The Journal of Experimental Biology 215, 2342-2346

doi:10.1242/jeb.066886

RESEARCH ARTICLE

Spectral sensitivity of phototaxis in the dinoflagellate *Kryptoperidinium foliaceum* and their reaction to physical encounters

Morten Moldrup[1,2] and Anders Garm[2,*]

[1]Academia Sorana, Akademigrunden 8, 4180 Sorø, Denmark and [2]Section of Marine Biology, Department of Biology, University of Copenhagen, Universitetsparken 4, 2100 Copenhagen Ø, Denmark

*Author for correspondence (algarm@bio.ku.dk)

SUMMARY

The dinoflagellate *Kryptoperidinium foliaceum* possesses one of the largest eyespots among the autotrophic dinoflagellates. Until now they were believed to be negatively phototactic using a non-opsin photopigment. Here we provide evidence that in newly established cultures they are positively phototactic and that the dynamic range of phototaxis is ~2.5 log units. Additionally, we find that the spectral sensitivity of the phototaxis agrees reasonably well with the absorption curve of a theoretical opsin, with a peak sensitivity around 500 nm. The sensitivity in the short wavelength end of the tested spectrum is unexpectedly low, but this is probably due to selective filtering. Interestingly, the phototaxis could be temporarily overruled by tactile stimuli. After physical contact with the light guide, the cells escaped the area, and we suggest that this may serve as predator avoidance.

Key words: behaviour, dinoflagellate, eyespot, predator avoidance, opsin.

Received 13 October 2011; Accepted 19 March 2012

INTRODUCTION

For years the apparent simplicity of phototaxis in unicellular systems has attracted scientific attention (e.g. Foster and Smyth, 1980; Gualtieri, 2000). Despite being studied for almost 200 years (Treviranus, 1817) it was only recently that a thorough understanding of this process was reported (reviewed by Hegemann, 2008). At present it is only within a few species of green alga (Chlorophyta) and euglenid (Euglenophyta) lines that we have an understanding at the molecular level, including which photopigments initiate the phototransduction (Hegemann, 2008). Both of these lineages predominantly inhabit freshwater systems (van den Hoek et al., 1995) and not much is known about the photobiology of the motile members of one of the ecologically most important marine protist lineages: the dinoflagellates.

Scientific effort has been directed at describing the many different eyespot complexes found in protists, with the aim to locate the photosensitive structure (e.g. Foster and Smyth, 1980; Gualtieri, 2000; Kateriya et al., 2004). All studies so far indicate that opsins are involved in the phototransduction in these organisms. The existence of the eyespot is a key element in (most of) the species' ability to orient themselves in a light gradient. The knowledge of how photosensitive structures support phototaxis, and indeed where they are located, remains sparse (Gualtieri, 2000). One of the reasons for this is the lack of an obvious model organism. The dinoflagellates are a composite group with autotrophic, mixotrophic and heterotrophic members. The autotrophic and mixotrophic members are not even uniform groups because they harbour a multitude of different symbionts (chloroplasts) originating from almost all other groups of autotrophic protists (Moestrup and Daugbjerg, 2007). Thus, no single species can be considered a good representative of the group as a whole. When examining the photobiology and phototaxis of dinoflagellates, one of the criteria for the choice of study species has been the ability to keep them in sustainable culture. Work on photophysiology has therefore been performed on autotroph dinoflagellates, which are typically the easiest to culture. Further, the study species needs to harbour an eyespot and have an appropriately large size. All these criteria are met in *Kryptoperidinium foliaceum* (Fig. 1). In fact, it exhibits one of the largest eyespots known within the dinoflagellates (Dodge and Crawford, 1969; Taylor, 1987).

For the above-mentioned reasons, *K. foliaceum* has been used as the experimental organism in several previous studies, including work performed on photobehaviour in dinoflagellates (Withers and Haxo, 1978; Horiguchi et al., 1999). Surprisingly, the results indicate that this species is not phototactic but rather photophobic, which seems counter-intuitive for a photosynthesising organism. However, the use of freshly isolated material from Øresund, Denmark, did recently allow us to conduct experiments showing positive phototaxis in *K. foliaceum* (M.M., Ø. Moestrup and P. J. Hansen, unpublished). In the present study we aim to investigate this phototaxis further. Using a behavioural assay we have studied the spectral sensitivity underlying phototaxis and found support for the presence of a single opsin with peak sensitivity in the blue–green part of the spectrum. Further, we observed a novel putative mechanical induced behaviour when this autotrophic dinoflagellate came into contact with an obstacle.

MATERIALS AND METHODS

Cultures

A culture of *Kryptoperidinium foliaceum* Lebour 1925 (MBL07; Fig. 1) was established using water from Elsinore Harbour. Cells were isolated from a sample of natural seawater with a drawn Pasteur pipette and transferred to a multi-dish well (24 wells) into 0.2 µm filtered seawater. The culture was kept in seawater based f/20 medium (Guillard, 1983) of approximately 34 p.s.u. This medium ensured that the cultures were never nutrient limited. Light (cool

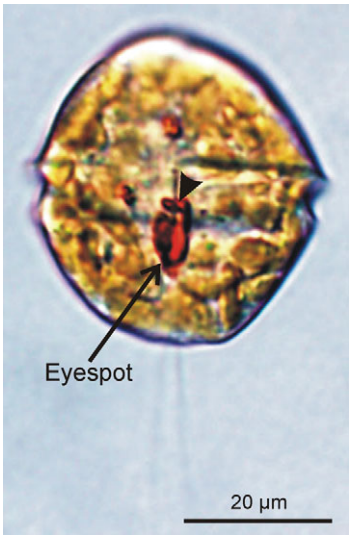

Fig. 1. A planozygote of the dinoflagellate *Kryptoperidinium foliaceum* (note the two flagella). The arrow indicates the eyespot, and the arrowhead indicates the anterior hook.

white, 20–100 μE m^{-2} s^{-1}) was provided following a light:dark cycle of 16 h:8 h. All culturing and experiments were performed at a temperature of 20±1°C.

Behaviour

To minimise temperature-generated convection, the air and culture temperature must be the same, so all behaviour experiments were performed using well-acclimated cultures (i.e. performed at culturing temperature). The culture (~2000 cells ml^{-1}) was pipetted into the spectrophotometry cuvettes that were used as the experimental containers. The cells in each cuvette were illuminated by an infrared (IR) LED (IR 204, Everlight, Odense, Denmark; peak=940 nm) from the side and their behaviour was recorded by an IR-sensitive digital video camera (uEye 1540-C, IDS Imaging, Obersulm, Germany) mounted on a dissecting microscope (Olympus SZR 61, Hamburg, Germany) placed in the IR light axis (Fig. 2).

The light stimulus was produced by a white super-bright LED (Luxeon III star, Philips, San Jose, CA, USA) and focused into a 1 mm light guide *via* a Linos Microbench system (Linos, Goettingen, Germany). The LED was controlled *via* an NI6229 A/D converter (National Instruments, Austin, TX, USA) and a custom-made program for LabView 8.5 (National Instruments). The optical bench housed neutral density filters (Linos, Goettingen, Germany) to control intensity in steps of 0.3 or 0.7 log units. The spectral composition was controlled by interference color filters with a half-width of 10 nm (F10-420.0-4-12.5M–F10-680.0-4-12.5M, CVI Laser, Albuquerque, NM, USA). The intensity dependence of the phototaxis was examined using white light regulated by the neutral density filters starting in the low intensity end. The maximum intensity was 1.1×10^{5} W sr^{-1} m^{-2} when integrated between 350 and 750 nm and measured at the tip of the light guide using a spectroradiometer (ILT900W, International Light Technologies, Peabody, MA, USA). The spectral sensitivity was examined using the colour filters in 10 or 20 nm steps from 420 to 680 nm. Equal quanta stimulation (6.1×10^{18} photons sr^{-1} s^{-1} m^{-2}) was ensured also using the spectroradiometer. The light stimulus was introduced into the cuvette from above *via* the light guide. The tip of the light guide was placed 10 mm above the bottom of the cuvette. Each experimental series (N=3) started with a response magnitude (RM)–log intensity (logI) curve followed by spectral sensitivity tests (19 filters in total), and ended with a second RM–logI curve. The second RM–logI curve was generated to ensure that experimental conditions had not changed during the experiment. Each stimulus lasted 10 min, and 5 min of darkness was introduced prior to each stimulus to remove any potential effects of previous stimuli (Fig. 3). The total duration of the protocol was ~550 min.

Analysis

The number of cells attracted to a predefined area in front of the light guide served as the measure for response magnitude of *K. foliaceum*. The final approximately 8 min of each stimulus period was recorded and three of the video frames equally spaced time-wise were frame grabbed. The cells in each picture were enumerated using ImageJ software (National Institutes of Health, Bethesda, MD, USA) (Fig. 4). The average cell count from the three pictures was then corrected for the number of cells present during darkness and used for further analyses. The relative sensitivity curve was obtained from transformation of the behaviourally recorded spectral data by the RM–logI curve. This is done to correct for nonlinearities between the behavioural response and the number of absorbed photons (for details, see e.g. Coates et al., 2006). Swim trajectories from single cells were automated using LabTrack 2.3 (BioRAS, Copenhagen, Denmark).

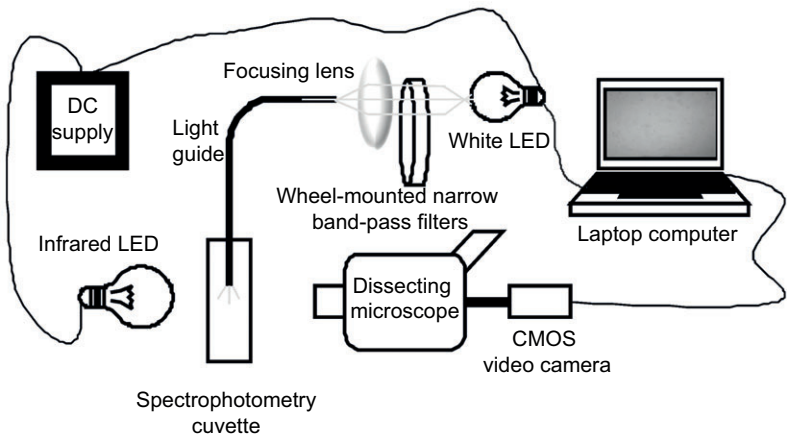

Fig. 2. Experimental setup. A computer-controlled LED illuminates a series of narrow bandwidth filters. From here the light is focused by a lens into a fibre light guide, which is inserted into a cuvette. This cuvette holds the algal culture, and the light guided behaviour of these is video recorded using infrared light.

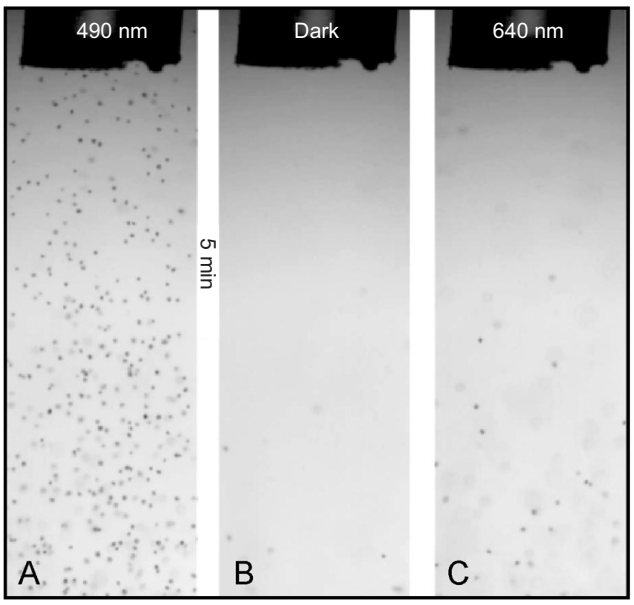

Fig. 3. Dispersal in darkness. (A) During light stimulation, the dinoflagellates gather in the light in front of the light guide. (B) After 5 min of darkness, the cells have dispersed and moved away from the area in front of the light guide. (C) A few of the cells are attracted when presenting light at 640 nm after the 5 min of darkness.

RESULTS

Behavioural observations

In darkness, the flagellates swam in what appeared to be random directions at a steady speed. The actual speed could not be determined because the tracking was performed in two dimensions (2-D). After turning on the light the cells displayed a quick behavioural change (within 100 ms), resulting in straight swimming towards the tip of the light guide. The approximately 2-D configuration of the phototaxis allowed for speed determination on a subset of the cells, which had a mean of 0.35 mm s^{-1}.

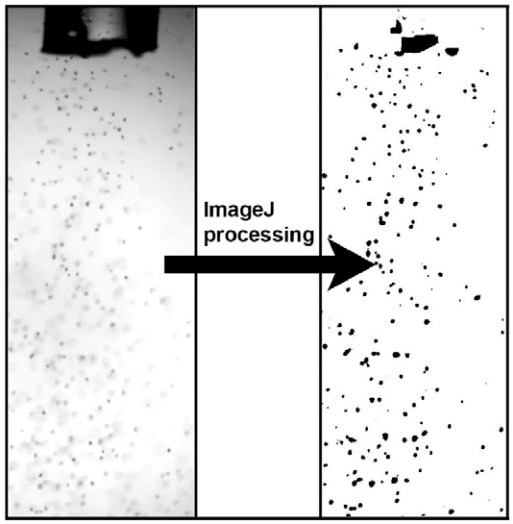

Fig. 4. Picture processing for enumeration. The picture is thresholded so that the individual cells gain the strongest contrast. The picture is then converted to a binary picture (as shown on the right) whereupon the 'particle analysis tool' was used in a configuration that excluded the large remnants of the light guide as well as single pixel noise only to enumerate real cells. This protocol was set up as a macro in the analyzing software ImageJ so that all frame-grabbed pictures of each series were treated equally.

Dynamic range

The dynamic range of *K. foliaceum* is here defined as the linear part of the RM–logI curve (Fig. 5A). This corresponds to a behavioural response at intensities between ~5×10^{1} and ~3×10^{4} W sr^{-1} m^{-2} (20 to 9000 µE m^{-2} s^{-1} when calculated as 500 nm photons) or ~2.5 log units. The behavioural response reached a maximum and saturated at ~$5\,10^{4}$ W sr^{-1} m^{-2}. No photoinhibition was observed within the used intensity range.

Spectral sensitivity

Peak sensitivity was found in the blue–green area around 500 nm. Using the standard least-square means method, a decent fit was found when comparing the behavioural data with a theoretical opsin absorption curve (Govardovskii et al., 2000) (Fig. 5B). The spectral sensitivity of *K. foliaceum* had the best fit to a 494 nm opsin (R^2=0.58, N=3). However, it is noteworthy that the obtained spectral sensitivity was somewhat lower than the theoretical opsin absorption curve in the short wavelength end and around 480 nm. No response was seen when stimulating with light above 600 nm. The data were also fitted to the absorption curve of the other possible photopigment,

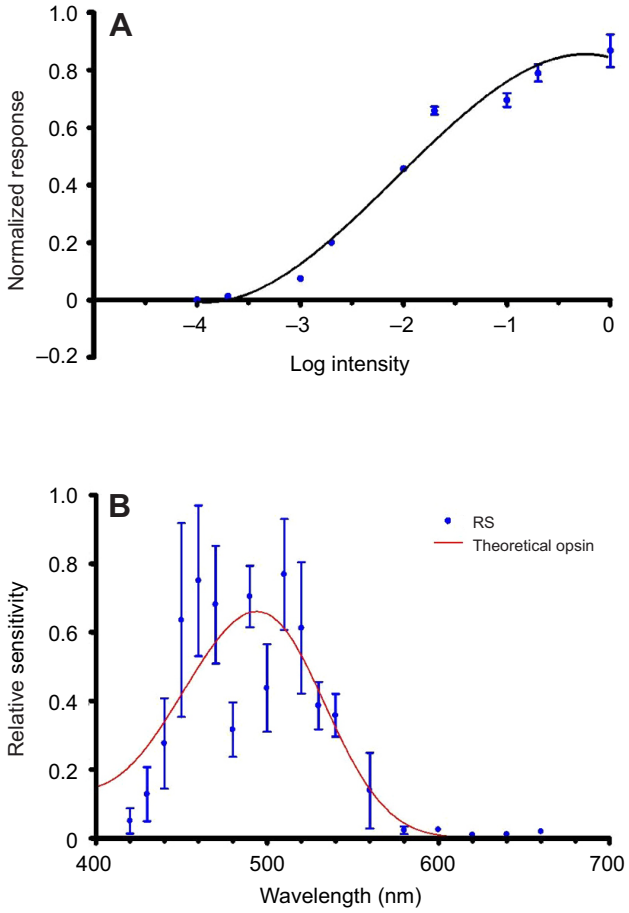

Fig. 5. Spectral sensitivity for phototaxis. (A) Normalized response of *Kryptoperidinium foliaceum* as a function of light intensity (I). I_{max}=1.1×10^{5} W sr^{-1} m^{-2}. (B) Relative sensitivity of phototaxis. The red line indicates the absorption curve for a theoretical opsin.

β-carotene, taken from the literature (Miller, 1934). This resulted in a rather poor fit with an R^2 value of 0.04.

Physical encounters

At the very end of their phototactic trajectory, the *K. foliaceum* cells bump into the light-emitting tip of the light guide. This physical encounter induces an escape response. When an encounter occurs the cells react almost instantaneously to this physical contact (40 ms spacing between dots; Fig. 6). The encounter overrides the positive phototaxis and the cells escape the light guide for variable length of time (Fig. 6). After a brief escape response (1–2 s), some cells resume positive phototaxis and for some cells the whole sequence of events repeats itself (Fig. 6A,B).

DISCUSSION

We have shown that the dinoflagellate *K. foliaceum* displays positive phototaxis and that they are attracted to white light at ecologically relevant intensities. In our behavioural experiments, the flagellates had a graded response with stronger light attracting more cells. The spectral sensitivity behind the phototaxis indicated that it is based on a single opsin with peak sensitivity around 500 nm. Interestingly, the phototaxis could be temporarily overruled by tactile stimuli. After physical contact with the light guide, the cells escaped the area with the high light intensity.

Spectral sensitivity

One way to establish which type of photopigment is responsible for the phototaxis is to compare the spectral sensitivity curve with the absorption curve of known photopigments. The half width and shape of the spectral sensitivity curve fits reasonably well with the theoretical absorption of an opsin with the peak sensitivity close to 500 nm (R^2=0.58). Still, at the short wavelength end of the spectrum the two curves differ from each other. One possible explanation is that this organism filters the light to protect itself from harmful UV radiation. Such high-pass filtering is known from visual systems throughout the animal kingdom (Lythgoe, 1979). The deviation from the theoretical curve could also be caused in large part by the variability typically present in behavioural assays.

The alternative photopigments known from *K. foliaceum* are chlorophylls *a* and *c*, fucoxanthin, diadinoxanthin, β-carotene, β-zeacarotene and γ-carotene. All of these photopigments have an absorption maximum in the 430–490 nm range (Withers and Haxo, 1975), but have differently shaped absorption curves. Chlorophylls *a* and *c*, fucoxantin and diadinoxanthin all have well-documented roles as primary or accessory pigments in light harvesting for photosynthesis and are found primarily in the chloroplasts (Withers and Haxo, 1975). They all have a secondary absorption peak in the orange/red part of the light spectrum, which is not supported by the data presented here (Fig. 5B) and we therefore exclude them as candidate photopigments supporting the phototaxis. The carotenoids (β-carotene, β-zeacarotene and γ-carotene) are found both in chloroplasts and, more importantly for this study, in the lipid globules that constitute the eyespot (Withers and Haxo, 1978). They have two local absorption maxima in the 425–490 nm range, but the maxima are too far down the short wavelength end to match the spectral sensitivity curve we found for *K. foliaceum* (indicated by the very low R^2 value, 0.04). The location of the carotenes makes it much more likely that they serve as directional shading pigments enabling the directional swimming associated with the phototaxis shown here. Even though the actual photosensitive structure of dinoflagellates has never been identified, it is a common and very likely hypothesis that it lies to the one side of and in close association with the eyespot (Gualtieri, 2000). The helical swim pattern of *K. foliaceum* will result in varying light intensity at the photosensitive structure during each turn because of the shading by the carotenoid droplets. This allows the flagellate to determine the direction of light. For the shading to be most efficient, the involved pigment has to have a high absorbance in the same part of the light spectrum as the photopigment.

Further support for an opsin being the photopigment behind phototaxis in *K. foliaceum* comes from the observations that this behaviour initiated more or less instantly when the light was switched on. From animal vision, which is always based on opsins (Land and Nilsson, 2002), it is known that opsin-based phototransduction is fast, allowing high temporal resolution (Burns and Arshavsky, 2005; Howard et al., 1984), which is necessary for the change in swimming direction in less than 100 ms observed here.

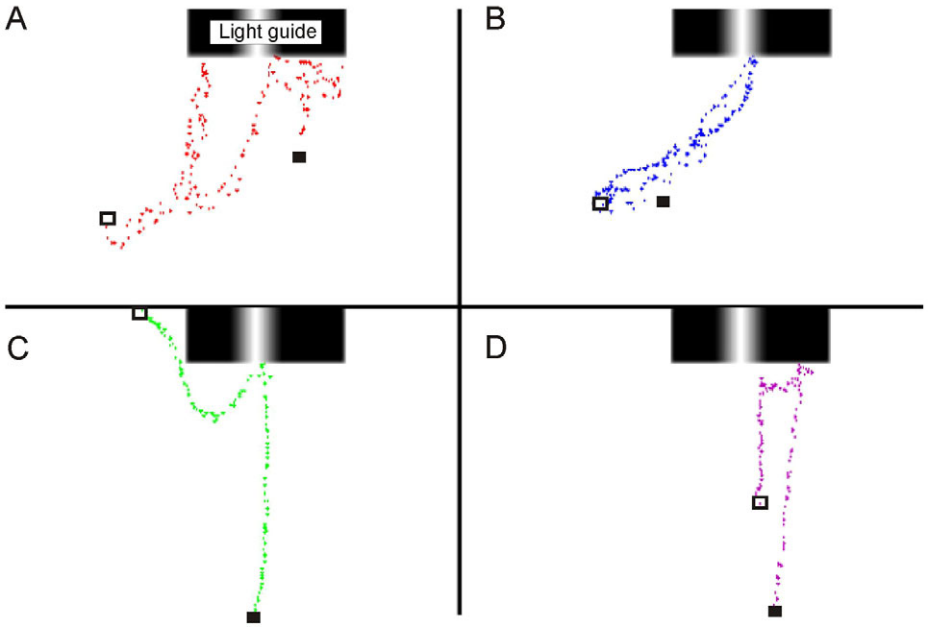

Fig. 6. Physical encounters. Tracked swimming of *Kryptoperidinium foliaceum* under a light guide. (A–D) Four physical encounters. Each dot is separated by 40 ms and the black squares indicate the start of the track. Open squares indicate end points.

However, nothing is known about the phototransduction in dinoflagellates. Lastly, all previous studies of photoreception behind phototaxis in protists have revealed the photopigment to be an opsin (Hegemann et al., 2001). Actually, the 494 nm peak we found is remarkably close to the 495 nm peak reported from purified membranes of the green algae *Chlamydomonas reinhardtii* (Beckmann and Hegemann, 1991), and could indicate that the two organisms have similar photobiology.

Horiguchi et al. found a similarly shaped spectral sensitivity response for the UTEX1688 strain of *K. foliaceum* (Horiguchi et al., 1999). Interestingly, they found the peak sensitivity to be at 430 nm and to support photophobic rather than phototatic behaviour. One interpretation of this could be that *K. foliaceum* employs a two-pigment system, one for phototaxis and one for photophobia, as has been reported for *Gyrodinium dorsum* (Forward, 1973). However, such a system is not supported by our results or the work of Horiguchi and colleagues (Horiguchi et al., 1999). We believe that the difference can be explained by a culture artefact, caused by long-term cultivation of the UTEX1688 strain of *K. foliaceum*, resulting at least in a degenerating eyespot and likely also in changes in the photosensitive structures (M.M., Ø. Moestrup and P. J. Hansen, unpublished).

Ecology

The natural environment of *K. foliaceum* is a shallow, protected brackish lagoon, inlet or bay. It is therefore important to consider their natural light environment. Such shallow-water habitats are often nutrient rich and therefore rich in planktonic organisms, giving the water a blue–green colour. This is well known, and it would, therefore, be prudent to anticipate that phototactic organisms living in such waters, e.g. *K. foliaceum*, are best suited to perceive light in this part of the spectrum, as this will enhance the photon absorption and the contrast.

Physical encounters

The phototactic behaviour is readily induced by light, but the swim trajectories of the cells revealed that another behaviour can substitute for phototaxis. When analyzing the videos it became apparent that the cells bumped into the light-emitting surface of the fibre light guide. This physical encounter with the light guide immediately changed the swimming direction of the cells, causing them to display escape behaviour. We can only speculate about the function of this behaviour, but it seems reasonable that natural encounters will normally be associated with possible predatory organisms, from which the dinoflagellates then try and escape. It is quite common for protists to react to a hydromechanical stimulus that exceeds a certain threshold, and this has been studied in a number of species (e.g. Jakobsen, 2001). In fact, the observed behaviour is not unlike that described for ciliates, where ciliary reversal occurs upon collision with a solid object (Naitoh and Eckert, 1973). The force of a direct encounter with an object, as seen in our experiments, probably exceeds that of any water-pressure-induced stimuli. The escape behaviour exhibited in the present study we expect only to be present in autotrophic dinoflagellates, as mixotrophic and heterotrophic species rely on the ability to sense, adhere to and engulf particles for feeding (Jacobson and Anderson, 1986; Hansen, 1991). If these ‘predatory’ mixotrophic and heterotrophic flagellates displayed the escape behaviour it would probably make them escape their often large prey item upon contact.

ACKNOWLEDGEMENTS

The authors appreciate the help offered by P. J. Hansen, University of Copenhagen.

FUNDING

This study was funded through a PhD grant for M.M. by the Faculty of Science, University of Copenhagen, and by the Carlsberg Foundation [grant no. 2010-01-0335 to A.G.].

REFERENCES

Beckmann, M. and Hegemann, P. (1991). In vitro identification of rhodopsin in the green alga *Chlamydomonas*. *Biochemistry* **30**, 3692-3697.
Burns, M. E. and Arshavsky, V. Y. (2005). Beyond counting photons: trials and trends in vertebrate visual transduction. *Neuron* **48**, 387-401.
Coates, M. M., Garm, A., Theobald, J. C., Thompson, S. H. and Nilsson, D. E. (2006). The spectral sensitivity of the lens eyes of a box jellyfish, *Tripedalia cystophora* (Conant). *J. Exp. Biol.* **209**, 3758-3765.
Dodge, J. D. and Crawford, R. M. (1969). Observations on the fine structure of the eyespot and associated organelles in the dinoflagellate *glenodinium foliaceum*. *J. Cell Sci.* **5**, 479-493.
Forward, R. B., Jr (1973). Phototaxis in a dinoflagellate: action spectra as evidence for a two-pigment system. *Planta* **111**, 167-178.
Foster, K. W. and Smyth, R. D. (1980). Light antennas in phototactic algae. *Microbiol. Rev.* **44**, 572-630.
Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. *Vis. Neurosci.* **17**, 509-528.
Gualtieri, P. (2000). Morphology of photoreceptor systems in microalgae. *Micron* **32**, 411-426.
Guillard, R. R. L. (1983). Culture of phytoplankton for feeding marine invertebrates. In *Culture of Marine Invertebrates* (ed. C. J. Berg Jr), pp. 108-132. Pennsylvania, USA: Hulchinson Ross.
Hansen, P. J. (1991). *Dinophysis* – a planktonic dinoflagellate genus which can act both as prey and predator of a ciliate. *Mar. Ecol. Prog. Ser.* **69**, 201-204.
Hegemann, P. (2008). Algal sensory photoreceptors. *Annu. Rev. Plant Biol.* **59**, 167-189.
Hegemann, P., Fuhrman, M. and Kaleriya, S. (2001). Algal sensory photoreceptors. *J. Phycol.* **37**, 668-676.
Horiguchi, T., Kawai, H., Kubota, M., Takahashi, T. and Watanabe, M. (1999). Phototactic responses of four marine dinoflagellates with different types of eyespot and chloroplast. *Phycol. Res.* **47**, 101-107.
Howard, J., Dubs, A. and Payne, R. (1984). The dynamics of phototransduction in insects. *J. Comp. Physiol. A* **154**, 707-718.
Jacobson, D. M. and Anderson, D. M. (1986). Thecate heterotrophic dinoflagellates – feeding behaviour and mechanisms. *J. Phycol.* **22**, 249-258.
Jakobsen, H. H. (2001). Escape response of planktonic protists to fluid mechanical signals. *Mar. Ecol. Prog. Ser.* **214**, 67-78.
Kateriya, S., Nagel, G., Bamberg, E. and Hegemann, P. (2004). “Vision” in single-celled algae. *News Physiol. Sci.* **19**, 133-137.
Land, M. F. and Nilsson, D.-E. (2002). *Animal Eyes*. Oxford: Oxford University Press.
Lythgoe, J. N. (1979). *The Ecology of Vision*. Oxford: Clarendon Press.
Miller, E. S. (1934). Absorption spectra of alpha and beta carotenes and leaf xanthophyll at room and liquid nitrogen temperatures. *Plant Physiol.* **9**, 179.
Moestrup, Ø. and Daugbjerg, N. (2007). On dinoflagellate phylogeny and classification. In *Unravelling the Algae: the Past, Present, and Future of Algal Systematics* (ed. J. Brodie and J. Lewis), pp. 215-230. Boca Raton, FL: CRC Press.
Naitoh, Y. and Eckert, R. (1973). Sensory mechanisms in *Paramecium*. II. Ionic basis of hyperpolarizing mechanoreceptor potential. *J. Exp. Biol.* **59**, 53-65.
Poulsen, L. K., Moldrup, M., Berge, T. and Hansen, P. J. (2011). Feeding on copepod fecal pellets: a new trophic role of dinoflagellates as detritivores. *Mar. Ecol. Prog. Ser.* **441**, 65-78.
Taylor, F. J. R. (1987). *The Biology of Dinoflagellates*. Oxford: Blackwell Scientific Publications.
Treviranus, L. G. (1817). *Vermischte Schriften Anatomischen und Physiologischen*, Vol. 2, pp. 71-92.
van den Hoek, C., Mann, D. G. and Jahns, H. M. (1995). *Algae: an Introduction to Phycology*. Cambridge: Cambridge University Press.
Withers, N. W. and Haxo, F. T. (1975). Chlorophyll c_1 and c_2 and extraplastidic carotenoids in the dinoflagellate *Peridinium foliaceum* Stein. *Plant Sci. Lett.* **5**, 7-15.
Withers, N. W. and Haxo, F. T. (1978). Isolation and characterization of carotenoid-rich lipid globules from *Peridinium foliaceum*. *Plant Physiol.* **62**, 36-39.