JOURNAL OF THE ROYAL SOCIETY Interface

A dynamic broadband reflector built from microscopic silica spheres in the 'disco' clam *Ctenoides ales*

Lindsey F. Dougherty, Sönke Johnsen, Roy L. Caldwell and N. Justin Marshall

J. R. Soc. Interface 2014 **11**, 20140407, published 25 June 2014

JOURNAL OF THE ROYAL SOCIETY Interface

rsif.royalsocietypublishing.org

Research

Cite this article: Dougherty LF, Johnsen S, Caldwell RL, Marshall NJ. 2014 A dynamic broadband reflector built from microscopic silica spheres in the 'disco' clam *Ctenoides ales*. *J. R. Soc. Interface* **11**: 20140407.
http://dx.doi.org/10.1098/rsif.2014.0407

Received: 17 April 2014
Accepted: 19 May 2014

Subject Areas:
biophysics

Keywords:
optics, silica, bivalve, reflection, *Ctenoides*, scattering

Author for correspondence:
Lindsey F. Dougherty
e-mail: lindseydougherty@berkeley.edu

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsif.2014.0407 or via http://rsif.royalsocietypublishing.org.

Royal Society Publishing

A dynamic broadband reflector built from microscopic silica spheres in the 'disco' clam *Ctenoides ales*

Lindsey F. Dougherty[1], Sönke Johnsen[2], Roy L. Caldwell[1] and N. Justin Marshall[3]

[1]Department of Integrative Biology, University of California Berkeley, Berkeley, CA 94720, USA
[2]Department of Biology, Duke University, Durham, NC 27708, USA
[3]Queensland Brain Institute, University of Queensland, Brisbane, Queensland 4072, Australia

The 'disco' or 'electric' clam *Ctenoides ales* (Limidae) is the only species of bivalve known to have a behaviourally mediated photic display. This display is so vivid that it has been repeatedly confused for bioluminescence, but it is actually the result of scattered light. The flashing occurs on the mantle lip, where electron microscopy revealed two distinct tissue sides: one highly scattering side that contains dense aggregations of spheres composed of silica, and one highly absorbing side that does not. High-speed video confirmed that the two sides act in concert to alternate between vivid broadband reflectance and strong absorption in the blue region of the spectrum. Optical modelling suggests that the diameter of the spheres is nearly optimal for scattering visible light, especially at shorter wavelengths which predominate in their environment. This simple mechanism produces a striking optical effect that may function as a signal.

1. Introduction

Structural coloration is common in dynamic visual displays by terrestrial and marine animals [1–5]. The spectral environment in which they live influences communication methods that use coloration [1,6–11]. In the ocean's euphotic zone, the 'disco' or 'electric' file clam *Ctenoides ales* (family Limidae) is found inside small crevices at depths of approximately 3–50 m. At these depths, the majority of wavelengths available for visual displays are in the blue-green range (400–500 nm) [12]. This is true even at shallow depths where long wavelengths have not yet attenuated (less than 15 m), as the crevices in which *C. ales* are found are dominated by horizontal light composed of short wavelengths (electronic supplementary material, file S1).

The flashing display on the mantle lip of *C. ales* has been mischaracterized as bioluminescence [13,14], although it is actually mediated by light scattered from photonic nanostructures [15]. Structures of this sort typically use materials that have high refractive indices relative to the substrate, such as collagen, chitin, keratin and guanine [16]. Silica also has a high refractive index ($n = 1.43$ at 589 nm) [16] but has only rarely been used as a biophotonic structure, such as in diatoms [17–19] and the weevil *Pachyrhynchus argus* [20]. Photonic nanostructures of any substance however can enhance reflectance, such as the ultrathin, aperiodic filaments in scales of *Cyphochilus* spp. beetles [21] and the bead-studded scales in the wings of certain pierid butterflies [22].

Within bivalves, studies of light manipulation are limited to the bioluminescence of the marine clams *Pholas dactylus* and *Gastrochaena* sp. [23], and the iridophores of the giant clam *Tridacna*, which are thought to scatter light towards symbiotic zooxanthallae [24]. *Ctenoides ales*, however, is the only known bivalve with a behaviourally mediated photic display. The fundamental characteristics of this display are described here to determine its potential as a signal. Ongoing studies of the function of the display are being conducted in the context of habitat-specific sensory ecology. In order to provide a

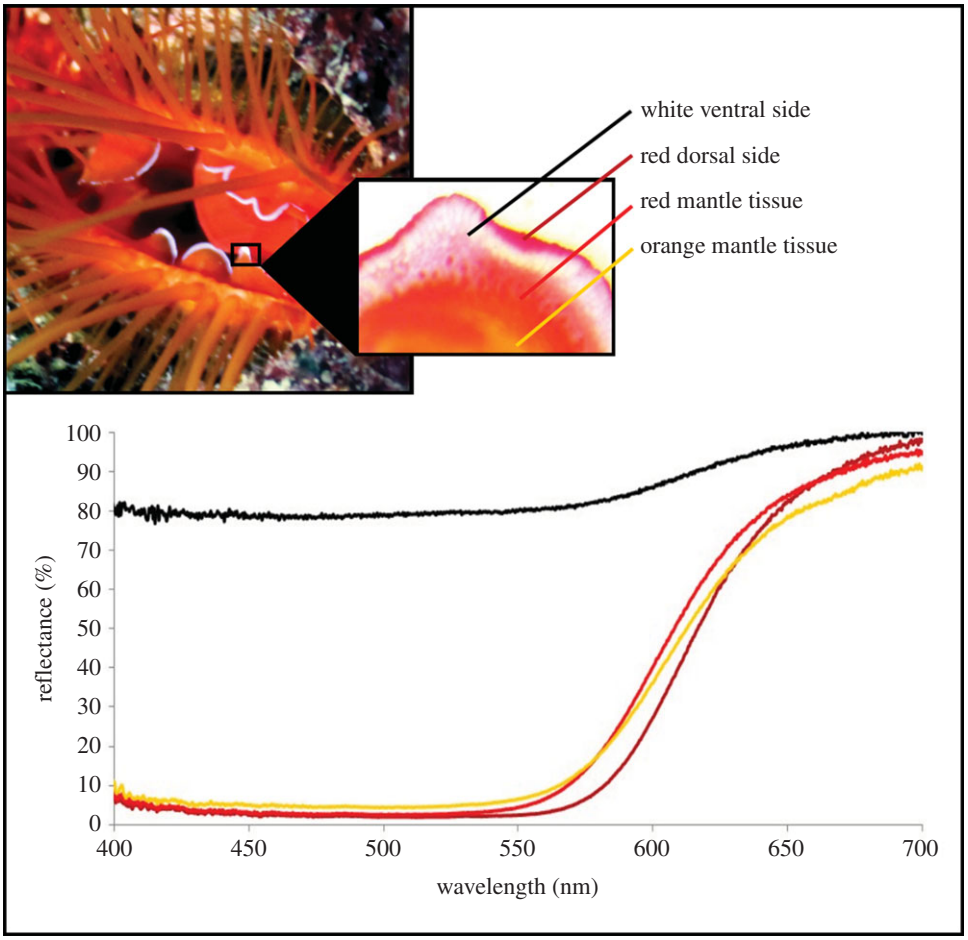

Figure 1. Spectrometry on mantle and lip tissue. Top: *C. ales* and microscope photograph of tissue (inset) showing points of measurement for spectrometry. Bottom: per cent reflectance for points of measurement.

preliminary comparative framework, we collected data from the morphologically and ecologically similar congener *Ctenoides scaber*, which does not flash.

2. Material and methods

We used five techniques to investigate the display of *C. ales*; spectrometry, high-speed video, transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS) and optical modelling. Spectrometry, high-speed video and TEM were used to provide a comparative framework on *C. ales* and *C. scaber*. Our prediction was that the display of *C. ales* would show differences in reflectance, ultrastructure and mantle lip movement when compared with *C. scaber*. EDS and optical modelling were not appropriate for comparison, as *C. scaber* had no distinctive ultrastructure to warrant further analysis. Specimens were observed in the laboratory and *in situ* in Australia (Lizard Island, 14°38′ S, 145°27′ E) and Indonesia (Lembeh Straight, 1°27′ N, 125°14′ E, and Kri Island, 0°34′ S, 130°40′ E). Laboratory work was conducted at ambient room temperature (23–26°C).

2.1. Spectrometry

Spectrometry was conducted using an Ocean Optics USB2000 (Ocean Optics, Dunedin, FL, USA) to measure reflection of the two distinct tissue sides of *C. ales* and to look for any similar distinctions in *C. scaber*. An Olympus SZX9 microscope (Olympus, Waltham, MA, USA) was used for magnification. A small portion of tissue (less than 1 cm^2) was excised from the mantle edge and placed on a white reflectance standard (WS-2; Ocean Optics) 50 mm away from the microscope objective. The standard and the tissue were submerged in salt water. The spectrometer, which used a Sony ILX511 linear silicon CCD array and fibre-optic cable, was mounted in the microscope and aimed straight down at the tissue. The Olympus LG-PS2 light microsource was oriented at a 45° angle to the tissue outside of the seawater and illuminated the tissue at 29.7° due to refraction (assuming a refractive index of seawater of 1.34). Results were analysed using OOIBase32 software (Ocean Optics). The measured area was small and thus had to be imaged through a microscope. Therefore, owing to the limitations of the microscope, the ultraviolet (UV) portion (300–400 nm) of the reflectance was not measured.

2.2. High-speed video

Black-and-white high-speed video was captured using the FASTCAM SA3 and analysed with FASTCAM Viewer software in order to analyse the inner mantle fold movement of *C. ales*. (Photron, San Diego, CA, USA). Images were taken at 1024 × 1024 pixel resolution at 1000 frames per second using a standard fluorescent bulb for illumination.

2.3. Transmission electron microscopy

Tissues from three *C. ales* specimens and one *C. scaber* specimen were fixed in 2.5% gluteraldehyde to examine differences in ultrastructure between the two species. Six tissue fragments from *C. ales* and two tissue fragments from *C. scaber* were examined. TEM was conducted using the Philips/FEI Tecnai 12 TEM (Philips, Hillsboro, OR, USA) at the Electron Microscopy Lab at the University of California at Berkeley, USA. Tissue was fixed with osmium tetroxide and sections were stained with uranyl acetate and lead citrate.

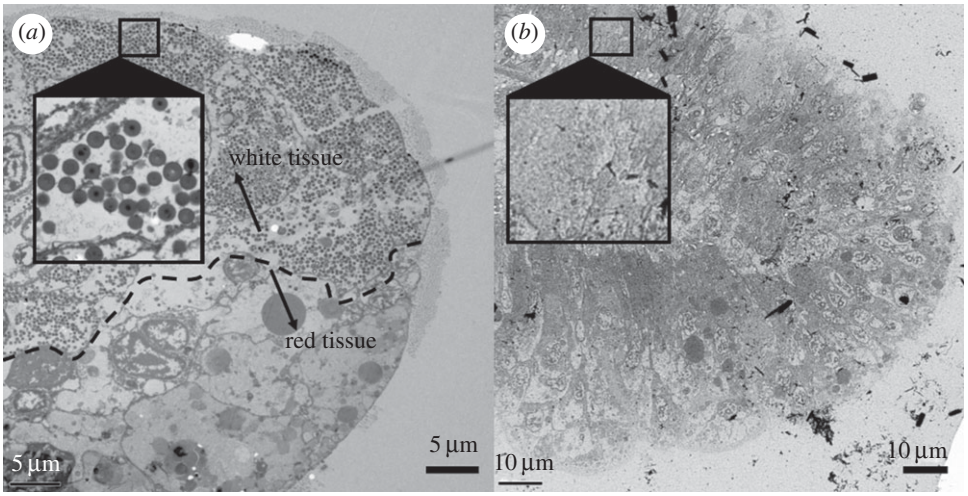

Figure 2. TEM species comparison. (*a*) TEM of *C. ales* inner mantle fold marginal edge showing electron-dense spheres (inset) in the white ventral side, and a lack thereof in the red dorsal side. (*b*) TEM of congener *C. scaber* lacks any similar electron-dense spheres.

2.4. Energy dispersive X-ray spectroscopy

A JEOL JEM2100 LaB_6 STEM analytical transmission electron microscope (JEOL, Peabody, MA, USA) fitted with a thin-window energy dispersive X-ray detector was used to conduct elemental analysis on tissue samples mounted on copper grids using spectral point acquisition. Analysis was done at the Centre for Microscopy and Microanalysis at the University of Queensland, Australia. Samples were analysed at an accelerating voltage of 200 kV in a bright field TEM at 600× magnification. The average diameter of the three-dimensional spheres was determined by fitting a histogram of the diameters of 176 circular sphere sections (measured from TEM images using Adobe Photoshop CS5.1) to a model that assumed the three-dimensional sphere diameters were normally distributed and that the spheres were randomly intersected by the section planes. The volume density of the spheres was determined by measuring the average area density from 25 square regions of interest (with average area of 9.26 μm^2) and then using standard stereological methods to convert this value to a volume density of 25 ± 3 spheres per μm^3.

2.5. Modelling light scattering from the spheres

Modelling was used to determine the angle-weighted scattering of the spheres. Methods are described in the electronic supplementary material. Briefly, we followed the methods of Bettelheim & Siew [25] to estimate the angle-weighted scattering from a dense collection of hard spheres as a function of the diameter of the spheres and their packing density.

3. Results and discussion

3.1. Morphology and spectrometry

Both inner mantle folds of *C. ales* have a unique marginal edge with two distinct sides (figure 1). The ventral side appears as a white band along the width of the tissue and is strongly scattering (more than 80% reflection over 400–550 nm). The dorsal side of the tissue, however, is red and is weakly scattering (less than 5% reflectance over 400–550 nm). This results in a roughly 16-fold difference in reflectance (figure 1), so the furling and unfurling of the mantle creates a highly dynamic signal. This is especially true at shorter wavelengths, which predominate in the clams' 3–50 m underwater crevice habitats [12]. Despite being thin (less than 25 µm), the white ventral side is optically thick, and therefore opaque. Spectrometry of the mantle tissue of the congener *C. scaber* did not show any optical asymmetry.

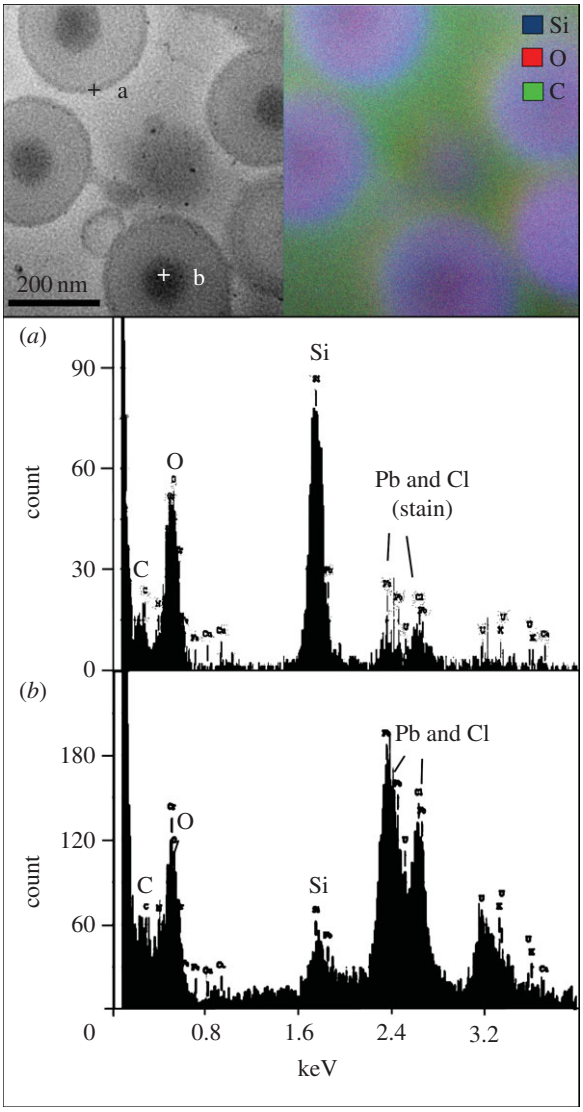

Figure 3. EDS elemental analysis shows the composition of the reflective spheres. Blue (silicon) and red (oxygen) combine to form the purple, amorphous silica spheres (SiO_2), while green (carbon) composes the underlying tissue. Both the outer shells (*a*) and the cores (*b*) of the spheres are composed of silica (silicon 1.70–1.80 keV, oxygen 0.40–0.60 keV).

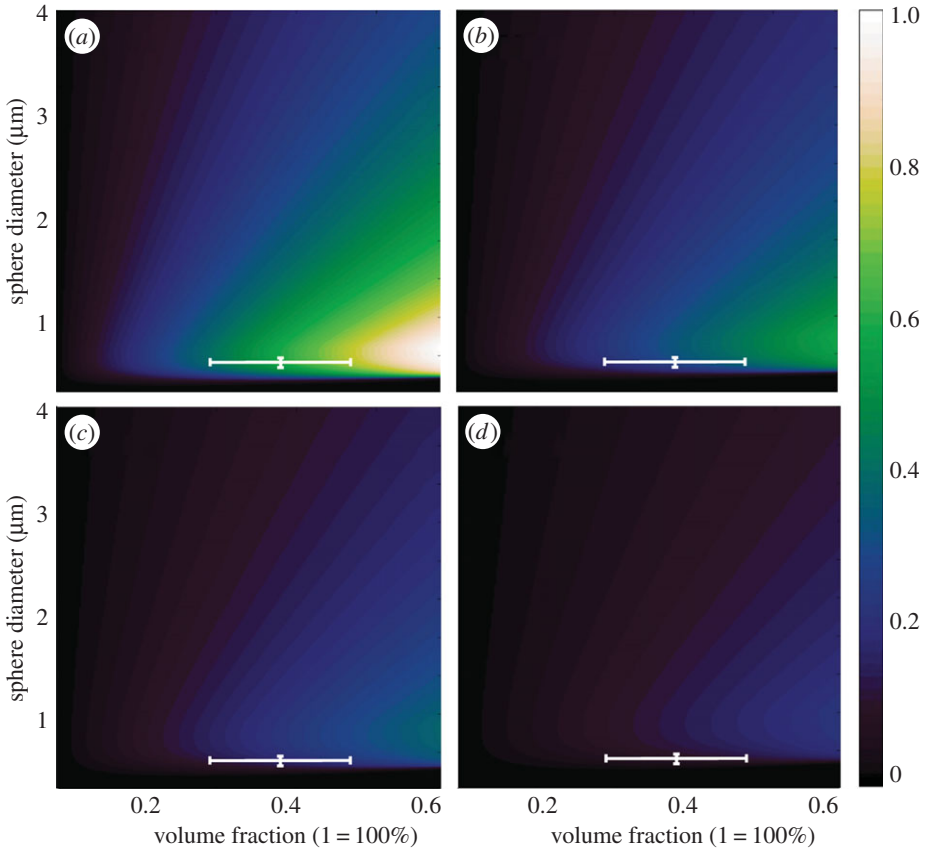

Figure 4. The effect of sphere diameter and density on the total amount of (*a*) 400, (*b*) 480, (*c*) 550 and (*d*) 650 nm angle-weighted scattered light from a dense collection of spheres (arbitrary units). The mean values (dots) and error bars show the range of the parameters found in *C. ales* tissue at four different wavelengths. The size of the spheres found in *C. ales* is close to optimal for maximal light scattering at 400 and 480 nm. Units are normalized to one for the maximum angle-weighted scattering for 400 nm light.

3.2. High-speed video

Black-and-white high-speed video (1000 frames per second) confirmed that the marginal edge unfurls and then furls back up in a wave-like motion (electronic supplementary material, file S2), similar to what was reported by Okubo *et al.* [15]. The unfurling motion exposes the highly reflective ventral side, and the furling motion exposes the poorly reflective dorsal side. The rapid transition creates the flashing appearance (electronic supplementary material, file S3). This pattern of movement occurs whenever the valves are open and infrared video shows that the movement also occurs in the dark. Although motions of the inner mantle folds help draw seawater into the gills for filter feeding [26–28], the motion of *C. ales* differs from typical mantle movement in that only the marginal edge of the inner mantle fold associated with the white band moves rapidly. This suggests that feeding or respiration is not the primary function [15]. No equivalent movement was seen in the congener *C. scaber*.

3.3. Transmission electron microscopy

TEM of *C. ales* showed that the tissue had two distinct sides: the ventral side of the mantle containing electron-dense spheres 0.30 µm ± 0.04 µm (mean ± s.d.) in diameter, and the dorsal side of the mantle, which did not (figure 2*a*). We measured 25 ± 3 spheres per μm^3, with a total volume fraction of 0.35 ± 0.1. *Ctenoides scaber* lacked any structures similar to those found in *C. ales* (figure 2*b*).

3.4. Energy dispersive X-ray spectroscopy

The spheres of *C. ales* were composed of amorphous silica (SiO_2), based on the presence of silicon (1.70–1.80 keV) and oxygen (0.40–0.60 keV) (figure 3). To the best of our knowledge, *C. ales* is the first animal to use silica as a scattering structure via intracellular nanospheres. Indeed, it is unusual to see silica secreted by animals for any purpose, except diatoms [17–19], sponges [29] and the weevil *Pachyrhynchus argus* [20]. Within molluscs, the only examples of silica secretion are in the radula in certain species of limpets and chitons [30].

3.5. Modelling light scattering from the spheres

We modelled the angle-weighted scattering of the dense collections of spheres using methods developed by Bettelheim & Siew [25] in order to determine how both sphere diameter and sphere packing influence angle-weighted scattering (electronic supplementary material, file S4). The true three-dimensional sphere diameter was 0.30 ± 0.04 µm (mean ± s.d.)—smaller than the 0.5–0.6 µm previously described by Okubo *et al.* [15]. The volume fraction of the spheres was 0.35 ± 0.1. This showed that the diameters of the spheres were close to the optimal value for scattering visible light, especially at shorter wavelengths (400 and 480 nm; figure 4). This broadband scattering creates a dynamic display at shorter wavelengths as the mantle is furled and unfurled. Similarly, many species of butterflies can create iridescent flashes as they fly using structural colours on one

side of their wings. These flashes have been suggested to increase signal efficacy [31–34].

3.6. Ecological function of the mantle display

The flashing of *C. ales* may serve a signalling function, possibly as a settlement cue. In field observations, 60% of specimens were found in groups of 2–4 ($n = 106$). Size differences suggested that settlement was asynchronous. Many species of bivalves possess light-sensitive eyes in the pediveliger stage, during which settlement occurs [35], so the display may be a cue for juvenile conspecifics.

Many other marine animals have the ability to detect the display of *C. ales*, as some crustaceans and fish have flicker fusion frequency thresholds from 13 to 75 Hz [36–39], which is well above the 2 Hz that *C. ales* flashes ($n = 7$). Preliminary looming trials, in which a stimulus was moved towards *C. ales*, showed that the flash rate roughly doubled for the 5 s following the stimulus (to 4 Hz, $n = 7$, $p = 0.003$, Mann–Whitney). This suggests that the display might serve an aposematic function.

If the 1-mm-wide flashing mantle edge is viewed from a distance of 100 mm, it subtends an angle of approximately 0.5°, which is visible to many reef fish, cephalopods or sharp-eyed crustaceans [40]. The flashing and movement, which widens the display beyond 1 mm, may also allow animals with less acute vision to view the display, especially in close proximity. Investigations into the behavioural function of the flashing are all ongoing.

Acknowledgements. The authors thank the Lizard Island Research Station, the Raja Ampat Research and Conservation Centre, Lembeh Resort, J. Auchterlonie, R. Templin and J. Drennan at the Center for Microscopy and Microanalysis at the University of Queensland, M. Zelman of Surface Optics Corporation (San Diego, CA, USA), D. Elias for high-speed video assistance and R. Zalpuri of the Electron Microscopy Lab, both of the University of California Berkeley.

Funding statement. This work was supported by the University of California Museum of Paleontology Palmer Fund, the NSF East Asia and Pacific Summer Institutes (EAPSI) Award, the Professional Association of Diving International (PADI) Foundation Award, the Animal Behavior Society Student Research Grant, the Conchologists of America Grant and the Lerner Gray Memorial Fund from the American Museum of Natural History. S.J. was supported in part by a grant from the Office of Naval Research (N00014-09-1-1053).

References

1. Mäthger MA, Denton EJ, Marshall NJ, Hanlon RT. 2009 Mechanisms and behavioural functions of structural coloration in cephalopods. *J. R. Soc. Interface* **6**, S149–S163. (doi:10.1098/rsif.2008.0366.focus)
2. Meadows MG, Butler MW, Morehouse NI, Taylor LA, Toomey MB, McGraw KJ, Rutowski RL. 2009 Iridescence: views from many angles. *J. R. Soc. Interface* **6**, 107–113. (doi:10.1098/rsif.2009.0013.focus)
3. Srinivasarao M. 1999 Nano-optics in the biological world: beetles, butterflies, birds, and moths. *Chem. Rev.* **99**, 1935–1961. (doi:10.1021/cr970080y)
4. Vukusic P, Sambles JR. 2003 Photonic structures in biology. *Nature* **424**, 852–855. (doi:10.1038/nature01941)
5. Hanlon RT, Messenger JB. 1996 *Cephalopod behaviour*. Cambridge, UK: Cambridge University Press.
6. Osorio D, Vorobyev M. 2008 A review of the evolution of animal colour vision and visual communication signals. *Vis. Res.* **48**, 2042–2051. (doi:10.1016/j.visres.2008.06.018)
7. Sweeney A, Jiggins C, Johnsen S. 2003 Polarized light as a butterfly mating signal. *Nature* **423**, 31–32. (doi:10.1038/423031a)
8. Johnsen S. 2001 Hidden in plain sight: the ecology and physiology of organismal transparency. *Biol. Bull.* **201**, 301–318. (doi:10.2307/1543609)
9. Cummings M. 2007 Sensory trade-offs predict signal divergence in surfperch. *Evolution* **61**, 530–545. (doi:10.1111/j.1558-5646.2007.00047.x)
10. Seehausen O *et al*. 2008 Speciation through sensory drive in cichlid fish. *Nature* **455**, 620–626. (doi:10.1038/nature07285)
11. Johnsen S. 2012 *The optics of life: a biologist's guide to light in nature*. Princeton, NJ: Princeton University Press.
12. Jerlov NG. 1976 *Marine optics*. Amsterdam, The Netherlands: Elsevier.
13. Okutani T. 1994 A new discovery of streak-like flashing luminescence in *Ctenoides ales*. *Jpn J. Malacol.* **53**, 57–59.
14. Mikkelsen PA, Bieler R. 2003 Systematic revision of the Western Atlantic file clams *Lima* and *Ctenoides* (Bivalvia: Limoida, Limidae). *Invert. Systemat.* **17**, 667–710. (doi:10.1071/IS03007)
15. Okubo S, Kurokawa T, Suzuki T, Funakoshi S, Tsujii T. 1997 On the mechanism of the streak-like flash on the mantle of *Ctenoides ales*. *Jpn J. Malacol.* **56**, 259–269.
16. Welch VL, Vigneron JP. 2007 Beyond butterflies—the diversity of biological photonic crystals. *Opt. Quant. Electron.* **39**, 295–303. (doi:10.1007/s11082-007-9094-4)
17. Fuhrmann T, Landwehr S, El Rharbi-Kucki M, Sumper M. 2004 Diatoms as living photonic crystals. *Appl. Phys. B* **78**, 257–260. (doi:10.1007/s00340-004-1419-4)
18. Wilson SD. 1966 A reflection-diffraction microscope for observing diatoms in color. *Appl. Opt.* **5**, 1683–1684. (doi:10.1364/AO.5.001683)
19. Noyes J. 2008 Light manipulation in a marine diatom. *J. Mater. Res.* **23**, 3229–3235. (doi:10.1557/JMR.2008.0381)
20. Parker AR, Welch VL, Driver D, Martini N. 2003 Opal analogue discovered in a weevil. *Nature* **426**, 786–787. (doi:10.1038/426786a)
21. Vukusic P, Hallam B, Noyes J. 2007 Brilliant whiteness in ultrathin beetle scales. *Science* **315**, 348. (doi:10.1126/science.1134666)
22. Stavenga DG, Stowe S, Siebke K, Zeil J, Arikawa K. 2004 Butterfly wing colours: scale beads make white pierid wings brighter. *Proc. R. Soc. Lond. B.* **271**, 1577–1584. (doi:10.1098/rspb.2004.2781)
23. Dubois R. 1877 Note sur la fonction photogénique chez le Pholas dactylus. *C. R. Soc. Biol.* **39**, 564–566.
24. Griffiths DJ, Winsor H, Luong-Van T. 1992 Iridophores in the mantle of giant clams. *Aust. J. Zool.* **40**, 319–326. (doi:10.1071/Z09920319)
25. Bettelheim FA, Siew EL. 1983 Effect of change in concentration upon lens turbidity as predicted by the random fluctuation theory. *Biophys. J.* **41**, 29–33. (doi:10.1016/S0006-3495(83)84402-6)
26. Galtsoff PS. 1964 The American oyster, *Crassostrea virginica* Gmelin. U.S. Fish Wildlife Serv. *Fish. Bull.* **64**, 1–457.
27. Morton JE, Yonge CM. 1964 Classification and structure of the Mollusca. In *Physiology of Mollusca*, vol. I (eds KM Wilbur, CM Yonge), pp. 1–58. New York, NY: Academic Press.
28. Owen G. 1966 Feeding. In *Physiology of Mollusca*, vol. II (eds KM Wilbur, CM Young), pp. 1–51. New York, NY: Academic Press.
29. Simpson TL, Volcani BE. 1981 *Silicon and siliceous structures in biological systems*. New York, NY: Springer.
30. Hua TE, Lee CW. 2007 Silica biomineralization in the radula of a limpet *Notoacmea schrenckii* (Gastropoda: Acmaeidae). *Zool. Stud.* **46**, 379–388.
31. Ghiradella H, Aneshansley D, Eisner T, Silberglied RE, Hinton HE. 1972 Ultraviolet reflection of a male butterfly: interference color caused by thin-layer

elaboration of wing scales. *Science* **178**, 1214–1217. (doi:10.1126/science.178.4066.1214)

32. Kemp DJ, Rutowski RL. 2007 Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution* **61**, 168–183. (doi:10.1111/j.1558-5646.2007.00014.x)
33. Vukusic P, Sambles JR, Lawrence CR, Wootton RJ. 2001 Now you see it—now you don't. *Nature* **410**, 36. (doi:10.1038/35065161)
34. Rutowski RL, Macedonia JM, Merry JW, Morehouse NI, Yturralde K, Taylor-Taft L, Gaalema D, Kemp DJ, Papke RS. 2007 Iridescent ultraviolet signal in the orange sulphur butterfly (*Colias eurytheme*): spatial, temporal and spectral properties. *Biol. J. Linnean Soc.* **90**, 349–364. (doi:10.1111/j.1095-8312.2007.00749.x)
35. Carriker MR. 1990 Functional significance of the peidiveligeir in bivalve development. In *The bivalvia: proceedings of a memorial symposium in honour of Sir Charles Maurice Yonge (1899–1986) at the 9th International Malacological Congress, 1986, Edinburgh, Scotland, UK* (ed. B Morton), pp. 267–282. Hong Kong: Hong Kong University Press.
36. Frank TM. 2000 Temporal resolution in mesopelagic crustaceans. *Phil. Trans. R. Soc. Lond. B* **355**, 1195–1198. (doi:10.1098/rstb.2000.0666)
37. Frank TM. 2003 Effects of light adaptation on the temporal resolution of deep-sea crustaceans. *Integr. Comp. Biol.* **43**, 559–570. (doi:10.1093/icb/43.4.559)
38. Horodysky AZ, Brill RW, Warrant EJ, Musick JA, Latour RJ. 2008 Comparative visual function in five sciaenid fishes inhabiting Chesapeake Bay. *J. Exp. Biol.* **211**, 3601–3612. (doi:10.1242/jeb.023358)
39. Horodysky AZ, Brill RW, Warrant EJ, Musick JA, Latour RJ. 2010 Comparative visual function in four piscivorous fishes inhabiting Chesapeake Bay. *J. Exp. Biol.* **213**, 1751–1761. (doi:10.1242/jeb.038117)
40. Land MF, Nilsson DE. 2002 *Animal eyes*. New York, NY: Oxford University Press.