The Journal of Experimental Biology 212, 3951-3960
Published by The Company of Biologists 2009
doi:10.1242/jeb.031559

# Multiple photoreceptor systems control the swim pacemaker activity in box jellyfish

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Accepted 9 September 2009

## SUMMARY

**Like all other cnidarian medusae, box jellyfish propel themselves through the water by contracting their bell-shaped body in discrete swim pulses. These pulses are controlled by a swim pacemaker system situated in their sensory structures, the rhopalia. Each medusa has four rhopalia each with a similar set of six eyes of four morphologically different types. We have examined how each of the four eye types influences the swim pacemaker. Multiple photoreceptor systems, three of the four eye types, plus the rhopalial neuropil, affect the swim pacemaker. The lower lens eye inhibits the pacemaker when stimulated and provokes a strong increase in the pacemaker frequency upon light-off. The upper lens eye, the pit eyes and the rhopalial neuropil all have close to the opposite effect. When these responses are compared with all-eye stimulations it is seen that some advanced integration must take place.**

Key words: cubomedusae, eyes, pacemaker, swim pulse, *Tripedalia*.

## INTRODUCTION

The presence of a central nervous system (CNS) and the level of neuronal processing in cnidarians have long been matters of dispute. In most zoological textbooks their nervous system is presented as a simple nerve net without condensations, implying only little processing (e.g. Lesh-Laurie and Suchy, 1991). Still, during the last decades Mackie and coworkers have convincingly shown that hydromedusae have a complex nervous system with a CNS performing several types of processing and integration (Mackie, 1971; Passano, 1976; Mackie and Meech, 1995a; Mackie and Meech, 1995b; Mackie and Meech, 2000; Mackie et al., 2003; Mackie, 2004). The main part of this CNS is a double ring nerve that encircles the bell-shaped body and it is here that several subsystems can be distinguished by their physiology and neurotransmitter profiles (Mackie, 2004). From our earlier neuroanatomical studies we have shown that another cnidarian group, the cubozoans (or box jellyfish) also have condensations in their nervous system, which probably qualify as a CNS (Garm et al., 2006; Skogh et al., 2006; Garm et al., 2007b). The CNS of box jellyfish differs from that of hydrozoans because there is only a single ring nerve and an additional part in each of their four sensory clubs, the rhopalia. This rhopalial nervous system is directly connected to the ring nerve making the box jellyfish CNS one coherent system (Garm et al., 2006). Still, so far it has not been shown what kind of processing and integration takes place in the box jellyfish CNS.

One way to evaluate to what level processing takes place in the CNS is by looking at the complexity of the sensory input and behavioural output. Here box jellyfish stands out in Cnidaria with their advanced visual system, comprising of 24 eyes of four morphologically distinct types (Berger, 1898; Yamasu and Yoshida, 1976). Further, two of the eye types are structurally similar to camera-type eyes of vertebrates (Laska and Hündgen, 1982; Nilsson et al., 2005). The rhopalial nervous system lies in direct connection with these eyes, and subsystems seem to interconnect the different types of eyes and the two sides of the bilateral symmetric rhopalium (Parkefelt et al., 2005; Skogh et al., 2006; Parkefelt and Ekström, 2009). What exact information the eyes register and how it is being processed by the rhopalial nervous system is largely unknown though.

Concerning box jellyfish behaviour, more and more evidence points towards the presence of an elaborate repertoire. It has been shown that at least some species have internal fertilisation of their eggs, which includes a proper mating behaviour (Werner, 1973; Lewis and Long, 2005). Another well-documented cubozoan behaviour is obstacle avoidance (Hamner et al., 1995; Matsumoto, 1995) and it has been shown that this behaviour is visually guided and involves true spatial vision (Garm et al., 2007a). A major part of these behaviours is the swim speed of the medusa, which is largely controlled by the rate of bell contractions. The bell contractions are in turn controlled by a central pattern generator situated in the rhopalial nervous system (Yatsu, 1917; Satterlie, 1979). This swim pacemaker system has a one-to-one relationship with the swim pulses (Satterlie, 1979) and is influenced by the visual input (Garm and Bielecki, 2008). Under constant light intensities the pacemaker frequency stays constant but if the rhopalium experiences a sudden increase or decrease in light intensity it has great impact on the pacemaker. A decrease in intensity induces a so-called shadow response with a steep increase in pulse frequency for a limited period of time (Garm and Bielecki, 2008), a behaviour also known from hydromedusae (Yoshida and Ohtsu, 1973; Arkett, 1985; Arkett and Spencer, 1986a; Arkett and Spencer, 1986b). An increase in the light intensity inhibits the pacemaker making the jellyfish sink and this is an important part in optimising the feeding behaviour for *Tripedalia cystophora* (Buskey, 2003; Garm and Bielecki, 2008).

Because the pacemaker system is involved in several of the box jellyfish behaviours it seems to be the ideal system to investigate how the visual input influences these behaviours. Among other things, it offers the possibility to examine if parts of the rhopalial nervous system integrate information from several eye types or if the modulations of the pacemaker are governed by a single eye type only. In the present study we examine the visual control of the swim pacemaker of the Caribbean box jellyfish *T. cystophora*. We record

the pacemaker signals while stimulating one eye at a time with white light of a range of different intensities. The results demonstrate that stimulating the upper lens eye (ULE), the pit eyes (PE) or the neuropil (NP) has similar effects whereas stimulation of the lower lens eye (LLE) has the opposite effect.

## MATERIALS AND METHODS

### Animals

Adult medusae (7–9 mm in bell diameter) of *Tripedalia cystophora* Conant 1897 were obtained from our cultures at the University of Copenhagen, Denmark. In the cultures the medusae are kept in a 200l tank with circulating seawater at 25‰ and about 28°C and fed SELCO (INVE Technologies, Dendermonde, Belgium)-enriched *Artemia* daily. They reach adult size in 2–3 months.

### 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 seawater. The seawater was kept at a temperature of 28±0.5 deg. using a Peltier element. In the Petri dish the rhopalium was held by a micropipette at the area of the crystal, which allowed for orienting the rhopalium such that there was access to all four eye types. Under a dissection microscope a glass suction electrode [for electrode details, see Derby (Derby, 1995)] was applied to the cut surface of the rhopalial stalk in the area of the epidermal nerve. The suction electrode was moved around until the regular activity pattern of the pacemaker was seen where after the rhopalium was left to dark adapt for 3 min.

A Linos microbench system was used for light stimulation. Light from an ultra bright white LED (Luxeon III star, Philips, San Jose, CA, USA) was focused into a quartz light guide, 50 µm in diameter. The light guide was arranged in the Petri dish such that the light shone directly into either one of the PE, the ULE, the LLE or one of the slit eyes (SE). Due to the small diameter of the light guide, direct light could be limited to the eye type of interest only. The maximum intensity was $1.1\times10^{5}\,W\,sr^{-1}\,m^{-2}$ when integrated between 350 and 750 nm and measured at the tip of the light guide (ILT900W spectroradiometer, International Lights Technologies, Peabody, MA, USA). The LED was controlled *via* a NI6229 A/D converter (National Instruments, Austin, TX, USA) and a custom made program for LabView 8.5 (National Instruments).

The electrophysiological experiments tested the response to sudden changes in light intensities covering a range of approximately 1.1 log units (from $8.7\times10^{3}\,W\,sr^{-1}\,m^{-2}$ to $1.1\times10^{5}\,W\,sr^{-1}\,m^{-2}$) in five steps. To ensure maximum health of the preparation, only one or two rhopalia were used from each medusa and only one eye from each rhopalium. The protocol for each eye contained five consecutive recordings and only data from preparations that lasted a full protocol were used. Each recording started with 1 min of darkness ($<1\times10^{-3}\,W\,sr^{-1}\,m^{-2}$) followed by 3 min of light and then 3 min of darkness, giving the protocol a total duration of 35 min. Due to some indications of long-term adaptations half of the trials were started from the low intensity end and the other half from the high intensity end. All four eye types were tested ($N$=10 for each eye type). Control recordings

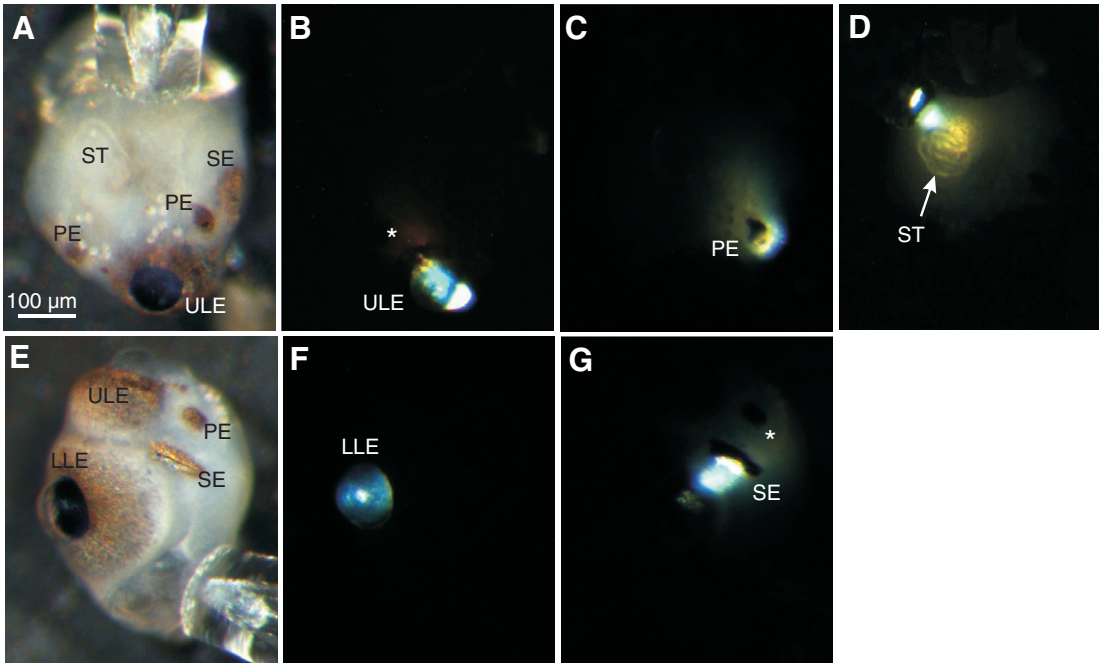


Fig. 1. Stimulus accuracy. The accuracy of the light stimulus varied with the different eyes. When working with the ULE, PE or NP the rhopalium was held by a micropipette at the back of the crystal (A) but in the case of the LLE and SE the micropipette was attached to the side of the crystal (E). When the 50 µm light guide was aimed at the ULE it is seen that here the pigment screen is not light proof and the NP is therefore also illuminated (B, asterisk). Illumination of the PE also allows light to reach the NP, because of its incomplete pigment screen and small diameter (C). When stimulating the NP at the base of the stalk (arrow) light scattered throughout most of the NP (D). The pigment screen of the LLE is completely light proof and stimulating this eye leaves the rest of the rhopalium in complete darkness (F). As for the PE, the small size and incomplete pigment screen of the SE causes the neuropil and the back side of the PE (G, asterisk) to be illuminated. LLE, lower lens eye; PE, pit eye; NP, neuropil; SE, slit eye; ST, stalk; ULE, upper lens eye. Scale bar in A applies to all subfigures.

were done (i) with the light guide aimed at the neuropil from above besides the stalk base (no eye was stimulated directly), which resulted in light scattered throughout most of the NP (Fig. 1D) and (ii) in 35 min of darkness. The neuropil was chosen as a control because leakiness of the PE and the ULE and the small size of the SE and PE result in illumination of the neuropil when working with these eyes (Fig. 1). For organisation of the NP see Skogh et al. (Skogh et al., 2006). A possible concern is heating of the preparation during the 3 min of stimulation but because we used a LED and light guide system heat transmission is minimal. Further, heating caused by photon absorption should be less than what they will experience under natural conditions where they are exposed to close to full sunlight (Stewart, 1996).

The recorded signals were amplified 1000 times (1700 differential AC amplifier from A-M systems, Carlsborg, WA, USA) and filtered through high- and low-pass filters in the amplifier (0.1 and 1000 Hz, respectively). The amplifiers 50 Hz notch filter was also used. All recordings lasted 7 min and were stored and analysed on a laptop using the NI6229 A/D converter and the program Igor Pro 6.03A (WaveMetrcs Inc., Lake Oswego, OR, USA) with a NeuroMatic add-on.

## RESULTS

### Characteristics of the pacemaker signal

When recording from the rhopalial stalk the pacemaker signal is not represented by discrete action potentials but rather by complex signals of long duration as described earlier (Garm and Bielecki, 2008). This is evident from the highly variable amplitude seen in Fig. 2A. In constant darkness the pacemaker has a mean frequency close to 1 Hz when measured over minutes. This frequency is rather variable and at times the activity occurs in bursts (Fig. 2B).

### Dark control

The pacemaker activity was recorded in darkness for 35 min in 7 min slots during control experiments. As said above the activity pattern changed and at times it was highly regular (Fig. 2A) but at other times the pacemaker would fire in bursts (Fig. 2B). Still, when the mean frequency was taken from 10 rhopalia and measured in 10 s

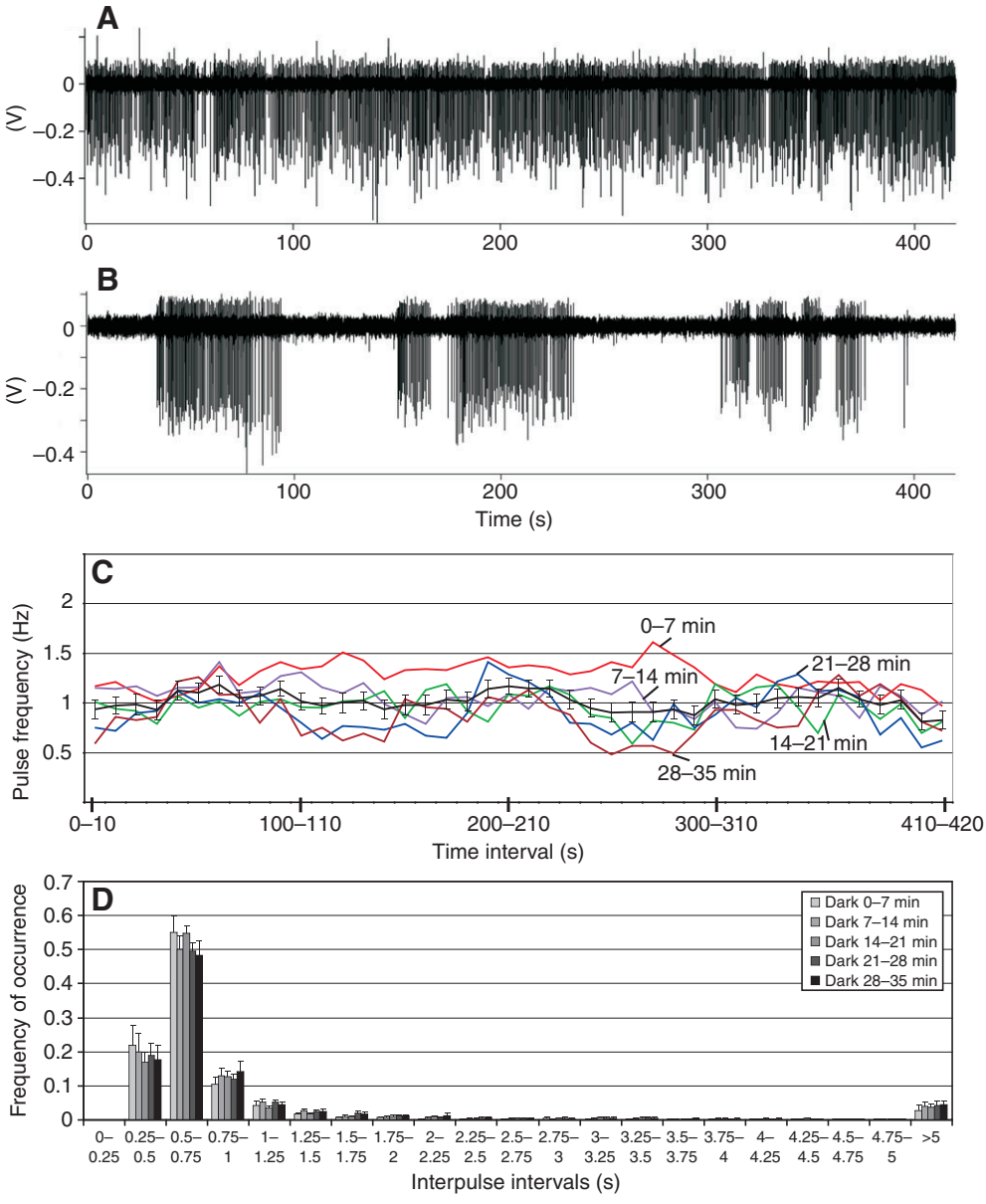


Fig. 2. Dark reference. The pacemaker activity was recorded in darkness ($<1\times10^{-3}$ W sr$^{-1}$ m$^{-2}$) for 35 min (=total time of the experimental protocol) in five slots of 7 min. Under these conditions the activity varied. In some cases the pacemaker showed a regular activity pattern (A) but in other cases strong bursting was seen (B). When the frequency is measured in 10 s intervals it is seen that the frequency tends to be highest, and most stable, in the first 7 min (C). The mean frequency is close to 1 Hz, the black line indicates the means ± s.e.m. When the stability is examined using the inter-pulse interval no significant differences were found between the five different time slots (D).

intervals the frequency stayed between 0.5 and 1.6 Hz (Fig. 2C). When the five slots of 7 min are compared it is seen that there is a tendency for the pacemaker to be most active during the first 7 min. The mean frequency during the first 7 min was approximately 1.25 Hz whereas during the last 7 min frequency was approximately 0.9 Hz but this difference was not significant [one-way analysis of variance (ANOVA), $F_{4,20}$=2.703, $P$>0.05]. The mean of the five 7 min slots is therefore used for comparison with the frequencies obtained under the different experiments conditions (Fig. 2C).

To examine if the regularity of the pacemaker activity is influenced by the time spent in darkness the inter-pulse intervals were measured in bins of 250 ms and compared between the five 7 min slots (Fig. 2D). This showed that the distribution of inter-pulse intervals does not differ between the different time periods spent in darkness.

### The lower lens eye

When the LLE was stimulated by the light guide it had major effect on the pacemaker activity (Fig. 3). The light-on had a strong and close to immediate inhibitory effect on the pacemaker (Fig. 3B). In the most extreme cases the pacemaker became almost silent during the 3 min of light stimulation (Fig. 3A). For all intensities the frequency during light was significantly lower than the dark reference (one-way ANOVA, $F_{5,102}$=206, $P$<0.0001, Tukey HSD *post hoc* $P$<0.001). The light-off response was the opposite and had an immediate and strong stimulatory effect on the pacemaker (Fig. 3C). This off-response not only restored the pacemaker frequency to the initial level but transiently overshot it (Fig. 3D, two tailed *t*-test, $P$<0.0024). In the strongest responses the frequency reached 2.4 Hz in the first 10 s after light-off. The light-off response lasted only for 10 s where after the frequency dropped below the dark reference (Fig. 3D). This drop was long lasting and the pacemaker frequency did not return to the level of the dark reference until about 2 min after light-off.

### The upper lens eye

Stimulation of the ULE had the opposite effect on the pacemaker as when stimulating the LLE. At light-on a fast and strong increase in the pacemaker frequency was seen (Fig. 4A,B). The initial effect typically exceeded 1.8 Hz but was brief and lasted for 10 s only. Over the next approximately 30 s the frequency declined to about 1.5 Hz, where it remained until light-off (Fig. 4D). For all intensities the frequency during light was significantly higher than the dark reference (one-way ANOVA, $F_{5,102}$=29.2, $P$<0.0001, Tukey HSD *post hoc* $P$<0.0001). During the light period the pacemaker activity is less variable, which is seen by the, in general, smaller standard error (Fig. 4D). At light-off the pacemaker activity immediately falls back to the level of the dark reference (Fig. 4C,D, one-way ANOVA, $F_{5,30}$=4.8, $P$<0.0024, Tukey HSD *post hoc* 0.15<$P$<1).

### The pit eye and the neuropil

Similar effects were seen in the pacemaker activity when stimulating either one of the PE (Fig. 5) or the NP at the base of the stalk (Fig. 6) although with slightly different magnitudes. These effects were again similar to what was seen for the ULE (compare Fig. 4 and Fig. 5). At light-on an increase in the pacemaker frequency was obtained but this did not peak until 10–20 s after light-on. A maximum of 1.9–2.1 Hz was typically seen for the NP

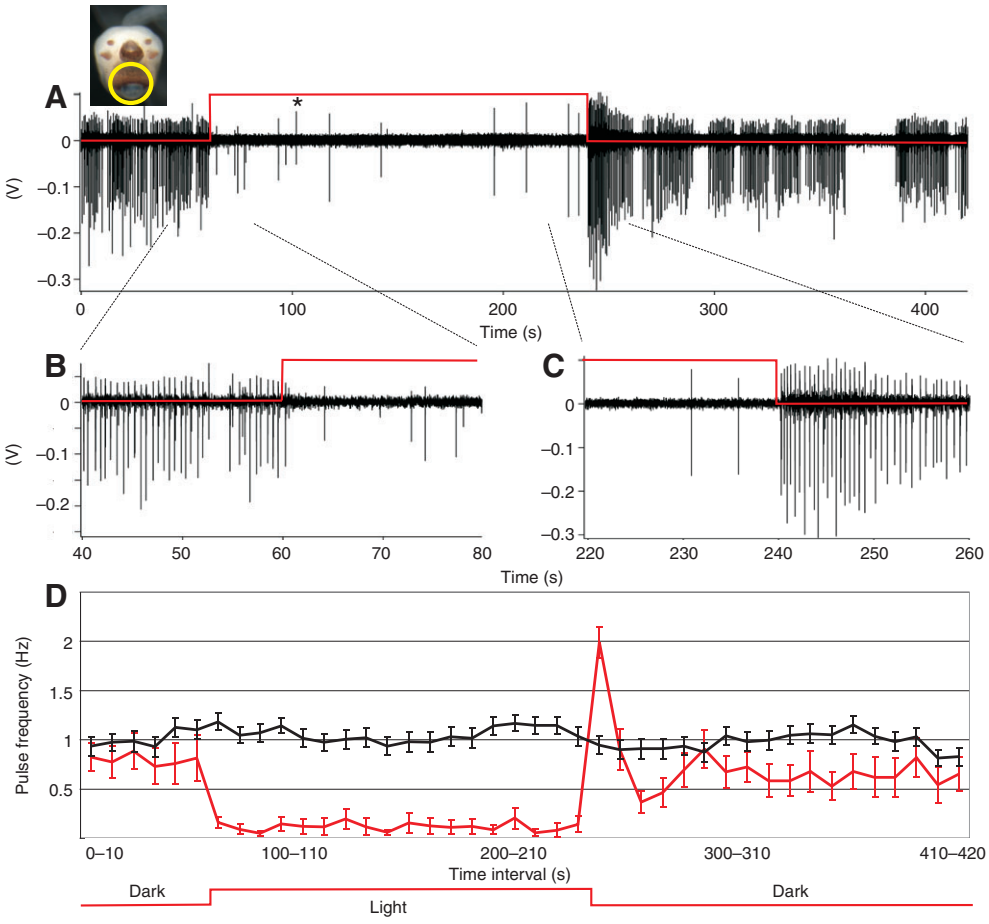


Fig. 3. Pacemaker activity when stimulating the lower lens eye (LLE). The pacemaker frequency is strongly influenced by stimulation of the LLE alone. Light-on causes a sudden decrease in the frequency and, in the most extreme cases, an almost complete stop of the pacemaker (A,B). Light-off, by contrast, causes an increase in the frequency (A,C). In both cases the response is almost immediate (B,C). The light-on response is long lasting whereas the light-off is transient and lasts for about 10·s (D). After the transient light-off response the frequency again drops below the level of the dark reference (black line in D). Note the very small error bars during light-on. The red line in A, B, C and under D indicates the stimulus pattern. The black line in D is the mean of the dark recordings and the error bars indicate ± s.e.m. The asterisk in A indicates activity of other non-pacemaker cells. Light intensity=$1.1\times10^{5}$ W sr$^{-1}$ m$^{-2}$ in A–C and $8.7\times10^{3}$ W sr$^{-1}$ m$^{-2}$ in D.

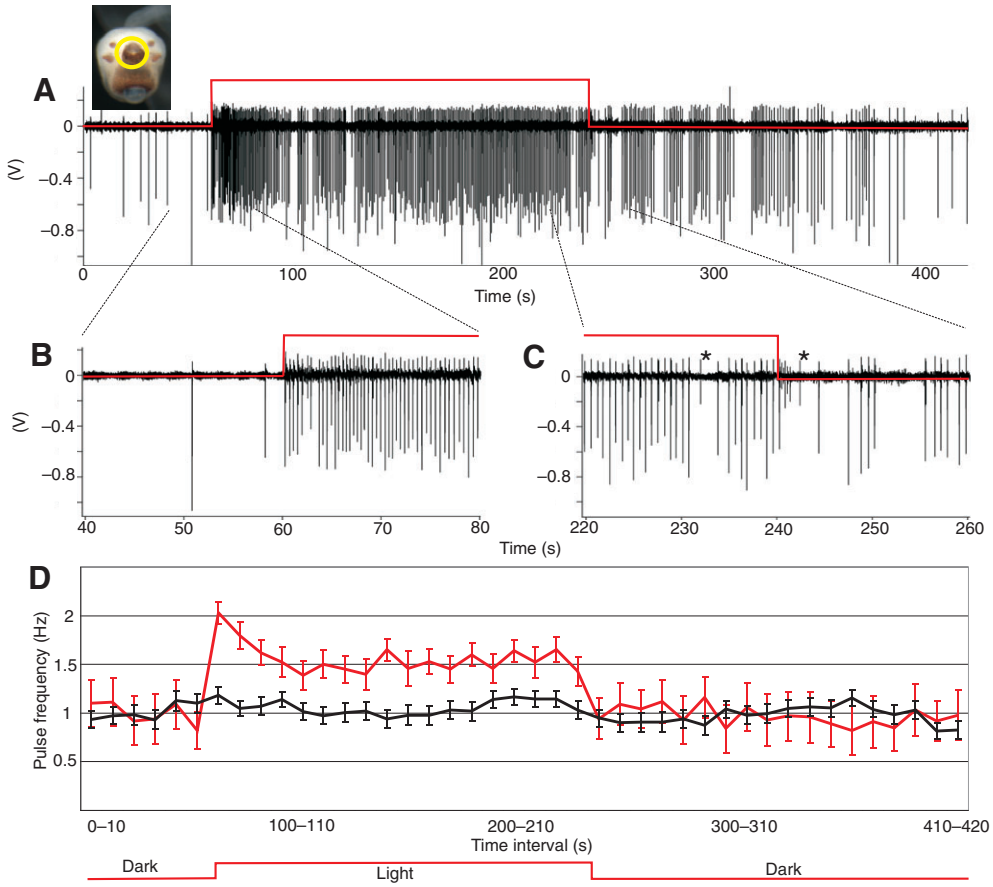


Fig. 4. Pacemaker activity when stimulating the upper lens eye (ULE). Stimulating the ULE causes effects on the pacemaker that are opposite to what was seen when stimulating the lower lens eye (LLE) (A and Fig. 2). At light-on the pacemaker frequency increases (A,B,D). This increase is immediate and culminates 0–10 s after the onset of the stimulus (2.1 Hz at $7.1\times10^4$ W sr$^{-1}$ m$^{-2}$ as shown in D) where after it stabilises at about 1.5 Hz for 2 min until light-off. At light-off the frequency falls back to the level of the dark reference (D). The red line in A, B, C and under D indicates the stimulus pattern. The asterisks in C indicate activity of other non-pacemaker cells. The black line in D is the mean of the dark recordings and the error bars indicate ± s.e.m. Light intensity=$7.1\times10^4$ W sr$^{-1}$ m$^{-2}$ for all subfigures.

and 1.7–1.9 Hz for the PE but the difference was not significant (two tailed *t*-test, $P$=0.17). As for the ULE the pacemaker frequency would drop to 1.4–1.6 Hz during the rest of the stimulus period. For all intensities the frequency during light was significantly higher than the dark reference (one-way ANOVA, $F_{5,102}$=39.9, $P$<0.0001, Tukey HSD *post hoc* $P$<0.0001). At light-off, the frequency decreased to about 0.3–0.6 Hz in the case of the PE and stayed lower than the dark reference during the 3 min of darkness (Fig. 5D, one-way ANOVA, $F_{5,102}$=52.4, $P$<0.0001, Tukey HSD *post hoc* $P$<0.0001). For the NP the light-off response takes the pacemaker to 0.5–0.9 Hz (Fig. 6A,C,D). For both the NP and PE the light-off caused some long term effects in the pacemaker frequency, which resulted in low pre-stimulus frequencies (Figs 5 and 6, one-way ANOVA, $F_{5,30}$=12.1, $P$<0.0001, Tukey HSD *post hoc* 0.0001<$P$<0.029).

### The slit eyes

The pacemaker response when stimulating the SE was more variable than when performing the other stimulations. Still, the average effect resembled that of stimulating the ULE, PE or NP (Fig. 7). Light-on in general caused an increase in the pacemaker frequency, normally peaking in the first 10 s (Fig. 7A,B,E). For all intensities the frequency during light was significantly higher than the dark reference (one-way ANOVA, $F_{5,102}$=28.3, $P$<0.0001, Tukey HSD *post hoc* $P$<0.0001). Light-off caused the frequency to fall back to about the level of the dark control (Fig. 7A,C,E). But in some of the trials a distinct off-response was missing (Fig. 7D). When compared with the ULE, PE or NP the magnitude of the pacemaker response was, in general, smaller when working with the SE (Fig. 8).

The pacemaker activity never exceeded 1.7 Hz and after the initial light-on response the frequency would typically settle around 1.3 Hz until light-off.

### Effects of the light intensity

All of the five different types of stimulations (four different eye types and NP) were tested with five different light intensities covering about 1.1 log units (Fig. 8). When stimulating the ULE, PE or SE no significant correlation was found between light intensity and magnitude of the light-on response, when measured as the mean frequency during the first 20 s after the stimulus onset (Fig. 8B,C,E, linear regression, $R^2$=0.07, 0.76 and 0.68, respectively). The light-on responses from these areas therefore seem to be all-or-nothing responses at least within the examined intensity range. Here it should be noted that saturation might have occurred and it could have been advantageous to have used a broader range of intensities to investigate this. A typical eye has a dynamic range of 2–2.5 log units when not taking adaptations into account. When working with the NP or the LLE there was a significant change with changing intensity (Fig. 8A,D). The higher the intensity the higher the mean swim pacemaker frequency in the first 20 s after light-on (linear regression, $R^2$=0.96 for NP and $R^2$=0.92 for LLE). This means for the LLE that the higher the light intensity the less the inhibition of the pacemaker. In the pre-stimulus situation there is some variation in the pacemaker frequency for the LLE. There is a trend for this variation to follow the intensity but this is not significant (linear regression, $R^2$=0.51). If, instead, the response is measured as the mean pacemaker frequency during the full 3 min of light a significant positive

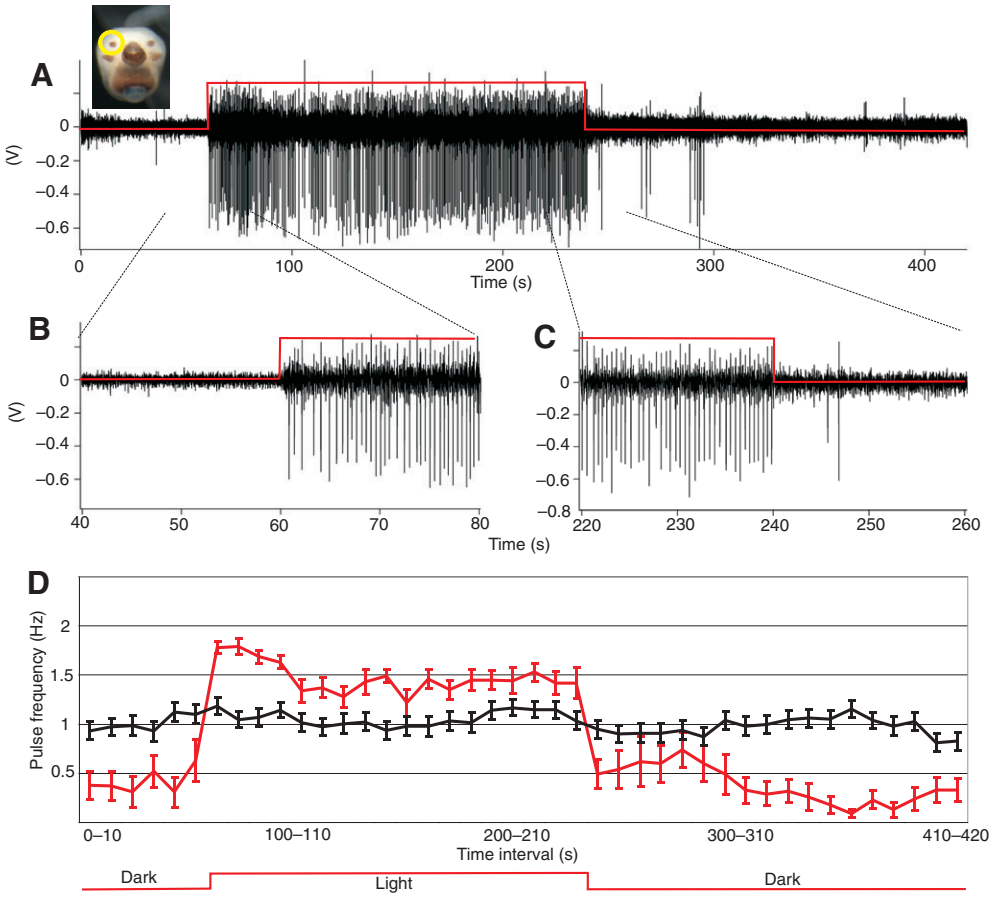


Fig. 5. Pacemaker activity when stimulating a pit eye (PE). Stimulating a PE has a large impact on the pacemaker frequency similar to when stimulating the upper lens eye (ULE) (A and Fig. 3). At light-on a strong increase is seen (A,B) whereas light-off has a strong and immediate inhibitory effect on the pacemaker (A,C). The light-on response has the strongest effect 10–20 s after light-on after which it decreases and stabilises at 1.4–1.5 Hz throughout the remainder of the light-on period (D). The light-off response causes a large drop in the frequency to about 1/3 of the dark level and this lasts for at least 3 min (D). The long-term influence of the light-off response also has the effect that the pre-stimulus frequency is lower than the dark reference. The red line in A, B, C and under D indicates the stimulus pattern. The black line in D is the mean of the dark recordings and the error bars indicate ± s.e.m. Light intensity=$1\times10^5$ W sr$^{-1}$ m$^{-2}$ for all subfigures.

correlation is seen with NP and PE (linear regression, $R^2$=0.92 for NP and $R^2$=0.85 for PE).

When considering the light-off response the situation is similar to the light-on response. With the LLE, ULE, SE and PE there is no significant correlation between the magnitude of the response and the light intensity when the magnitude is measured either as the mean pacemaker frequency in the first 20 s after light-off or as the mean frequency during the full 3 min after light-off ($R^2$<0.64). With NP there was a positive correlation between the intensity and the mean pacemaker frequency in the first 20 s after light-off ($R^2$=0.86). Interestingly, this again shows that the larger the relative change in intensity the less the inhibition.

## DISCUSSION

There are many interesting questions concerning vision in cubomedusae. How did this complex visual system evolve? Why are so many different eye types needed? What visual cues do they pick up? What visually guided behaviours do they support? How is the visual information translated into these behaviours? Answers are beginning to appear to some of these questions and in the present paper we have looked further into how the visual input to individual eyes is integrated in the rhopalial nervous system and influences one of the important behavioural parameters of the medusae; their rate of swim contractions.

### Multiple effectors

Interestingly, the results we present indicate that all four eye types are involved in modifying the swim pacemaker frequency. To our surprise not only stimulating the eyes but also stimulating the general NP of the rhopalium influenced the pacemaker. The question arises whether some of the results are artefacts caused by light escaping the stimulated eye and possibly reaching other photosensitive areas. Unfortunately this cannot be ruled out. As shown in Fig. 1 imperfection of the pigment screens along with very small eye size has the effect that it was not possible to stimulate the ULE, PE, SE and NP without having stray light affecting the others or at least the NP. Only in the case of the LLE will no light escape the eye. With respect to this it is also noteworthy that the ULE, PE, SE and NP provoke similar responses from the swim pacemaker. One way to evaluate which of these are true responses, and which might be caused by problems with the stimulation, is to look for differences in the response magnitudes and unique characteristics in the detailed responses.

The influence by ULE is probably valid because the light-on response seen here has a very distinct peak in the first 10 s, which was significantly higher than the following 10 s (two-tailed *t*-test for paired observations, $P$<0.01 for $8.7\times10^3$ and $9.6\times10^4$ W sr$^{-1}$ m$^{-2}$, $P$<0.1 for the other three intensities). This difference was not seen for any of the other stimulations (two-tailed *t*-test for paired observations, 0.27<$P$<0.87). The PE is probably also its own effector, because when stimulating this eye, the strongest and most consistent off-response is seen resulting in significantly lower frequencies for this eye at all of the tested intensities than for ULE, SE or NP (one-way ANOVA, $F_{3,68}$=15.7–70.5, $P$<0.0001, Tukey HSD *post hoc* $P$<0.0005). Further, the light-on response caused by stimulating the NP in general has the largest amplitude (although not statistically significant but in a few cases) and shows the clearest intensity dependence (highest $R^2$ values, see above), which in our

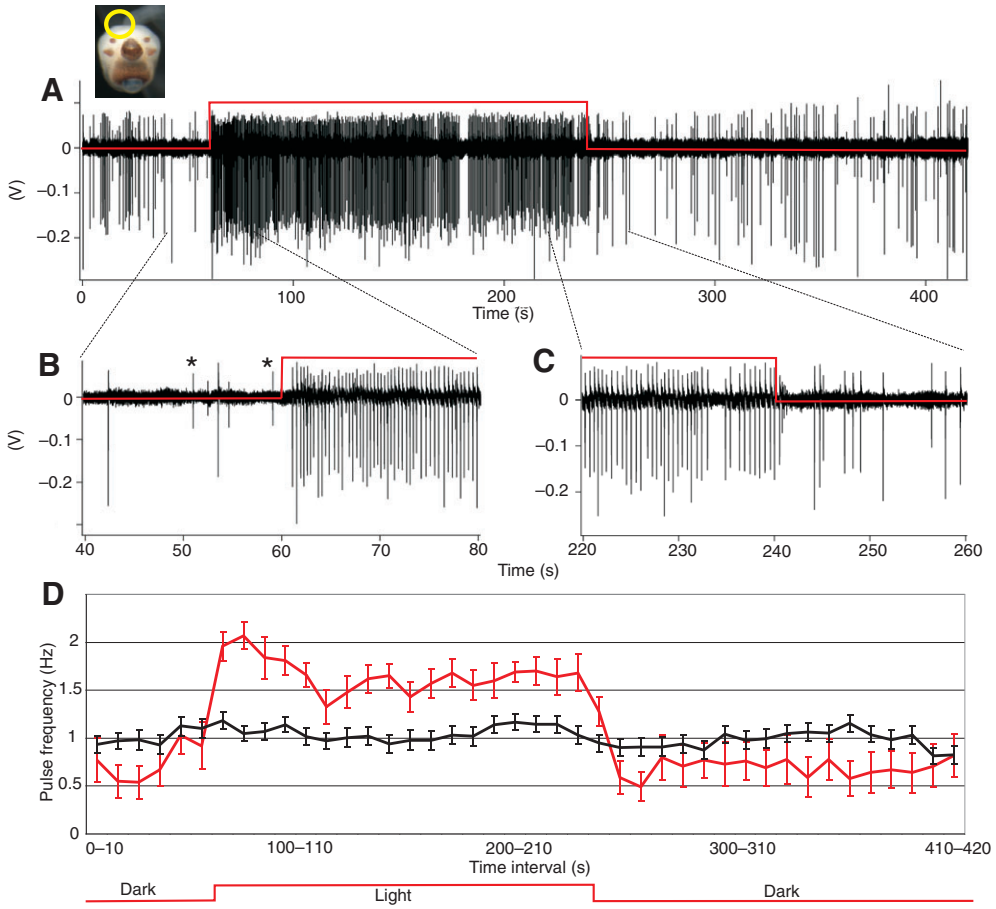


Fig. 6. Pacemaker activity when stimulating the neuropil (NP). Stimulating the NP has a large impact on the pacemaker similar to when stimulating a pit eye (PE) (compare with Fig. 5). At light-on the strongest increase is seen (A,B) whereas light-off has a strong and immediate inhibitory effect on the pacemaker (A,C). Like for PE the light-on response peaks 10–20 s after light-on after which it stabilises at 1.4–1.5 Hz (D). The light-off response causes a drop in the frequency to just below the dark level (D). Similar to the PE long-term influence of the light-off response causes a pre-stimulus frequency lower than the dark reference. The red line in A, B, C and under D indicates the stimulus pattern. The black line in D is the mean of the dark recordings and the error bars indicate ± s.e.m. The asterisks in B indicate activity of other non-pacemaker cells. Light intensity=$8.7\times10^{3}$ W sr$^{-1}$ m$^{-2}$ in A–C 8 and $1.1\times10^{5}$ W sr$^{-1}$ m$^{-2}$ in D.

opinion authenticate stimulations in this area. Stimulating the SE, however, produced some more dubious results. The responses were highly variable and did not appear to have any unique features. Also, the response amplitude, measured as the amount of change in the swim pacemaker frequency, was the smallest for this eye. We therefore believe that the areas that modify the pacemaker upon light stimulation are the LLE, ULE and PE and probably also NP. The effects seen from the SE are artefacts caused by stray light stimulating the ULE, PE, NP or a combination of them.

The above conclusion also matches what is known about the visual fields of the different eyes. When comparing the data available on the optics of *T. cystophora* (Nilsson et al., 2005; Garm et al., 2008) it becomes clear that the ULE and PE must have vastly overlapping visual fields and the same goes for the LLE and the SE. It makes good sense, therefore, that the effects of stimulating either the ULE or the PE are similar, because it is close to impossible under natural conditions to stimulate the one eye type without stimulating the other. The LLE and SE also have vastly overlapping visual fields. If the effects seen from the SE are not artefacts, these eyes will have more or less directly opposite effects on the pacemaker counteracting each other constantly, which would seem like an inappropriate and unlikely arrangement.

### Extraocular photoreception

The putative effects mediated by the NP came as a surprise and are admittedly not easily explained. Using the same logic as above there will be a constant conflict between the input to the swim pacemaker system provided by the LLE and the NP, because a large part of the light illuminating the NP will originate from within the large visual field of the LLE. Further, the effects mediated by NP are similar to those mediated by the ULE and the PE. Why have rather similar effects located in three different places? We do not have any good answers at this stage but the fact that the strongest intensity dependency is found for the NP causes us to believe that the effects are real.

Extraocular photoreceptors in the nervous system are commonly found throughout the animal kingdom, not least in cnidarians (Ohtsu, 1982; Arendt et al., 2004; Taddei-Ferretti et al., 2004). In *Hydra* such photoreceptors also modify a pacemaker system controlling body contractions (Taddei-Ferretti et al., 2004). One of the interesting things about extraocular photoreceptors is that they have a tendency to make use of other photopigments than those conventionally used in vision. In vertebrates melanopsin is such an extraocular photopigment (Kumbalasiri and Provencio, 2005), and a special ‘cnidops’ opsin clade has been found in cnidarians (Plachetzki and Oakley, 2007). Recently, two research groups have characterised opsins from two different species of cubomedusae, *T. cystophora* and *Carybdea rastonii* (Koyanagi et al., 2008; Kozmic et al., 2008). In both cases only a single opsin was found and interestingly they were only found in direct association with the retinas of the two lens eyes. Such an expression pattern is supported by our own immunofluorescence data (Ekström et al., 2008). This could be taken as evidence for a lack of photoreception in the NP but because these studies also failed to find any opsins in connection with the PE and SE we believe that additional undiscovered photopigments are at play. The fact that the effects on the swim pacemaker mediated by the PE and NP are intensity-dependent will be used in the near future to determine the spectral sensitivity of

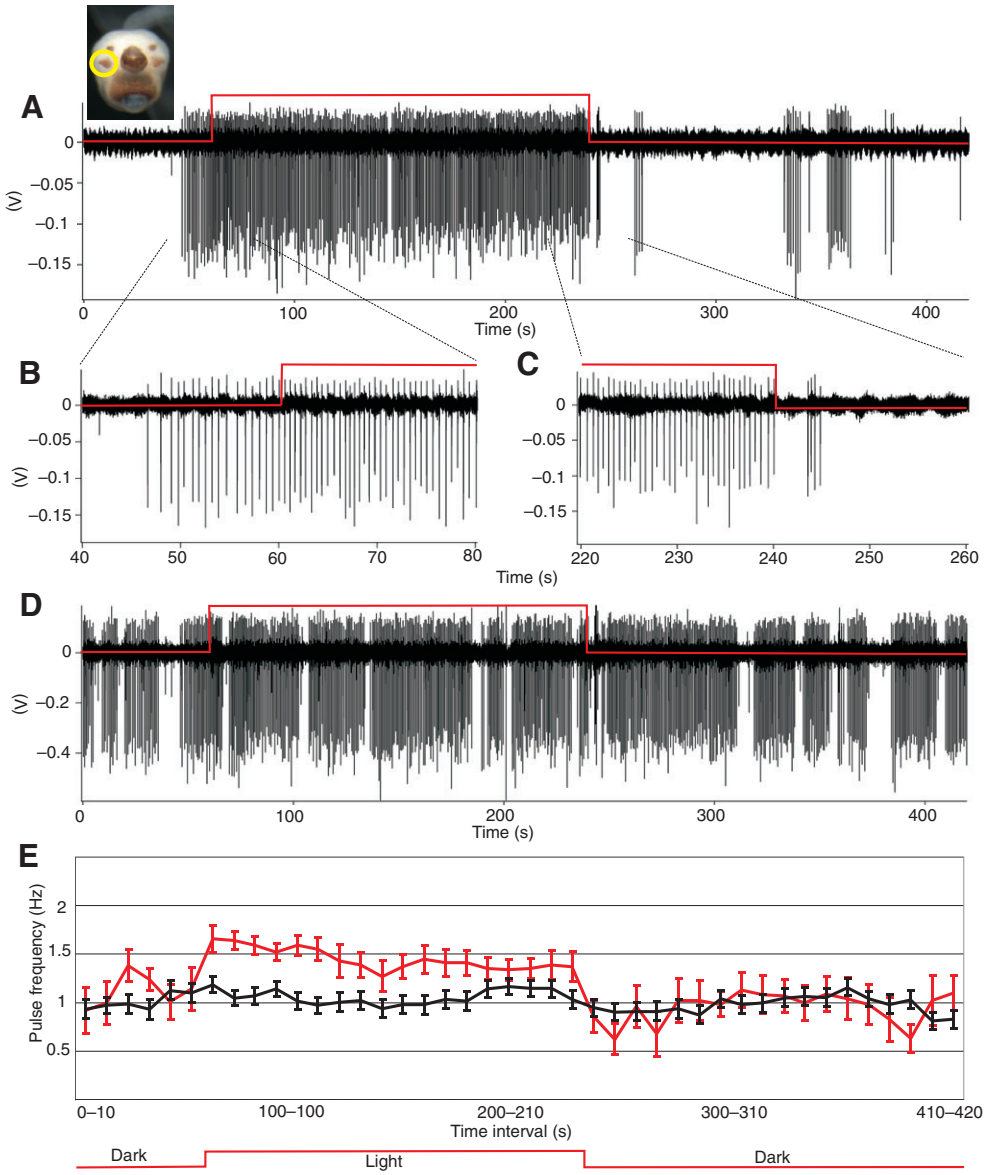


Fig. 7. Pacemaker activity when stimulating a slit eye (SE). Stimulating a SE has similar effect on the pacemaker as stimulating the upper lens eye (ULE) or the pit eye (PE) but more variable (A,D). Light-on stimulates the pacemaker frequency (A,B,E). Light-off has an inhibitory effect in some cases (A,C) but in other cases it has but little effect (D). In A and B a spontaneous burst started about 15 s before the stimulus and the onset of the stimulus caused a further increase in the activity. The light-on effect culminates in the first 10 s and at light-off the pacemaker frequency falls back to the level of the dark reference (E). The red line in A–D and under E indicates the stimulus pattern. The black line in E is the mean of the dark recordings and the error bars indicate ± s.e.m. Light intensity=$1.1\times10^{5}$ W sr$^{-1}$ m$^{-2}$ for all subfigures.

the underlying photoreceptors and thereby shed light on the nature of their photopigments.

### Complex swim pacemaker control

Being cnidarians cubomedusae belong to the group of animals first in evolution to possess a true nervous system. Even though their CNS holds several thousand nerve cells the number of computational units is probably a lot lower due to redundancy (Skogh et al., 2006; Garm et al., 2007b). It is therefore of great interest how such a relatively sparse CNS is able to handle the information provided by the far from simple visual system. Earlier work has suggested that one mechanism used is strong filtering in the periphery, which reduces the amount of information passed on to the CNS (Nilsson et al., 2005; O'Connor et al., 2009). Still, the neuroanatomy suggests that fairly complex information processing and integration does happen in the rhopalial nervous system (Parkefelt et al., 2005; Parkefelt and Ekström, 2009).

The results presented here support the idea that visual integration takes place in the rhopalial nervous system. Three of the four eye types and probably also the NP have impact on the pacemaker activity and all with at least slightly different characteristics. Recent work showed that applying a light stimulus to the entire rhopalium resulted in light-on and light-off responses resembling what is found here when stimulating the LLE alone (Garm and Bielecki, 2008). There are important differences, though, because the light-on response for the LLE alone is much stronger (the pacemaker activity decreases more) and the light-off is more brief and bi-phasic. This demonstrates that the visual control of the pacemaker is not a mere hierarchy between the stimulated eyes or a sum of their individual inputs. What kind of interactions is taking place we cannot say at this point. To look further into this it is necessary to identify which cells in the rhopalial nervous system make up the swim pacemaker and map how they connect with the different eye types.

### Visual ecology of the swim pacemaker control

The swim pacemaker frequency is an important part of controlling the swim speed of the medusae and therefore the swim pacemaker control is behaviourally important. One of the central behaviours here is the so-called shadow response or shadow reflex, which is known from both cubomedusae and hydromedusae (Yoshida and

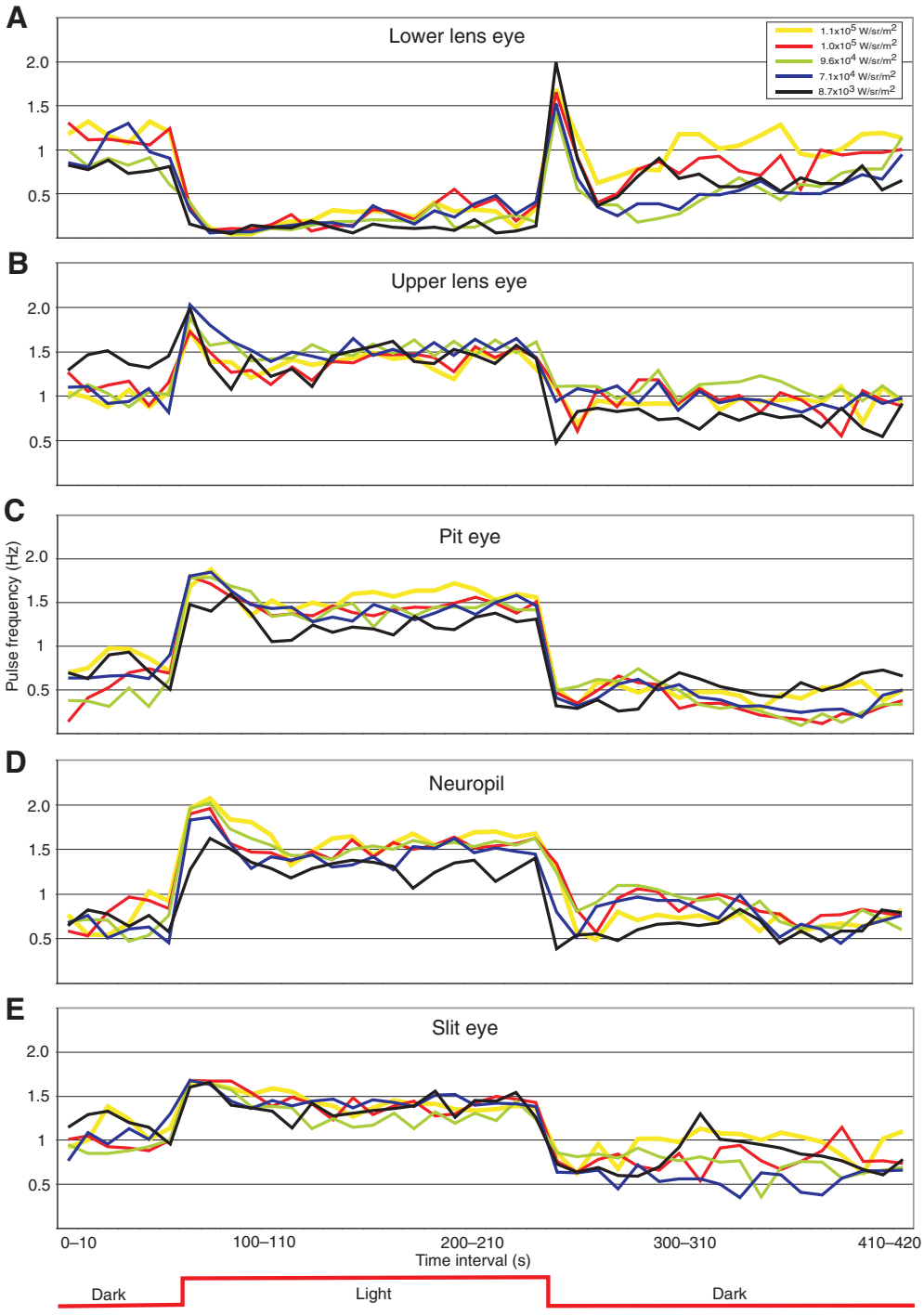


Fig. 8. Effects of different light intensities. In all five different types of stimulations five different intensities were tested covering ~1.1 log units. When the LLE or NP was stimulated the response magnitude during light was correlated with the intensity of the light stimulus (A,D). The stronger the light the higher the mean pacemaker frequency during the first 20 s after light-on. In the case of the PE, ULE and SE, no such correlation is seen (B,C,E, see text for details). In the case of the entire light-on period a positive correlation between amplitude and intensity was found for the NP and PE. The situation is similar for the light-off response where a correlation between the frequency change and stimulus intensity is again seen for the NP. LLE, lower lens eye; PE, pit eye; NP, neuropil; SE, slit eye; ULE, upper lens eye.

Ohtsu, 1973; Arkett, 1985; Arkett and Spencer, 1986a; Garm and Bielecki, 2008). Upon a sudden drop in light intensity the medusae increase the pulse frequency for a shorter (hydromedusae) or longer period of time (cubomedusae). In hydromedusae this may function as predator avoidance or control of the diurnal migration (Anderson and Mackie, 1977; Arkett and Spencer, 1986a) but in cubomedusae it has been shown to help optimise their feeding behaviour (Stewart, 1996; Garm and Bielecki, 2008). What we have shown here is that the eye type mainly governing this behaviour is the LLE. The swim pacemaker is also involved in the feeding behaviour by slowing down when experiencing an increase in light intensity, which prolongs the time the medusae stay in the light shaft where they feed (Garm and Bielecki, 2008). From our results it is again evident that this part of the feeding behaviour is largely controlled by the LLE. Hence, all of the cubozoan behaviours so far proven to be visually guided are controlled by the LLE (present results) (Garm et al., 2007a). To better understand the cubozoan visual system it is now important to find out what roles the other eye types play in the behavioural control of the medusae. The obvious place to start is to reveal the behavioural significance of the swim pacemaker control by the ULE and PE shown here.

The authors appreciate the constructive comments offered by several members of the Vision Group, Lund University and we truly thank Assoc. Prof. Poul Bennekou, University of Copenhagen, for the generosity with his equipment. A.G. would also like to acknowledge the grant # 272-07-0163 from the Danish Research Council (FNU).

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