series of neutral density filters were applied to the light beam in steps of 0.3 or 0.4 log units with inter stimulus intervals of 1 min starting at the lowest intensities. The maximum intensity was 3.2×10^5 W/m^2/sr. It was measured at the tip of the light guide with a radiometer (IL1700 from International Lights, Peabody, MA, USA) including an infrared blocking filter (700 nm low pass filter). This intensity equals 3.6×10^{21} photons/s/sr/m^2 when adjusting for the spectrum of the light source (measured with a S2000 spectrophotometer from Ocean Optics, Dunedin, FL, USA) and the relative sensitivity of a 500 nm receptor (Govadovskii 2000). For the spectral sensitivity a series of interference color filters with half-widths of 10–12 nm (CVI Laser, LLC,

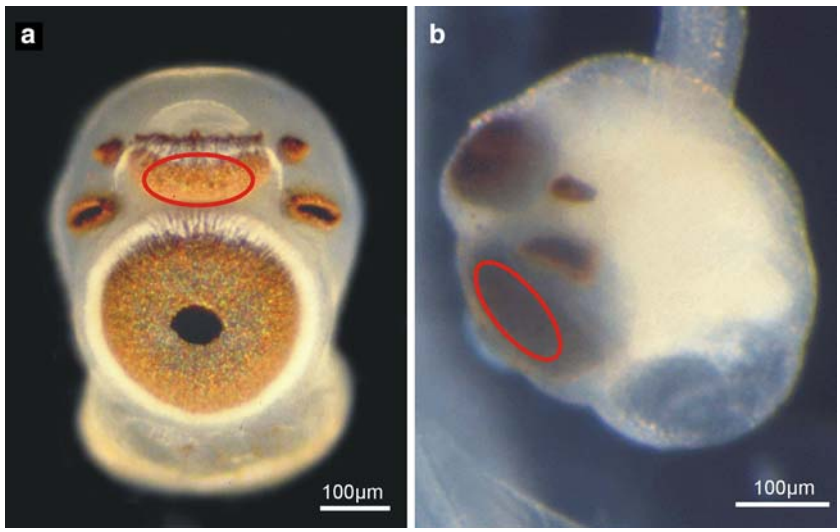

Fig. 2 Rhopalia showing the areas where the suction electrodes were placed (red ovals) on the upper lens eye (**a**) and on the lower lens eye (**b**). These areas were chosen, since the retina here comes closest to the surface of the rhopalium ensuring minimum interference from non-retinal cells

Albuquerque, NM, USA) were applied to the light beam in steps of 10 or 20 nm. The filters spanned 360–700 nm. Equal quantity stimulations (5.6×10^{19} photons/s/sr/m^2) were ensured using variable polarization filters and the inter-stimulus intervals was 1 min. After the series of color filters the V–log I curve was re-measured with 1 log unit steps to ensure that the sensitivity of the eye had not changed.

To check for the presence of more than one receptor type in the lower lens eyes a series of selective adaptation experiments were performed. The eyes were stimulated with a mixture of two light beams and one of them (the adaptation light) constantly presented light at either 530 nm (*T. cystophora*) or 580 nm (*Chiropsalmus sp.*) to the eye at 5.6×10^{19} photons/s/sr/m^2. The other presented 40 ms flashes of colored light as described above.

The signals were amplified 1,000 times (DAM 50 amplifier from World Precision Instruments, Sarasota, FL, USA) and filtered via high- and low-pass filters in the amplifier (1 and 300 Hz, respectively). In the case of *T. cystophora* the signals were additionally filtered through a 60 Hz notch filter (World Precision Instruments, Sarasota, FL, USA). The signals from *Chiropsalmus sp.* were filtered through a 50 Hz filter (Hum Bug from Quest Scientific, Vancouver, Canada). The recordings were stored and analyzed manually on a laptop using an A/D converter (12 bit USB module from Data Translation, Marlboro, MA. Software: Scope version 2.2.0.30 from Data Translation).

Results

The recorded signals

The photoreceptors of both the lower and the upper lens eye of both species responded with graded receptor-like potentials to the stimuli (Fig. 3). The signals recorded from *T. cystophora* were in general biphasic (Fig. 3a) whereas in *Chiropsalmus sp.* they were often triphasic (Fig. 3c), but in both species they became monophasic at low stimulus intensities. All the data analyses (V–Log I curves, time-to-peak and spectral sensitivity) were performed on the peak of the first phase, which always had by far the largest amplitude

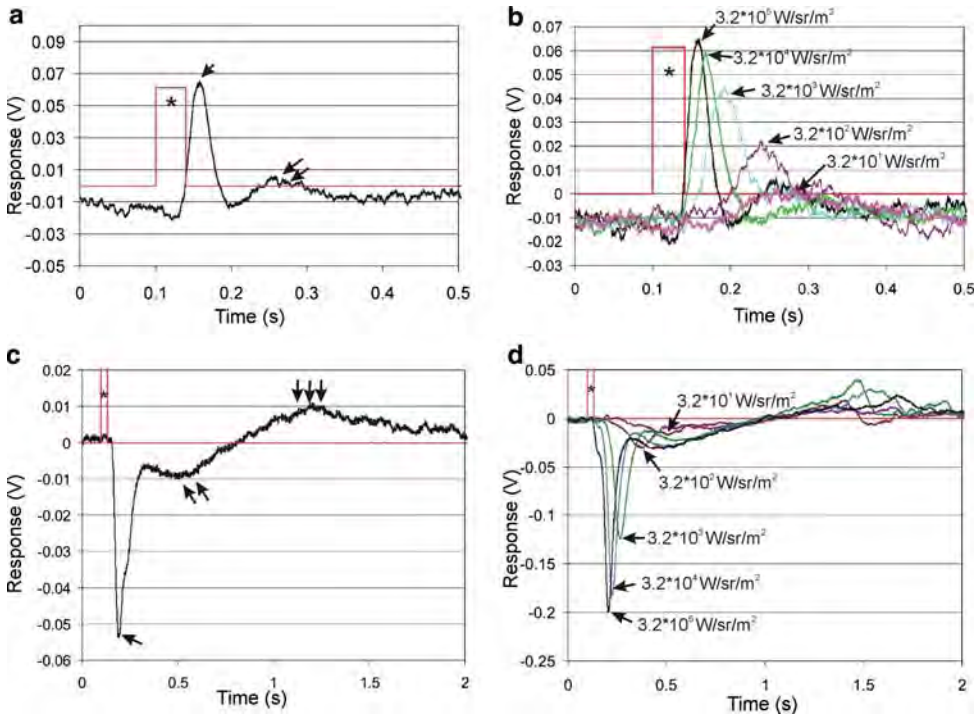

Fig. 3 Typical signals from the two lens eyes of both *Tripedalia cystophora* (**a**, **b**) and *Chiropsalmus sp.* (**c**, **d**) when stimulated with 40 ms flashes of white light. *Asterisks* indicate stimuli. **a** Typical response from an upper lens eye of *T. cystophora* when stimulated at full intensity. The response is biphasic. The first phase (*single arrow*) is considered the receptor potential and normally has positive amplitude. The second phase (*double arrow*) has much smaller amplitude and peaks a few hundred ms after the stimulus. **b** The same cells as in A stimulated over 4 log units of light intensities. The response becomes smaller and slower with decreasing light intensity. **c** Response from the lower lens eye of *Chiropsalmus sp.* In this species the signals are in most cases triphasic with a negative first phase. The additional third phase (*triple arrow*) has positive amplitude and can appear as late as 2 s after stimulation. **d** A series of recordings from an upper lens eye of *Chiropsalmus sp.* stimulated with light intensities covering 4 log units

and we consider the true photoreceptor response. This conclusion is supported by our morphological data. The vast majority of neurons are situated in the back of the rhopalium relatively far from the retinas. The areas where the suction electrodes are situated have a very dense receptor population but holds very few possible neurons (Skogh et al. 2006). It is therefore very unlikely that the strong and consistent first phase is produced by cells other than the photoreceptors. As further evidence, if the electrode is situated a little off the retina, closer to the population of neurons, only weak or no response to light is recorded. It is not known whether the additional phases are produced by the photoreceptors or by higher order neurons. The last phase of *Chiropsalmus sp.* was extremely slow and long lasting: the entire signal could be as long as 2 s. Interestingly, the third phase became shorter with lower intensities (Fig. 3d). Since the recordings were performed with extracellular electrodes it is not known whether the receptors respond with depolarization or hyper-polarization. In *T. cystophora* all but one series had positive amplitudes for both phases (Fig. 3a, b) whereas in *Chiropsalmus sp*. the two corresponding phases had negative amplitudes in all but two series (Fig. 3c, d). The additional third phase in *Chiropsalmus sp*. always had opposite polarity to the two first phases (Fig. 3d).

Dynamic range (V–log I curves)

The upper lens eye of both species have about the same dynamic range spanning approximately 3 log units from about 8×10^1 to 8×10^4 W/sr/m^2. The dynamic range is here defined as the linear part of the V–Log I curve (Figs. 4a, 5a). The dynamic range of the upper lens eye of *Chiropsalmus sp.* is less starting at 2×10^2 W/sr/m^2 (Fig. 5a). The V–Log I curve of the lower lens eye of *T. cystophora* starts at 2.3×10^2 W/sr/m^2 but is still linear at the highest stimulus intensities indicating that the photoreceptors are not saturated (Fig. 4a). The dynamic range may therefore be shifted towards higher intensities for this eye. Since the part of the dynamic range included in the stimulus intensity range spans 3 log units (Fig. 5a) it is also likely that the dynamic range of the lower lens eye of *T. cystophora* is broader than the others.

Response speed

Both eyes of both species have slow photoreceptors measured both as the half-width and the time-to-peak of the response (Figs. 4b, c, 5b, c, 6) with the eyes of *T. cystophora* being up to twice as fast as those of *Chiropsalmus sp.* At maximum intensities the time-to-peak was 47 ± 1 ms (mean ± SEM, $n = 10$) and 61 ± 3 ms (mean ± SEM, $n = 8$) for the lower and upper lens eyes of *T. cystophora* respectively (Fig. 4c). The minimum half-width was similar for both eyes, 27 ± 0.7 and 30 ± 1.4 ms (Fig. 4b, c), with flash duration having little or no influence (Fig. 6). In *Chiropsalmus sp.* the minimum time-to-peak was 73 ± 4 ms (mean ± SEM, $n = 12$) for the lower lens eye and 81 ± 3 ms (mean ± SEM, $n = 9$) for the upper lens eye (Fig. 5b). They also had longer half-widths than *T. cystophora*, 55 ± 3.4 ms and 59 ± 3.7 (mean ± SEM, $n = 7$ and 6) for the lower and upper lens eye respectively (Fig. 5b, c). In all cases both parameters are strongly dependent on the intensity of the stimulus, with lower intensities giving a slower and wider response. The time-to-peak and the half-width of the responses seem to be independent of the adaptational state since light adapted (to room light intensities) and dark adapted eyes have similar response dynamics (Fig. 7). Typical room light irradiance has been reported to be 1×10^{17} photons/s/sr/m^2 at 555 nm (Land 1981). In our experiments the room light clearly affects the eyes since the response amplitude is reduced likely due to the decline in intensity contrast. Although 1×10^{17} photons/s/sr/m^2 is about 1 log unit below the dynamic range, it is important to remember that this is a measure of irradiance. The way the eyes are arranged in our set-up (eyes pointing up-wards), the eyes perceive not only irradiant light, but the lamps are included in the visual field as well. This will produce higher light intensities.

Spectral sensitivity

In both species the two types of lens eyes have a similar spectral sensitivity peaking in the blue-green region close to 500 nm (Figs. 4, 5). Comparing the response to a theoretical absorption curves for opsins (Govadovskii et al. 2000) a good match is generally seen. The spectral sensitivity curve of the upper lens eye of *T. cystophora* fits the α-band of a 504 nm opsin ($R^2 = 0.988$, $n = 10$) and the upper lens eye of *Chiropsalmus sp.* a 510 nm opsin ($R^2 = 0.991$, $n = 6$) (Figs. 4d, 5d). The lower lens eye of *T. cystophora* displays a small secondary peak in the near UV range (380 nm) and the best match is to a full opsin (α + β-band) peaking at 512 nm ($R^2 = 0.965$, $n = 15$) (Fig. 4c). The secondary peak could be part of the β-band of the opsin. The spectral sensitivity of the lower lens eye of *Chiropsalmus sp.* matches the α-band of a 497 nm opsin ($R^2 = 0.959$, $n = 10$) but is broadened especially in the deep blue region around 420 nm (Fig. 5c).

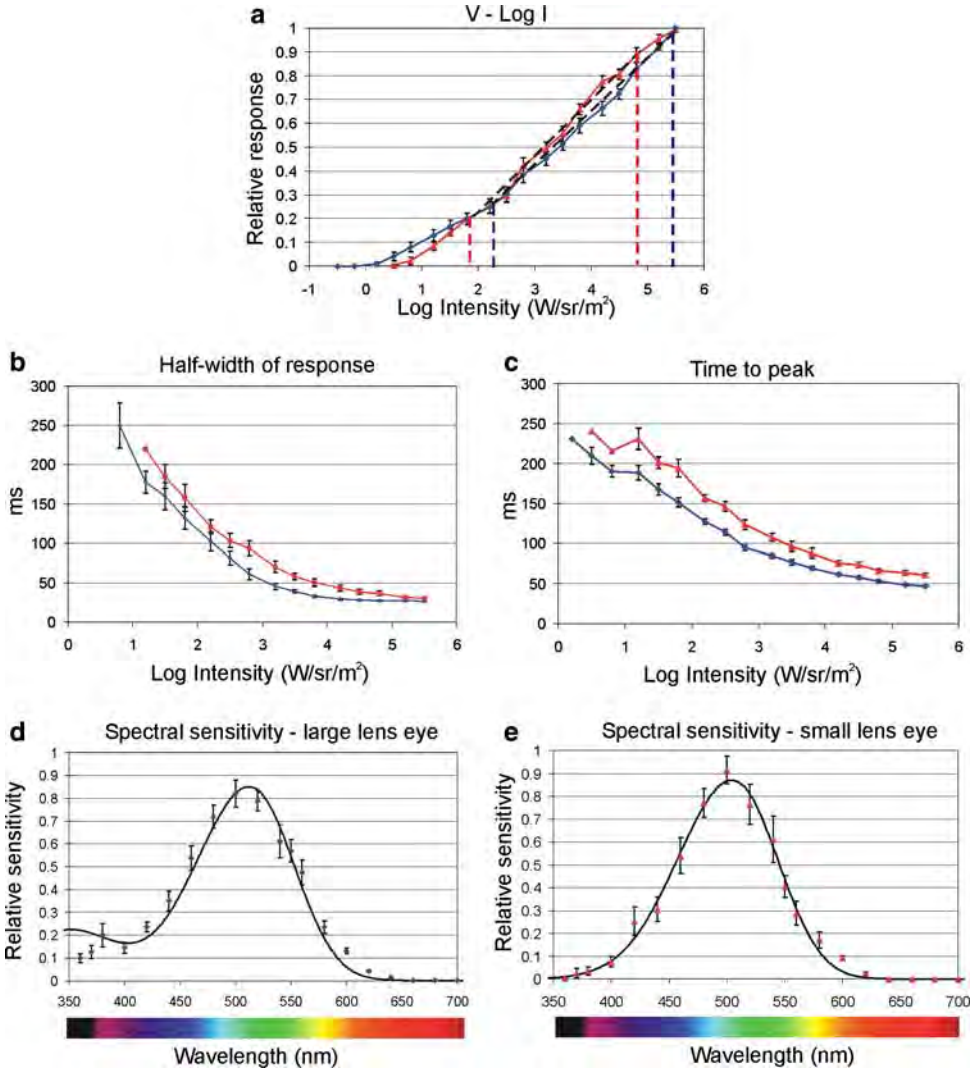

Fig. 4 Response dynamics of the lens eyes of *Tripedalia cystophora*. *Error bars* indicate SEM *Red* upper lens eye, *blue* lower lens eye. **a** The dynamic range of the two eyes determined from the linear part of the V–Log I curves covers about 3 log units ($n = 10$ and 8 for the lower and upper lens eyes, respectively). The upper lens eyes come close to saturation at the highest intensities, as indicated by the flattening V–Log I curve. The lower lens eye did not reach saturation within the stimulus range. **b** From the half width of the response it is seen that both eyes have slow photoreceptors with minimum value about 30 ms ($n = 8$ and 7 for the lower and upper lens eyes, respectively). **c** The time-to-peak shows that both lens eyes have slow phototransduction ($n = 10$ and 8 for the lower and upper lens eyes, respectively). The lower lens eye is the fastest and the minimum time-to-peak was 47 and 61 ms for the lower and the upper lens eye, respectively. **d**, **e** Both lens eyes have their spectral sensitivity peaking in the blue-green area close to 500 nm ($n = 15$ and 8 for the lower and upper lens eyes, respectively). When compared to a theoretical opsin template (*solid lines*) the "least squares fit" for the lower eye is to a 512 nm opsin including the β-peak ($R^2 = 0.965$), whereas the upper lens eyes has the "least square fit" to a 504 nm opsin without the β-peak ($R^2 = 0.988$)

The spectral sensitivity of the lower lens eyes differs from the opsin absorption curve in the UV or blue region. This could be caused by the presence of two or more populations of photoreceptors with different opsins. To examine this possibility selective adaptation experiments were performed on five lower lens eyes of *T. cystophora* using a 530 nm adaptation light and on four lower lens eyes of *Chiropsalmus sp.* using a 580 nm adaptation light (Fig. 8). These experiments will adapt long wavelength receptors but leave short wavelength receptors, such as the possible UV-receptor, with unaltered sensitivity. The spectral sensitivity curves produced by the selective adaptation were very similar to the normal spectral sensitivity curves in *Chiropsalmus sp.* (Fig. 8a). For the lower lens eye of *T. cystophora* the selective adaptation curves displayed much variation (large error bars) due to three out of five of the eyes being completely adapted. Still, it is seen that the peak at 380 nm is likewise affected by the adaptation light and can not be detected (Fig. 8b).

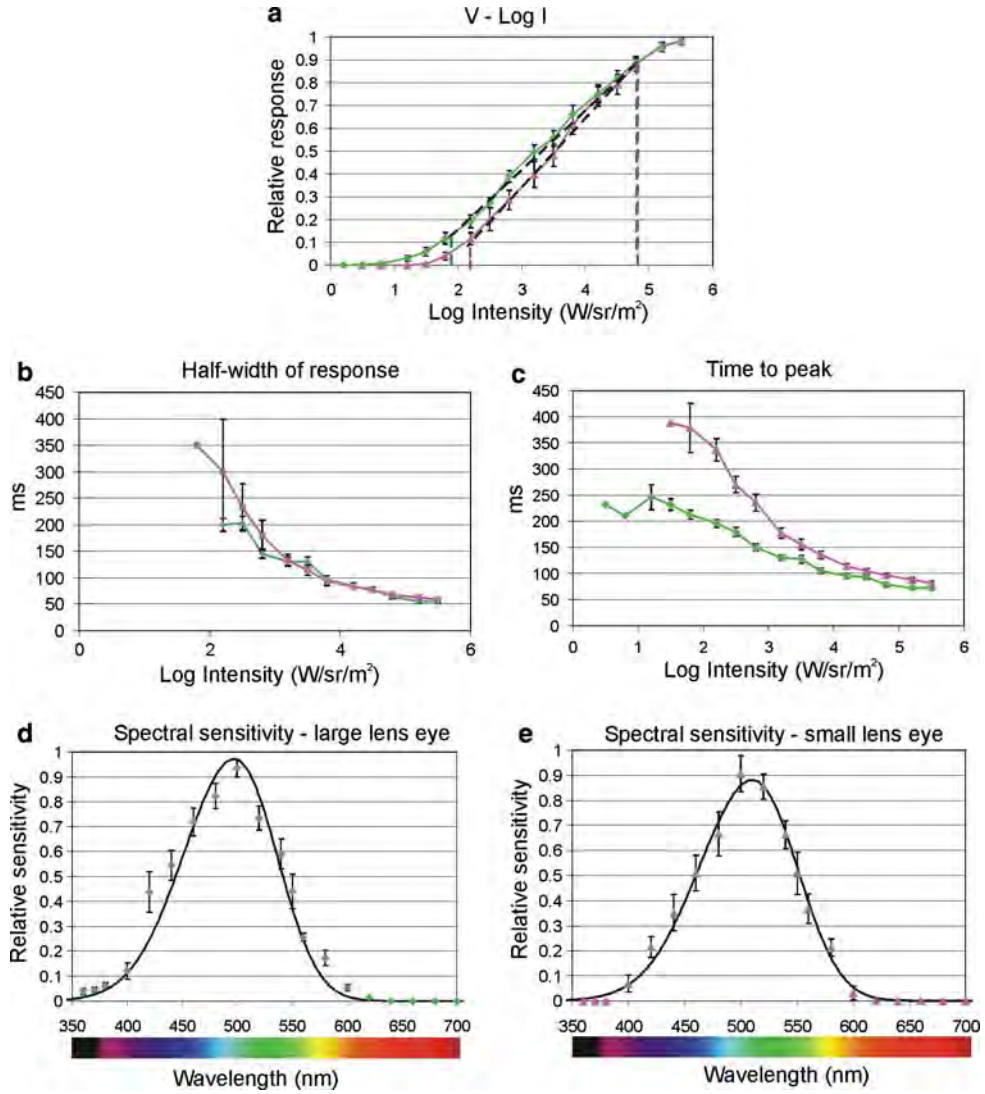

Fig. 5 Response dynamics of the lens eyes of *Chiropsalmus sp.* (*green* lower lens eye, *purple* upper lens eye), error bars indicate S.E.M. **a** The V–log I curves are rather similar even though the lower lens eye has a slightly wider dynamic range spanning about 3 log units ($n = 9$ and 12 for the upper and lower lens eye, respectively). The flattening of the curves at the high intensities indicates that the photoreceptors are close to saturation. **b** From the half width of the response it is seen that *Chiropsalmus sp.* has photoreceptors even slower than *T. cystophora* with minimum values about 55 ms ($n = 7$ and 6 for the lower and upper lens eyes respectively). **c** The two eyes differ in the time-to-peak of the receptor potential ($n = 9$ and 12 for the upper and lower lens eye, respectively). The lower lens eye is faster and has a minimum time-to-peak of 72 ms at maximum intensity (3.2×10^5 W/sr/m^2). At the lower intensities the differences increase and at 200 W/sr/m^2 the lower lens eye is close to twice as fast. **d**, **e** The spectral sensitivity of the two lens eyes is similar and peaks around 500 nm ($n = 6$ and 12 for the upper and lower lens eye, respectively). When compared to a theoretical absorption curve of the α-peak of a 510 nm opsin (*solid lines*) the data from the upper lens eye has a very good fit, $R^2 = 0.991$. The spectral sensitivity of the lower lens eye has the least squares fit to a 497 nm opsin, but with a poorer match, $R^2 = 0.959$, which could be due to the presence of two populations of photoreceptors with different opsins

Discussion

When trying to make functional interpretations of eye physiology it is crucial to keep in mind what visual environments the eyes are used in. The visual fields are important factors, and here special adaptations seem to have evolved within cubomedusae. Each of the eye-bearing rhopalia hangs from the bell in a flexible stalk and holds a large crystal in the distal part (Yamasu and Yoshida 1976). The weight of the crystal makes the stalk bend when the medusa re-orientates and this system ensures that the eyes are always oriented in the same way relative to gravity (unpublished results). The upper lens eye, therefore, looks straight upwards through Snell´s window and into the terrestrial environment. The lower lens eye, on the other hand, looks obliquely downwards and monitors the underwater world. Also, *T. cystophora* and

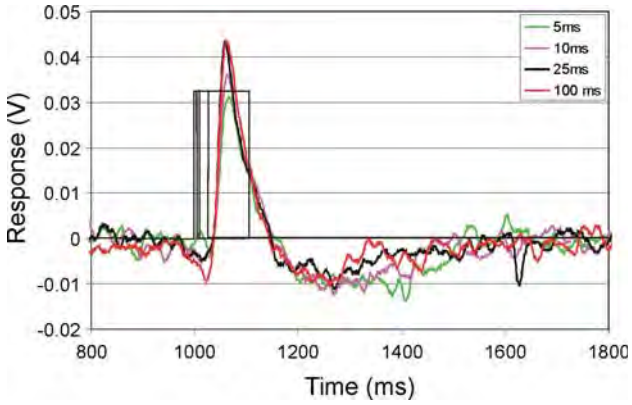

Fig. 6 When stimulating the lens eyes of *Tripedalia cystophora* with high intensity flashes of different durations (5, 10, 25, and 100 ms) it is seen that the responses have a similar half-width of about 30 ms. The longer flashes induces larger amplitudes and slightly shorter times to peak. The *black squares* indicates the stimuli

Chiropsalmus sp. live in very different habitats. The mangroves where *T. cystophora* is found form a visually complex habitat especially under water, whereas the sandy beaches inhabited by *Chiropsalmus sp.* (Gordon and Seymour 2004) have few visual cues. These differences are likely to be reflected in the physiological properties of the eyes.

Dynamic range

We find that the cubomedusan lens eyes have dynamic ranges covering about 3 log units of light intensity. A similar dynamic range is found for the photoreceptors of the hydromedusa *Polyorchis penicillatus* recorded with similar techniques (Weber 1982a). Because we have used extracellular "mass" recordings this range may differ from the dynamic range of a single receptor, since all cells adding to the signal are not stimulated equally. From the pore diameter of the electrode (5–10 μm) we estimate that we are recording from 10 to 15 cells at a time. We believe that this effect is negligible in our experiments though. The light guide we used to stimulate the eyes had a diameter (500 μm) larger than the pupils of both lens eyes, which should ensure a fairly homogeneous stimulation of the retina. Also, at least in *T. cystophora* the photoreceptors have very broad receptive fields (Nilsson et al. 2005), and 10–15 adjacent receptors will have vastly overlapping visual fields ensuring nearly identical stimulation. In

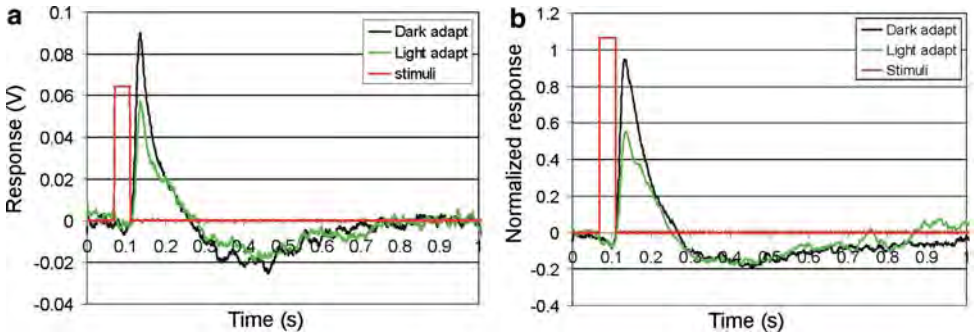

Fig. 7 When stimulated at high intensities light and dark adapted eyes of *Tripedalia cystophora* show impulse responses with very similar dynamics. **a** Examples of recordings from the same eye light or dark adapted. **b** Averaged responses from five eyes. Both the time-to-peak and the half-width are very similar for light and dark adapted eyes

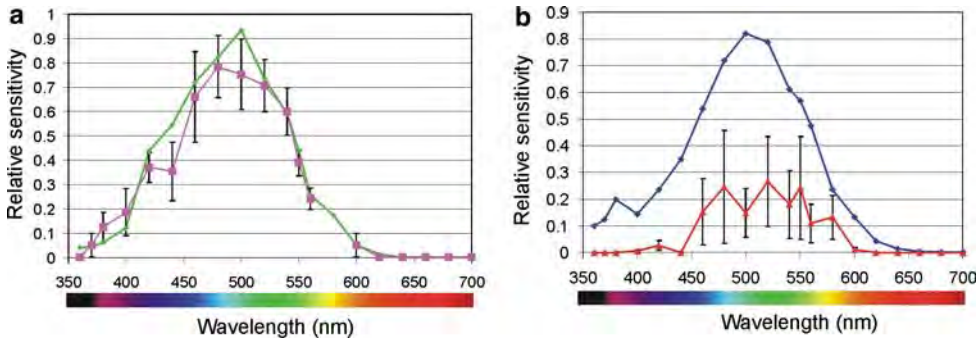

Fig. 8 To check for the presence of more than one receptor population in the lower lens eyes selective adaptation experiments were performed. *Error bars* indicate SEM. **a** In *Chiropsalmus sp.* the spectral sensitivity was tested using a constant adaptation light at 580 nm (*purple line*, $n = 4$). This curve is not significantly different from the normal spectral sensitivity curve (*green line*), which indicates the presence of a single receptor population. **b** In *T. cystophora* the spectral sensitivity was tested using a 530 nm adaptation light (*red line*, $n = 5$). The UV-peak found in the normal spectral sensitivity (*blue line*) is also affected by the adaptation light and is not detectable. The reason for the low relative sensitivity values and large error bars is that in three of the five series the eyes were completely adapted and gave null-responses to all the colored flashes

support of such artifacts having little effect, the typical dynamic ranges obtained from intracellular recordings of single cells of other invertebrates are in the range of 2.5 log units (Laughlin 1981).

T. cystophora and *Chiropsalmus sp.* live in shallow waters in the tropics, and in such environments the mean daylight irradiace is about 1×10^{20} photons/s/sr/m^2 at 555 nm (Land 1981). This fits well with the dynamic range we find for the lens eyes (1×10^{18}–1×10^{21} photons/s/sr/m^2 at 500 nm), since typical natural scenes vary 2–3 log units in intensity. It is interesting and unexpected that the results suggest that the lower lens eye of *T. cystophora* has its dynamic range shifted towards higher intensities. Since this eye observes the darker underwater world it seems more intuitive that it should be more sensitive than the upper lens eye. At present we have no good explanation for why this is so. It should be noted that we have measured the dynamic range of a dark adapted eye. A light adapted eye might have the dynamic range shifted to higher intensities but our results indicate that the adaptational state has little effect on the dynamics of the responses (Fig. 7). The obtained dynamic ranges support our preliminary behavioral observations that the two species of cubomedusae are active at daytime only, since the mean irradiation in even bright moon light (1×10^{14} photons/s/sr/m^2 at 555 nm; Land, 1981) is far below the threshold of the dark adapted photoreceptors. *Chiropsalmus sp.* is found on open beaches and its upper lens eye will therefore look directly up at the sky through Snell's window and receive not only reflected light but also light coming directly from the sun or the moon. This will provide the photoreceptors with much higher light intensities possibly allowing the moon to be detected at night.

Response speed

Both species seem to have eyes with slow photoreceptors indicated by the minimum half-widths of 27 ms for *T. cystophora* and 55 ms for *Chiropsalmus sp.* and minimum time-to-peak of 47 and 71 ms, respectively. This agrees well with results from the eyes of the hydrozoan jellyfish *Polyorchis penicillatus*, where the minimum time-to-peak is 50–60 ms and the half-width is about 70 ms (Weber 1982a). In comparison, slow insects eyes such as dark adapted cockroach or cricket eyes have half-widths of about 25 ms and times to peak of 41 and 43 ms, respectively (Howard et al. 1984). When light adapted, these insects eyes nearly double their speed (Howard et al. 1984), but interestingly we did not find this effect in the jellyfish eyes adapted to room light levels (Fig. 7). The mechanisms behind this lack in dynamics is not known but it might indicate that the cubomedusae apply a narrow band-pass filter to their temporal resolution. The half-width provide the better measure of temporal resolution, since the time-to-peak results from the speed of the phototransduction and does not directly determine the speed of vision, which may be further modified by properties of the nervous system. We may have underestimated the speed of vision in our experiments, since the mass recordings introduce a population artifact, similar to what was described for the dynamic response. Again, we estimate that this has minimal effect, which this is supported by the almost flat time-to-peak curves at the highest stimulus intensities, especially for the lower lens eye of *Chiropsalmus sp.*

Normally, slow vision is an adaptation either for dim light or for high acuity (Warrant et al. 2004; Warrant and Locket 2004), but since the medusae are living under tropical sunlight conditions and have poor spatial resolution (Nilsson et al. 2005) we believe there is another explanation. The slow responses will act as a temporal low-pass filter removing any fast moving objects from the vision of the jellyfish (Coates and Theobald 2004). This would be a parallel to earlier results showing that the spatial performance of the lens eyes of *T. cystophora* is also low-pass filtered (Nilsson et al. 2005). We believe that such filters are crucial elements of the visual system of cubomedusae because the limited central nervous system of the jellyfish is unlikely to be able to handle large amounts of visual information.

It is interesting that the speed of vision clearly differs between the two species, and it may correlate with differences in their habitat and behavior. *Chiropsalmus sp.* lives off sandy beaches where the visual environment is rather homogeneous and their eyes will therefore experience only very slow changes as they move around. *T. cystophora* lives in between mangrove roots where they perform rapid turns (180° in two bell contractions, unpublished results). Their visual environment thus changes faster, which possibly generates the need for less slow receptors.

Spectral sensitivity

Both the upper and lower lens eyes of *T. cystophora* and *Chiropsalmus sp.* have spectral sensitivities that peak in the blue-green region (497–512 nm). The spectral curves of the upper lens eyes closely fit ($R^2 = 0.988$, 0.991) the theoretical opsin template (Govadovskii et al. 2000) and this support that the photopigments are opsins (Coates et al. 2006). This is also suggested by results from the eyes of the hydromedusae *Polyorchis penicillatus* and *Sarsia tubulosa* (Weber 1982a, b). In

contrast, another non-bilaterian photosensitive system found in the parenchymella larvae of demosponges uses flavins or carotenoids as photopigments (Leys et al. 2002).

The present results strongly support our earlier results (Coates et al. 2006) that the upper lens eyes have only a single photopigment and here we find no sign of a long wavelength shoulder (Coates et al. 2006). The spectral sensitivity curves of the lower lens eyes on the other hand deviate somewhat from the opsin template. In both species the peak is too broad, especially in *Chiropsalmus sp.*, and additionally in *T. cystophora* there are indications of a small peak in the near UV-range not directly matching a β-peak. In both cases this could be due to the presence of additional photoreceptor populations. To test this we performed a series of selective adaptation experiments on the lower lens eyes. The spectral sensitivity curves from these experiments either had the same shape as those from non-adapted eyes (*Chiropsalmus sp.*, Fig. 8a) or lacked the near-UV peak (*T. cystophora*, Fig. 8b). This suggests that the recorded signals originate from a single population of photoreceptors and that the lower lens eyes of both species are therefore also likely to be color-blind.

Self-screening in the 35–50 μm long outersegments (Yamasu and Yoshida 1976; Laska and Hündgen 1982; Nilsson et al. 2005) probably explains some of the broadening of the curves, but since we do not know the absorption coefficient we cannot estimate the magnitude of this effect. In a recent paper we have shown that when assuming a typical invertebrate absorbance coefficient ($k = 0.0067\ \mu m^{-1}$) self screening has very little effect on the spectral curve (Coates et al. 2006). The β-peak in the opsin spectral sensitivity is completely or partly lacking in the cubozoan lens eyes. We believe that this due to UV-filtering in the eyes to prevent UV-damage to the eyes. Such a filter could be situated in the lenses.

We have shown that the dynamic ranges of both lens eyes of cubomedusae fits their day active lifestyle and that these eyes are slow and probably have color-blind vision, which agrees well with their relatively sparse central nervous system (Passano 1982; Mackie 2004; Garm et al. 2006; Skogh et al. 2006). It has been suggested that color vision first evolved in animals inhabiting shallow water to improve contrast (Maximov 2000). Lens properties of surface ripples make intensity contrast in such areas vary greatly over time and therefore color contrast provides a much more stable picture. It is in such habitats that all known species of cubomedusae are found including the two species examined here. Apparently, the visual tasks that the cubomedusae perform in these habitats do not depend on such contrast enhancement. The low spatial resolution and low temporal resolution of the lens eyes may offer an alternative way to reduce intensity flicker caused by the surface ripples.

Acknowledgments We would like to thank Peter Ekström, Megan O'Connor, and Linda Parkefelt, Lund University, for commenting on the manuscript. Dan Nilsson acknowledges the grant nr 621–2002-4873 from the Swedish Research Council, Anders Garm likewise acknowledges grant nr 21-04-0423 from the Danish Research Council and Melissa Coates acknowledges the NSF pre-doctoral fellowship program. All the experiments comply with the "Principles of animal care", publication No. 86-23, revised 1985 of the National Institute of Health, and also with the current laws of Puerto Rico.

References

Berger EW (1898) The histological structure of the eyes of Cubomedusae. J Comp Neurol 8(3):223–230

Buskey EJ (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 Institute Universität Wien 1(2):1–56

Coates MM (2003) Visual ecology and functional morphology of Cubozoa (Cnidaria). Integra Comp Biol 43:542–548

Coates MM, Garm A, Theobald JC, Thompson SH, Nilsson DE (2006) The spectral sensitivity in the lens eyes of a box jellyfish, *Tripedalia cystophora*. J Exp Biol 209:3758–3765

Coates MM, Theobald JC (2004) Optimal visual parameters for a cubozoan jellyfish in the mangrove environment. In: Society for Integrative and Comparative Biology Annual Meeting Abstracts. New Orleans. pp 316

Derby CD (1995) Single unit electrophysiological recordings from crustacean chemoreceptor neurons. In: Spielman AI, Brand JG (eds) Experimental cell biology of taste and olfaction. Current techniques and protocols. CRC, New York pp 241–250

Garm A, Ekström P, Boudes M, Nilsson DE (2006) Rhopalia are integrated parts of the central nervous system in box jellyfish. Cell Tissue Res 325:333–343

Gordon MC, Seymour J (2004) Growth and age determination of the Tropical Australian Cubozoan *Chiropsalmus Sp.* Hydrobiologia 530/31:339–345

Govadovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K (2000) In search of the visual pigment template. Visual Neurosci 17:509–528

Hamner WM, Jones MS, Hamner PP (1995) Swimming, feeding, circulation and vision in the australian box jellyfish, *Chironex fleckeri* (Cnidaria, Cubozoa). Mar Freshw Res 46:985–990

Hartwick RF (1991) Observations on the anatomy, behaviour, reproduction and life cycle of the cubozoan *Carybdea sivickisi*. Hydrobiologia 216/217:171–179

Hertwig O, Hertwig R (1878) Das Nervensystem und die Sinnesorgane der Medusen. Monographisch Dargestellt Universität Jena

Howard J, Dubs A, Payne R (1984) The dynamics of phototransduction in insects. J Comp Physiol A 154:707–718

Kinsey B (1986) Barnes on box jellyfish. James Cook University, Townsville, pp 1–95

Land MF (1981) Optics and vision in invertebrates. In: Autrum H (ed) Comparative physiology and evolution of vision in invertebrates, Vol VII/6B. Springer, Berlin, pp 472–592

Larson RJ (1976) Cubomedusae: feeding, functional morphology, behaviour, and phylogenetic position. In: Mackie GO (eds) Coelanterate ecology and behaviour. Plenum, New York pp 237–245

Laska G, Hündgen M (1982) Morphologie und Ultrastruktur der Lichtsinnesorgane von *Tripedalia cystophora* Conant (Cnidaria, Cubozoa). Zool J Anat 108:107–123

Laughlin SB (1981) Neural principles in the peripheral visual systems of invertebrates. In: Autrum H (ed) Comparative physiology and evolution of vision in invertebrates, Vol VII/6B. Springer, Berlin, pp 135–280

Lewis C, Long TAF (2005) Courtship and reproduction in *Carybdea sivickisi* (Cnidaria: Cubozoa). Mar Biol 147:477–483

Leys SP, Cronin TW, Degnan BM, Marshall JN (2002) Spectral sensitivity in a sponge larva. J Comp Physiol A 188:199–202

Mackie GO (2004) Central neural circuitry in the jellyfish *Aglantha*: a model "simple nervous system". Neuro-Signals 13:5–19

Martin VJ (2002) Photoreceptors of cnidarians. Can J Zool 80:1703–1722

Martin VJ (2004) Photoreceptors of cubozoan jellyfish. Hydrobiologia 530/531:135–144

Matsumoto GI (1995) Observations on the anatomy and behaviour of the cubozoan *Carybdea rastonii* Haacke. Mar Freshw Behav Physiol 26:139–148

Maximov VV (2000) Environmental factors which may have led to the appearance of colour vision. Philos Trans R Soc Lond B Biol Ser 355:1239–1242

Nilsson DE, Coates MM, Gislén l, Skogh C, Garm A (2005) Advanced optics in a jellyfish eye. Nature 435:201–205

Passano LM (1982) Scyphozoa and Cubozoa. In: Shelton GAB (eds) Electrical conduction and behaviour in "simple" invertebrates. Clarendon, Oxford pp 149–202

Pearse JS, Pearse VB (1978) Vision in cubomedusan jellyfish. Science 199:458–458

Raffaele P (2005) Killers in paradise. Smithsonian, pp 80–87

Shorten MO, Devenport J, Seymour J, Cross MC, Carrette TJ, Woodward G, Cross TF (2005) Kinematic analysis of swimming in Australian box jellyfish—*Chiropsalmus* sp. and *Chironex fleckeri* (Cubozoa, Cnidaria, Chirodropidae). J Zool (Lond) 267(4):371–380

Singla CL (1974) Ocelli of hydromedusae. Cell Tissue Res 149:413–429

Singla CL, Weber C (1982) Fine structure of the ocellus of *Sarsia tubulosa* (Hydrozoa, Anthomedusae). Zoomorphology 100:11–22

Skogh C, Garm A, Nilsson DE, Ekström P (2006) The bilateral symmetric rhopalial nervous system of box jellyfish. J Morphol 267:1391–1405

Stewart SE (1996) Field behavior of *Tripedalia cystephora* (class Cubozoa). Mar Freshw Behav Physiol 27(2–3):175–188

Takasu N, Yoshida M (1984) Freeze-fracture and histofluoresence studies on photoreceptive membranes of medusan ocelli. Zool Sci (Tokyo) 1:367–374

Toh Y, Yoshida M, Tateda H (1979) Fine structure of the ocellus of the hydromedusan, *Spirocodon saltatrix*. I. Receptor cells. J Ultrastruc Res 68:341–352

Warrant EJ, Kelber A, Gislén A, Greiner B, Ribi W, Wcislo WT (2004) Nocturnal vision and landmark orientation in a tropical halictid bee. Curr Biol 14(15):1309–1318

Warrant EJ, Locket AN (2004) Vision in the deep sea. Biol Rev 79:671–712

Weber C (1982a) Electrical activities of a type of electroretinogram recorded from the ocellus of a jellyfish, *Polyorchis penicillatus* (Hydromedusae). J Exp Zool 223:231–243

Weber C (1982b) Electrical activity in response to light of the ocellus of the hydromedusan, *Sarsia tubulosa*. Biol Bull 162:413–422

Yamamoto M, Yoshida M (1980) Fine structure of ocelli of an anthomedusan, *Nemopsis dofleini*, with special reference to synaptic organization. Zoomorphology 96:169–181

Yamasu T, Yoshida M (1973) Electron microscopy on the photoreceptors of an anthomedusa and a scyphomedusa. Pub Seto Mar Biol Lab 20:757–778

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