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Bioluminescence of deep-sea coronate medusae (Cnidaria: Scyphozoa)

Abstract Bioluminescence is the production of visible light by a living organism. The light commonly appears as flashes from point sources (involving one or more cells, usually described as photocytes) or as a glandular secretion. A visible flash usually involves synchronous light emission from a group of cells or, if from a single-celled organism such as a dinoflagellate, from a group of organelles. The number of cells (or organelles) responding synchronously is the main determinant of the flash intensity. Bioluminescence is a common phenomenon in many deep-sea animals and is widespread among the Cnidaria. In this paper, we compare and contrast in situ and laboratory recordings of the bioluminescent responses of specimens of the deep-sea scyphozoans *Atolla wyvillei*, *Atolla vanhoffeni*, *Atolla parva*, *Nausithoe rubra*, *Paraphyllina intermedia*, *Periphyllopsis braueri* and *Periphylla periphylla*. Displays in all seven species consist of localised flashes and propagated waves of light in the surface epithelium. The first few single waves propagate at rates of up to 60 cm s^{-1} but subsequent ones in any sequence of stimuli gradually decrease in speed. After several single wave responses, a subsequent stimulus may elicit multiple waves that persist for several seconds. Following such a frenzy, the specimen becomes temporarily refractory to further stimuli, but if rested will recover its normal responses and may produce further frenzies. The dome area, situated above the coronal groove, of the genera *Paraphyllina, Periphylla,* and *Nausithoe* is covered with luminescent point sources. Such point sources are generally absent from the dome of species of *Atolla*. Captured specimens of *A. parva* also produce secretory bioluminescence, corroborating prior in situ observations of this ability. Secretory bioluminescence in *P. periphylla* takes the form of scintillating particles released from the lappet margins. We did not observe secretory displays in specimens of any other species in the laboratory, but one instance of apparent secretory luminescence was recorded in situ in a specimen of *A. wyvillei*.

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Introduction

The bioluminescent responses of deep-sea animals have become much better documented and appreciated as a result of the development of methods for their capture that minimise mechanical damage and/or physiological shock (Wild et al. 1985; Widder 2002). Delicate gelatinous animals are particularly susceptible to mechanical damage; the responses of specimens taken in standard nets, even if equipped with a protective closing codend, rarely match the complexity of those observed in situ from submersibles or Remotely Operated Vehicles (ROVs). The responses of several specimens of *Atolla wyvillei* taken in midwater trawls equipped with a closing cod-end have been described previously. Waves of bioluminescence travel round the aboral surface of the animal in a variety of patterns (Herring 1990). Inevitably these experimental animals had still suffered damage in contact with the net, prior to reaching the relative protection of the cod-end.

Recent studies on the ecology of the gelatinous fauna of the deep sea have derived much of their information from direct in situ observations of the animals, using manned submersibles and remote observations from ROVs (Pugh 1989; Larson et al. 1991). Capture methods are now available from both types of platform for the recovery of intact single specimens and their transfer to ship (or shore) laboratories. We have used these techniques to examine the bioluminescent responses of a variety of animals (e.g. Herring et al. 1992; Widder et al.

1992a; Johnsen et al. 1999) and report here the results of observations of specimens of two widespread genera of coronate scyphozoans, *Atolla* and *Periphylla,* and three much less common coronates, *Nausithoe rubra*, *Paraphyllina intermedia* and *Periphyllopsis braueri*. A preliminary account of the responses of some specimens of *Periphylla periphylla* has been given previously (Herring et al. 1997).

Materials and methods

Specimens of *Atolla* spp. ($n=23$) and *Periphylla periphylla* ($n=21$) were captured individually with the Johnson Sea-Link (JSL) manned submersible during cruises between 1989 and 1999 in the western North Atlantic and in 1991 in the western Mediterranean (Alboran Sea). Large specimens of *P. periphylla* ($n=84$) were obtained during two cruises of the R/V ''Håkon Mosby'' in Lurefjord, Norway, in 1994 and 1995. In this fjord, individuals undertake a diel vertical migration from deep water that may bring them to the surface on moonless nights (Youngbluth and Bamstedt 2001). Individual specimens were collected in buckets from the surface during such nights (using an inflatable dinghy) and stored on deck wrapped in opaque black polythene until required for experiment. A single specimen of *Periphyllopsis braueri* and two of *Paraphyllina intermedia* were collected in the Bahamas in 1989, and single specimens of *Nausithoe rubra* and *Paraphyllina intermedia* were collected in the Gulf of Mexico in 1995 and in the Bahamas in 1996, respectively. These specimens were collected with the JSL's detritus samplers, which are 11 l clear acrylic cylinders with hydraulically activated sliding lids. Medusae were transferred in their samplers from the submersible to a shipboard cold laboratory for temporary storage and were subsequently placed in crystallising dishes containing cooled seawater (8–12°C) for transfer to the ship's darkroom.

Mechanical stimuli, using a mounted steel needle, were given by hand (a glass probe produced similar responses). Electrical stimuli (20 V, 5 ms) were delivered through Pt electrodes connected to a Grass square-wave stimulator. Bioluminescent responses to the stimulation were recorded with a Dage ISIT image-intensified videocamera or Intevac GenIISys image intensifier system and CCD videocamera. The images were analysed on a frame-by-frame basis. The conduction rates of propagated responses were determined from the time taken for a luminous wave to travel at least half the circumference of any particular specimen. The circumference was calculated from the diameter measured from lappet tip to lappet tip.

Some specimens were stimulated in situ by being manoeuvred into a horizontal acrylic cylinder (24 cm diameter) mounted on the submersible and with 1.8-mm mesh nylon screen across its rear end. The submersible was then driven gently forward so that the animal struck the mesh. The results of the impact were recorded using a Sub Sea Systems underwater Silicon Intensified Target (SIT) video camera focused on the screen, and compared with the responses obtained in the laboratory. Specimens were then collected for identification by means of a suction sampling device connected to the proximal end of the acrylic cylinder. Specimens were drawn down into numbered collection buckets mounted in a carousel on the lower work platform of the submersible (illustrated in Widder et al. 1992b).

The experimental procedures employed for studying the effects of temperature and light are given in the relevant paragraph of the Results.

Results

Atolla spp.

Three species of *Atolla* were examined, *A. wyvillei*, *A. vanhoffeni* and *A. parva*. Responses were recorded from 17 specimens of *A. wyvillei* (size range 20–174 mm diameter), three specimens of *A. vanhoffeni* (63–113 mm) and three of *A. parva* (27–50 mm). The flash patterns and propagation were fundamentally similar in all three species, but showed considerable variation, both between specimens and in any one specimen at different stages of stimulation (Fig. 1).

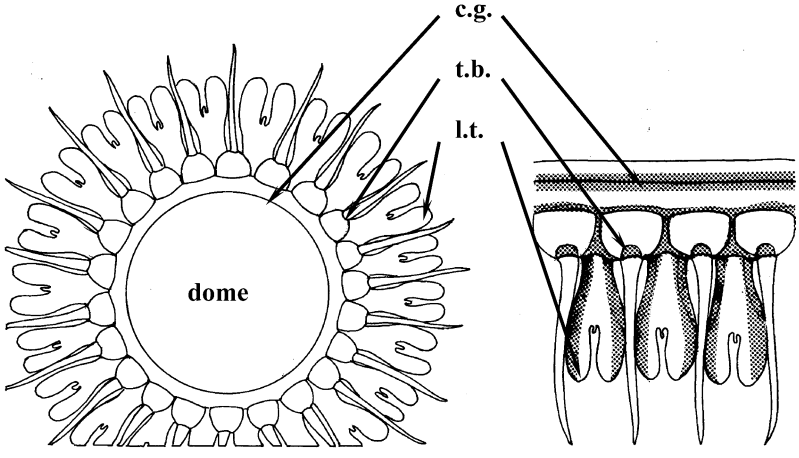


Fig. 1 *Left* Diagrammatic view of the aboral or exumbrellar surface of an *Atolla wyvillei* (not to scale) showing the main feaures noted in the text, and *right* an enlarged view of a typical region with the main bioluminescent areas shaded (*c.g.* coronal groove, *t.b.* temtacle base, l.t. lappet tip; modified from Herring 1990)

Mechanical stimulation, equivalent to contact with another animal, was provided by the touch of a mounted needle in the region of the coronal groove, the tentacle bases or the lappet margins (Fig. 1). Stimuli were repeated at variable intervals of between 2 and 10 s, long enough to allow the completion of one response before another was initiated. A gentle first stimulus often produced only a limited local flash response lasting about 500 ms (Herring 1990), particularly when the lappets were stimulated. Subsequent stimuli usually resulted in an increase in the intensity of the local bioluminescence and propagation of the response as a wave of bioluminescent flashes moving over the upper (exumbrellar) surface. A stronger first stimulus would usually initiate a propagated wave immediately. Stimulation at the lappet margins resulted in a wave that propagated radially inwards until it reached the tentacle bases and/or coronal groove, where the wave divided to continue in both clockwise and anticlockwise directions. Propagation initiated at the coronal groove or the tentacle bases was primarily circumferential, with radial extensions down the lappets as the main wave reached each of their inner margins. Propagation of the bioluminescence was often disjunct, with dark regions between the illuminated ones. Bioluminescence was not usually observed on the central portion of the exumbrellar surface (or dome) encircled by the coronal groove, but mechanical stimulation within this area did result in the appearance of bioluminescence at the peripheral sites noted above. In a few specimens of *Atolla spp* discrete spots or patches of luminescence were observed in this region, particularly in one *A. vanhoffeni* (Fig. 2).

Bioluminescence was never observed from any of the tentacles in laboratory specimens nor did tentacle stimulation ever initiate bioluminescence elsewhere.

Multiple responses

The propagated wave noted above was unpolarised, travelling in both clockwise and anticlockwise directions. It could continue for any distance, ranging from a few mm to half the circumference (Fig. 3).

In the latter case the two bioluminescent waves usually disappeared at the point where the two components met, typically on the opposite side of the exumbrellar surface, 180° from the start position. In some cases propagation in one of the directions failed after a short distance. In these circumstances the other wave often continued to the point of failure of the first one. In a very few cases propagation failed altogether in one direction and the wave in the other direction first continued all the way round and then made repeated circuits of the exumbrellar surface (up to 11 circuits were recorded from a single wave) (Fig. 4).

Following repeated stimulation many specimens were finally induced into a "frenzy" of bioluminescent responses. In the simplest of these a single stimulus initiates repeated bioluminescent waves from the same

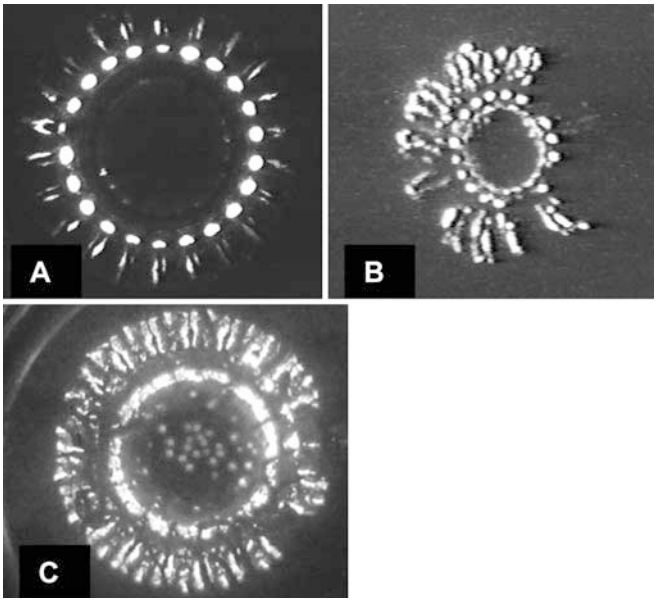


Fig. 2 Representative bioluminescence images (viewed from the exumbrellar surface) of **a** *A. wyvillei* (90 mm diameter), **b** *A. parva* (32 mm diameter) and **c** *A. vanhoffeni* (61 mm diameter). Typical images from all three species show luminescence down the lappets, at the tentacle bases and in the coronal groove region. This image of *A.vanhoffeni* also shows some bright sources present within the area circumscribed by the coronal groove

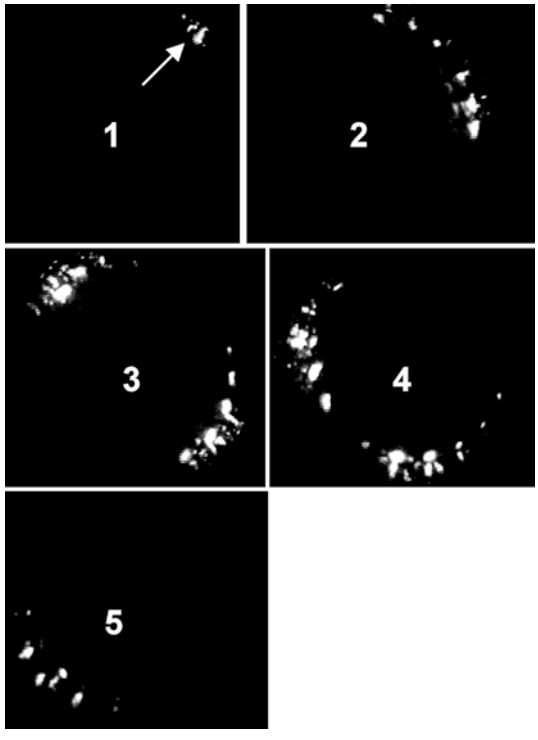


Fig. 3 Unpolarised propagation of clockwise and anticlockwise waves from a single stimulus to a 47-mm diameter *A. vanhoffeni*. Frame intervals are 330 ms. The stimulus was applied at the point indicated by the arrow in frame *1*, where luminescence first appeared. Two luminescent waves propagated away from the stimulus site in frames *2–4* and met directly opposite the point of stimulation in frame *5*

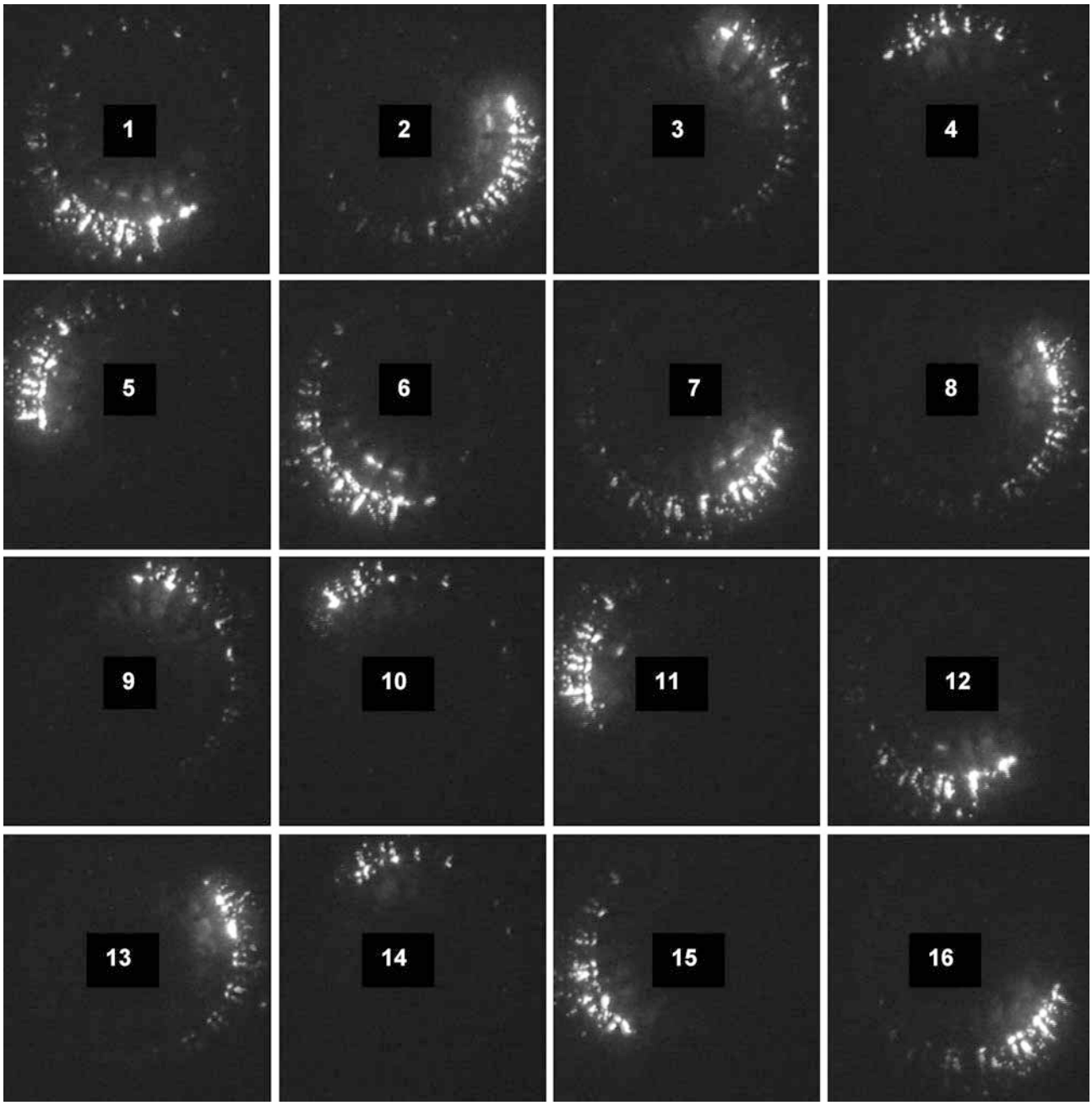


Fig. 4 Three circuits of an anti-clockwise luminous wave in the same specimen as Fig. 2. Frames *1–11* are at intervals of 330 ms, frames *12–16* follow at 660 ms intervals as the propagation rate slowed

source, the second and subsequent waves commencing at intervals of a few hundred ms, before the light from the previous wave is entirely extinguished (Fig. 5). More complex frenzies arose from instances in which secondary waves were initiated from a new source (or sources), usually (but not invariably) displaced 180° from the original stimulus and first visible on the lappet margin or at a tentacle base. In a few instances a wave travelled for short distances along the coronal groove in the opposite direction to one moving simultaneously around the tentacle bases.

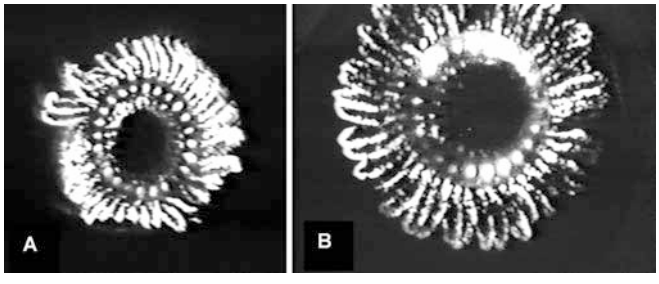


Fig. 5 Appearance of two specimens of *A. wyvillei* viewed from the exumbrellar surface, **a** 50 mm diameter and **b** 66 mm diameter, during bioluminescent frenzies. Note the outline of the lappets and the bright spots at the tentacle bases, where increased brightness in **b** indicates the passage of one of the luminous waves superimposed on the decaying luminescence from the previous one

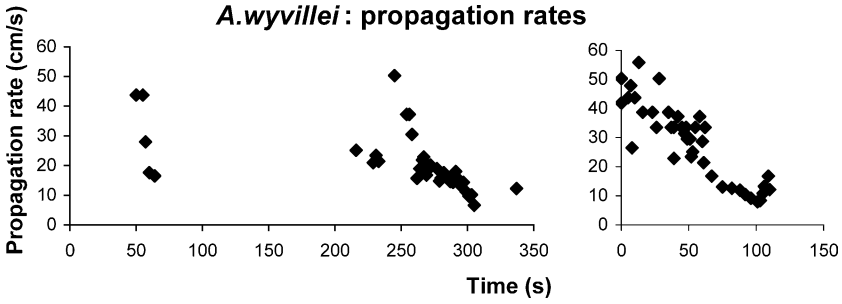


Fig. 6 Reduction in the propagation rate of single waves in a 200-mm diameter *A. wyvillei* during a series of stimuli. The x-axis label ("time (s)") indicates the elapsed time from the start of the particular series. In the first series a stimulus at time 0 elicited only a very short propagation distance. There is a rapid reduction in rate over the first 5 effective stimuli (given between 50 and 64 s), a slight increase in rate over the next 4 stimuli (at 216s-233 s), and a further large increase and subsequent fall from 249 s. A 10-min rest ensued before the second series of stimuli, by which time the rate had recovered to its original value before declining again

Combinations of repeated waves, varying pathways of propagation, and the spontaneous appearance of new sources generated very complex patterns of bioluminescence, which circulated rapidly over the exumbrellar surface for periods of up to 10–15 s.

Following such a frenzy the specimen would become temporarily refractory to further stimuli for a period of up to a few minutes, but if rested would recover its normal responses and produce further frenzies.

Propagation rates

The first few single waves propagated at rates of 40–60 cm s^{-1} (at 10–14°C) but subsequent ones in any sequence of stimuli gradually decreased in speed, the last one or two (before failure) declining to less than 5 cm s^{-1}. After a break in the stimulus sequence of a minute or more the propagation rate often recovered to a rate approaching the original speed (Fig. 6).

We have recorded more than 200 flash (wave) responses from a single specimen. The cycle of stimuli and pauses can be repeated on many occasions before the animal fails to respond altogether, a state usually preceded by a condition in which local flashes still appeared but propagated responses were no longer produced. During the passage of the first few waves of a series the observed intensity gradually increased at each site but later in the sequence the propagated responses became dimmer and increasingly disjunct.

Although the propagation rate in any sequence of stimuli showed an overall decline, the regular decline only applies strictly to waves resulting from the continued stimulation of one region. If the stimulus point was moved elsewhere the first few responses from the new site sometimes had an enhanced propagation rate compared to the reduced rate at the previous site. In the case of a continuously circling wave the propagation rate also declined gradually with time (Fig. 7), but the early

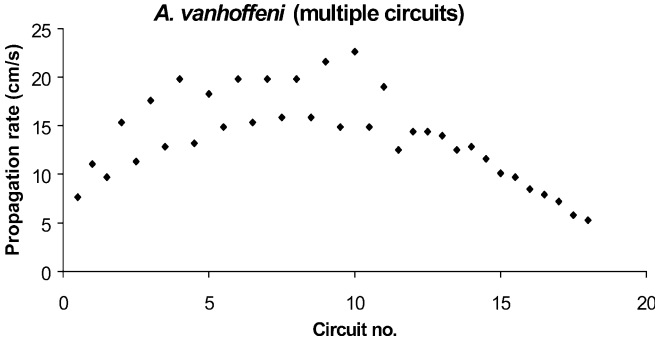


Fig. 7 Propagation rate measured during 18 circuits of a single luminous wave in *A. vanhoffeni* (61 mm diameter). Data points represent the rates for each half circuit. The rate increases gradually and then declines over a total time of 27 s. During the first 11 circuits the rates differ over each half of the animal; later they become indistinguishable

circuits of a frenzy sometimes showed a brief increase, perhaps indicating short-term facilitation.

We have recorded a wide range of propagation rates in specimens of *A. wyvillei* but have not observed any size-related differences. Rates in the smaller species *A. vanhoffeni* and *A. parva* fell within the range observed in *A. wyvillei*. The intensities of flashes were not recorded (and varied as described above) but we have not noticed any consistent differences between animals of different sizes.

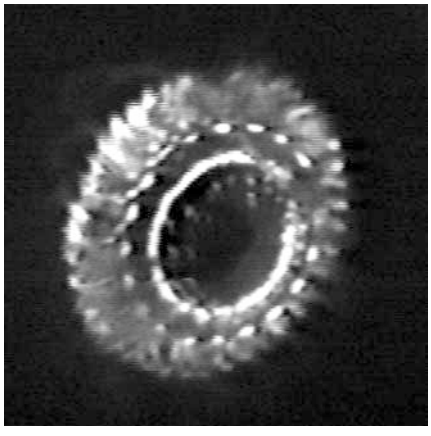


Fig. 8 A swimming specimen of *A. parva* (50 mm diam) luminescing at the same time. The lappets, tentacle base spots and coronal groove are clearly visible

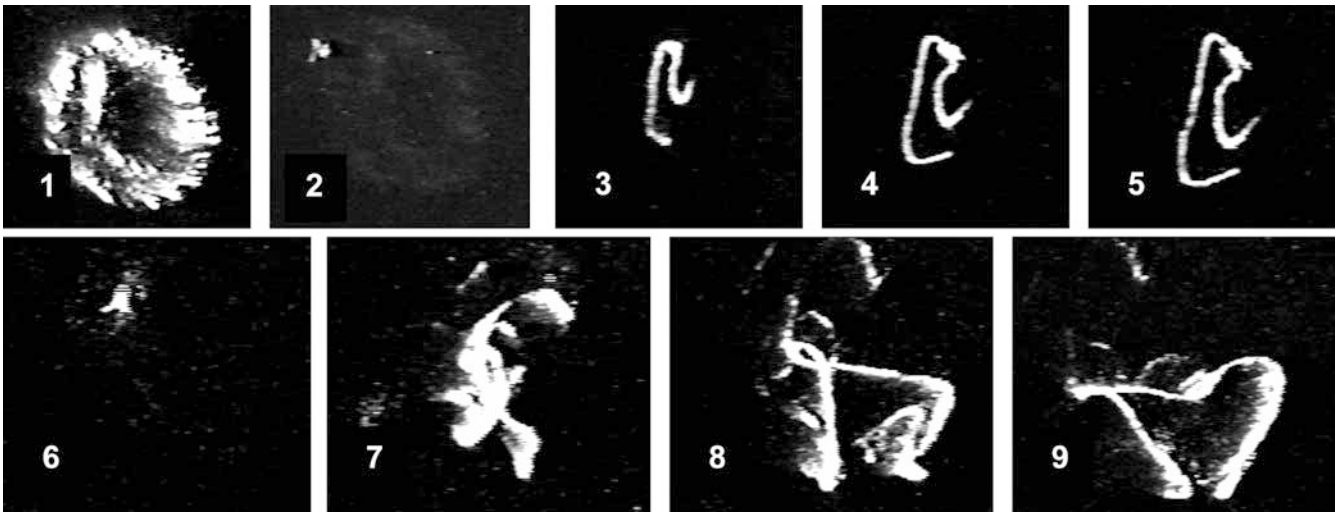


Fig. 9 Two episodes of secretion by a luminescing specimen of *A. parva* (36 mm diam). Frame intervals at top (*1–5*) are 1,320 ms, 1,320 ms, 1,320 ms and 2,640 ms ; the second sequence (*6–9*) is at intervals of 1,320 ms. A whole body flash (*1*) precedes the secretion in the first sequence

The pathway for the spread of bioluminescence in any one specimen tended to be consistent over a series of stimulation periods (Herring 1990). The most frequently observed pathway involved only the tentacle base regions (e.g. Fig. 2a); propagation along the coronal groove was relatively infrequent. Involvement of the lappets was usually associated with luminescence at the tentacle bases, but occasionally occurred independently.

Several specimens began swimming, either spontaneously or in response to the mechanical stimulus. Some of these luminesced at the same time (Fig. 8), but the two processes (the propagation of muscle contraction for swimming and the propagation of the luminescent waves) were asynchronous and appeared to be physiologically independent.

Secretory bioluminescence

None of our laboratory specimens of *A. wyvillei* or *A. vanhoffeni* exhibited any form of luminescence other than the intracellular flashes and propagated waves described above, but two specimens of *A. parva* produced additional secreted bioluminescence, substantiating prior in situ observations of this ability (Widder et al. 1989). The bioluminescence appeared in the form of one or two discrete thin streamers of light from one point on the lateral margin and lasted for several tens of seconds as a coherent luminous thread, gradually decaying and distorting in the experimental vessel (Fig. 9). One of the specimens responded twice with a secretion but the precise source of emission was not discernible. We also observed an apparent in situ secretion from the tentacles of one specimen of *A. wyvillei* (see In situ stimulation, below).

Nausithoe rubra

Bioluminescence in the specimen of *Nausithoe rubra* was similar to that of the *Atolla* spp., except that the dome above the coronal groove was luminescent. Stimulation at the lappet margins initiated a wave that propagated radially inwards until it reached the coronal groove, where the wave divided to continue in both clockwise and anticlockwise directions. At approximately the point where the two waves met, opposite the site of stimulation, the luminescence crossed the coronal groove and spread over the dome as a wave (Fig. 10).

Paraphyllina intermedia

The luminescence of *Paraphyllina intermedia* was more clearly composed of bright point sources than that of

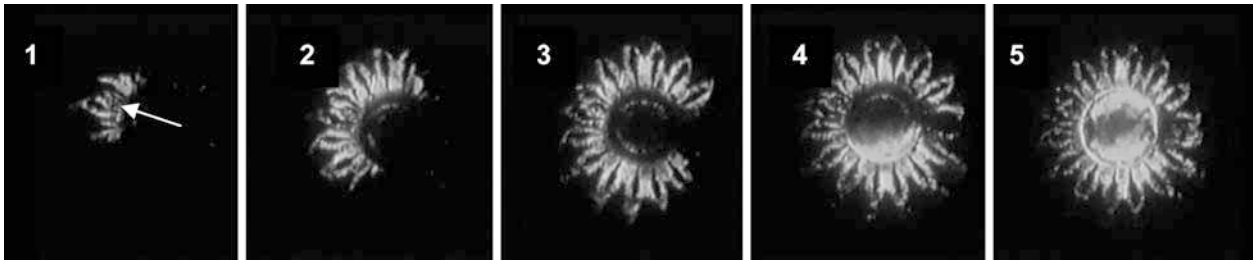


Fig. 10 Unpolarised spread of a wave of bioluminescence in *Nausithoe rubra*, from the point of stimulation indicated by the arrow, round the lappets, within the coronal groove and over the central dome. Frame intervals are 200 ms

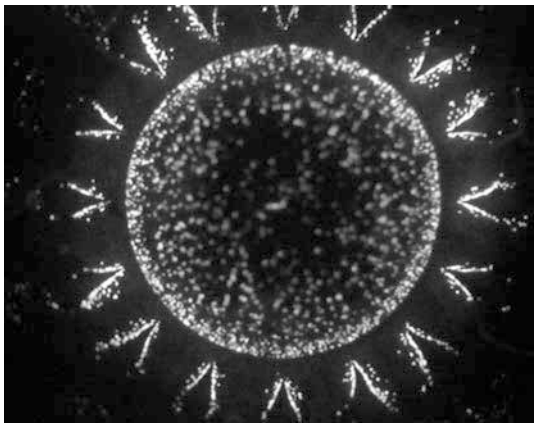


Fig. 11 Appearance of a luminescing specimen of *Paraphyllina intermedia* (coronal groove diameter 50 mm). The edges of adjacent lappet bases produce a V-shaped pattern and luminous sources are spread over the whole of the dome

Atolla spp. or *Nausithoe rubra*. A mechanical stimulus in the coronal groove region elicited bioluminescence down the outer margins of each lappet, so that the junction between adjacent lappets produced a V-shaped pattern (Fig. 11). The luminescence spread as a wave both radially and across the exumbrellar surface and a strong stimulus resulted in a series of such waves.

Periphyllopsis braueri

The specimen responded to a touch with local luminescence that consisted of a large number of rapidly pulsing sites. Stroking the exumbrella produced persistent lines of luminescence. Sticky luminous material remained attached to the probe and appeared to be shed into the water, flashing as separate particles for periods of tens of seconds. The general impression was that the responses were almost identical to those of *Periphylla periphylla* (below).

Periphylla periphylla

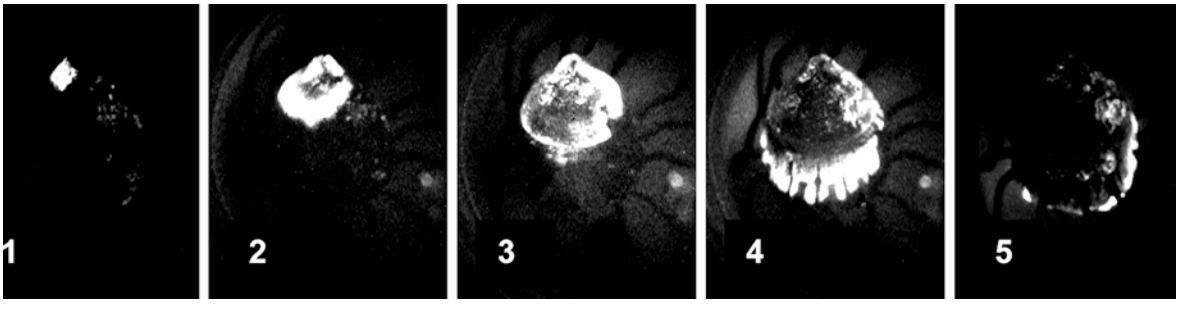
Our specimens of *P. periphylla* collected from the Atlantic and Mediterranean (21 specimens) were relatively small, with a coronal groove diameter of up to 35 mm. Those from Lurefjord (84 specimens) were considerably larger; most had a coronal groove diameter of >100 mm and the largest reached 134 mm. The bioluminescent responses were very similar over the entire size range. They differed from those of species of *Atolla* primarily in that (1) the conical dome situated above the coronal groove was also luminescent, and (2) scintillating particles were released from the lappet margins.

The basic response to a mechanical or electrical stimulus at any point on the dome or lappets was a local flash that propagated radially as a wave of luminescence. Hundreds of such responses can be obtained from a single specimen in good condition. Waves initiated on the dome crossed the coronal groove and travelled down the lappets without hindrance, and vice versa (Fig. 12).

The primary wavefront was of uniform intensity, but is followed very closely by a second wavefront of brighter point sources that persist for about 1 s. The first few stimuli induced increasingly bright responses that reach a maximum; further stimuli resulted in a gradual decline in observed intensity. The propagation rate ranged from <5–50 cm s^{-1} and during the course of repeated electrical stimuli (20 V, 5 ms) at rates of 0.5–1 s^{-1} the speed of conduction rapidly declined.. This decline could be temporarily reversed by a period of rest lasting about a minute (Fig. 13). Over an extended series of stimuli a gradual disruption in the early uniformly radial propagation appeared, resulting in erratic and disjunct propagation pathways.

A single stimulus could elicit multiple waves, either from the original point of stimulus or from unstimulated new sources. The coronal groove could become either a total barrier to propagation, so that waves progressed either around the dome or around the lappets, or a partial one, in which case waves propagated across it only at a specific point. After numerous stimuli a "frenzy" of wave propagation could occur, with multiple waves propagating around all or part of the exumbrellar surface and often continuing for several seconds. Different specimens exhibited every variety of luminescent response noted above. In some, the first few stimuli resulted in waves that were hardly visible until they

Fig. 12 Lateral view of the propagation of a luminous wave in *P. periphylla* (coronal groove diam. 134 mm) from a stimulus just below the dome apex (*1*) to the lappet margins (*4,5*). Frame intervals are 240 ms



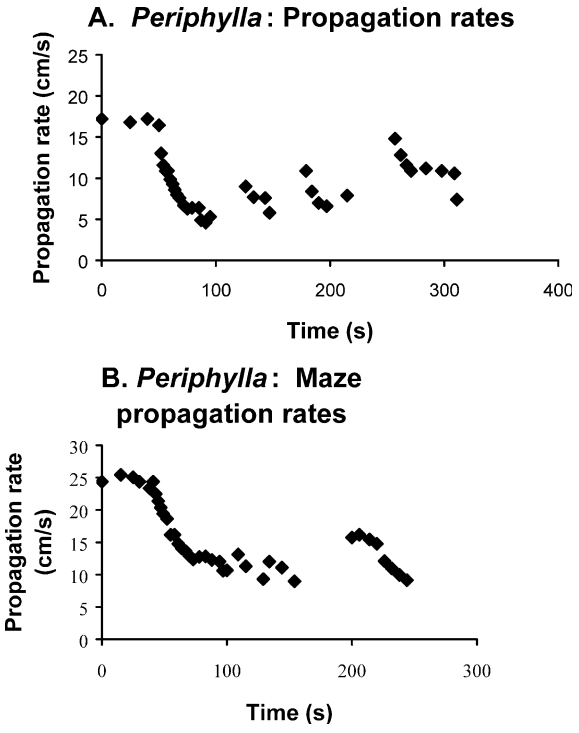


Fig. 13a, b Varying propagation rate of luminous waves in *P. periphylla* initiated by successive stimuli. **a** propagation around a whole animal (90 mm diameter); **b** propagation over a 345-mm maze pathway marked on an isolated portion of a dome (see Fig. 16). At each pause between stimuli the rate partially recovers from its previous decline

reached the lappet margins. In others, a wave could be induced that was largely restricted to the coronal groove and extended out from it down particular lappets (Fig. 14).

The first waves of luminescence usually left a few sites in their wake scintillating brightly but briefly. With successive stimuli the scintillating sites increased in number and duration. When an early wave reached the lappet margins there was a very bright and persistent scintillating response at the margins (Fig. 15 1–5). If the specimen was free-swimming this bright luminescence was usually released in the form of a cloud of scintillating particles left in the animal's wake (Fig. 15 6–10).

Fig. 14 Propagation sequence of a wave elicited in a splayed specimen of *P. periphylla* by a stimulus at the site indicated in (*1*) by the *arrow*. The wave is largely confined to the coronal groove (104 mm diameter) and to the margins of 3–4 lappets (*4,5*). Frame intervals are 400 ms

Each particle flashed repeatedly at a rate that rapidly declined from a maximum frequency of more than 5 s^{-1} to less than 1 s^{-1} but which could continue for several minutes.

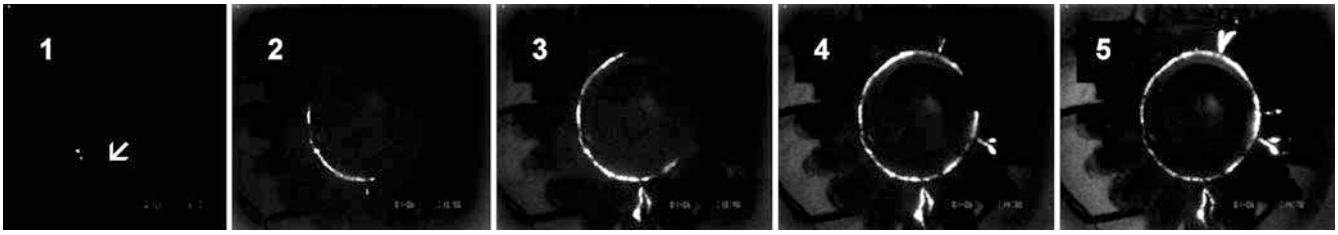
Any damage to the epithelial surface resulted in longer scintillation at the site when a wave passed over the region. A surface cut became visible as two parallel lines of scintillating sites with a dark centre; after repeated stimulation the two sides became less distinct. A wave of luminescence would not propagate across a cut surface, however superficial the incision. As a result, it was possible to incise a maze on the surface of the dome and then stimulate a wave of luminescence to travel along the defined pathway (Fig. 16). Similarly, stimuli within a box or other closed area of incisions only propagated within it (Fig. 17).

Exposure to high intensity light (e.g. daylight) reduced the light emission after subsequent stimuli and could block conduction of a bioluminescence wave altogether. An opaque black mask with a narrow slit in it was placed over one specimen and the dome illuminated for 10 min through the slit with a fibre optic illuminator. The conduction of a subsequent wave of luminescence was apparently unhindered by the illuminated region but light emission from it was blocked and the region remained dark (Fig. 18). The specimen was re-tested several times over a period of 3–4 h, but the region never regained its luminescent ability. As previously noted (Herring et al. 1997) luminescence could also be blocked by local application of isotonic $MgCl_2$, but this inhibition was reversible.

Temperature effects

One Lurefjord animal was cut into four quadrants and each quadrant immersed for 20 min in seawater at a different defined temperature (the highest one was above the normal environmental range for Lurefjord, but at the limit of that for Mediterranean specimens). The quadrants were separately stimulated and the propagation rates of the first few responses determined from a video recording. The results (Fig. 19) demonstrated that an increase in temperature had a positive effect on the propagation rate, at least in the short term. A second animal, tested over a similar temperature range, gave qualitatively very similar results (not shown) but the data are insufficient for statistical analysis.

Temperature also affected the secretions from the lappet margins. The frequency of scintillation in the particles increased with a rise in water temperature and



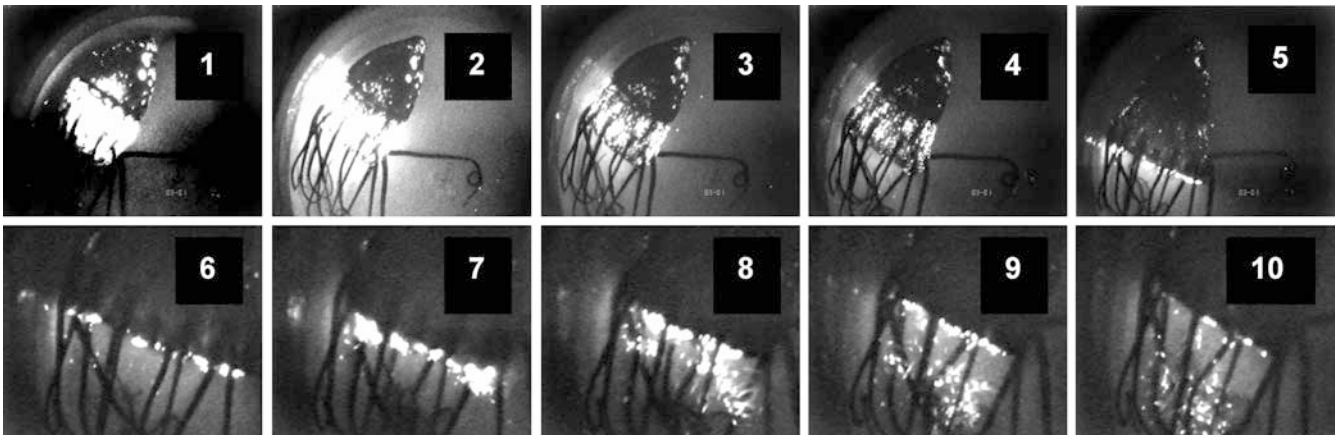


Fig. 15 Lateral view of the bioluminescence response of a free-swimming *P. periphylla* (coronal groove diameter 70 mm) to a single stimulus on the dome 200 ms before frame *1*. Frames *1–5* (200 ms intervals) show the passage of the main wave down to the lappets. Frame *6* (1 s later) shows a persistent bright scintillation at the lappet margins. Frames *7–10* start 1 s later at frame intervals of 200 ms and show the expulsion of scintillating particles from the lappet margins combined with a swimming pulse (frames *6–10* are slightly enlarged relative to frames *1–5*)

the luminous lifetime of the particles decreased equivalently.

In situ stimulation

The responses of in situ oceanic specimens of both *Atolla* spp. and *Periphylla periphylla* to impact on a mesh screen were recorded with a SIT videocamera (as described in Materials and methods), and the records examined for comparison with the behaviour of animals stimulated in the laboratory. Both genera responded to the impact with single or multiple propagated waves of luminescence and although precise measurements were impracticable, the propagation rates (about 30 cm s^{-1}) and patterns were very similar to those elicited in the laboratory by a strong stimulus. ROV observations of larger Lurefjord specimens of *P. periphylla* also confirmed the equivalence of the *in situ* and laboratory responses (P. Flood, unpublished observations).

The only different response we recorded was that of one *A. wyvillei*, in which impact on the screen produced not only the propagated circular waves of light but also the appearance of secretory luminescence from several sites within the tentacle mass.

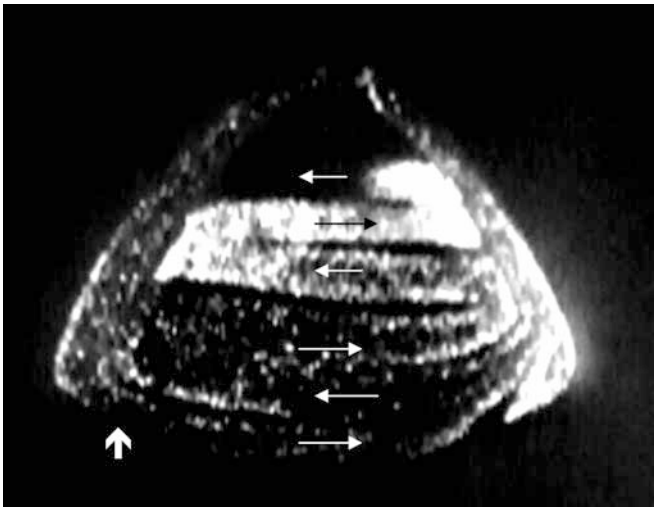


Fig. 16 Portion of the dome of a *P. periphylla* with a series of superficial incisions resulting in a wave of luminescence initiated at bottom left following a sinuous propagation pathway, as indicated by the *arrows*. The stimulus point is indicated by the *heavy arrow* at lower left

Discussion

The bioluminescent responses of the four genera are fundamentally similar in that a propagated wave of light spreads radially from the point of stimulation over some or all of the exumbrellar surface of the animal. The variation in the spread is clearly a function of ''condition'', incorporating both physiological history (including previous stimulation) and the degree of epithelial integrity. In scyphozoans the neuroeffector pathways are believed to operate through the medium of a diffuse nerve net (Passano and Passano 1971; Passano 1982; Josephson 1974; Arai 1997) and the non-polar and diffuse spread of the luminous wave is in accord with this hypothesis. Recent antibody labelling of scyphozoan nerve nets indicates two separate nets, one FMRFamide positive and the other labelled by a neuron-specific monoclonal antibody (R.Marx, personal communication). The multiple circuits observed in some specimens are reminiscent of the ''entrapped circuit waves'' of muscle contraction demonstrated in the scyphozoan *Cassiopea xamachana* by A.G. Mayer in the early 1900s (see Passano 1982) and in the hydrozoan *Stomotoca atra* by Mackie (1975). The visible luminescence is the integral of the spread of conduction and the luminescent potential of the epithelium at different locations. Nevertheless, there do appear to be preferred circuits, at least in species of *Atolla*, in that separate

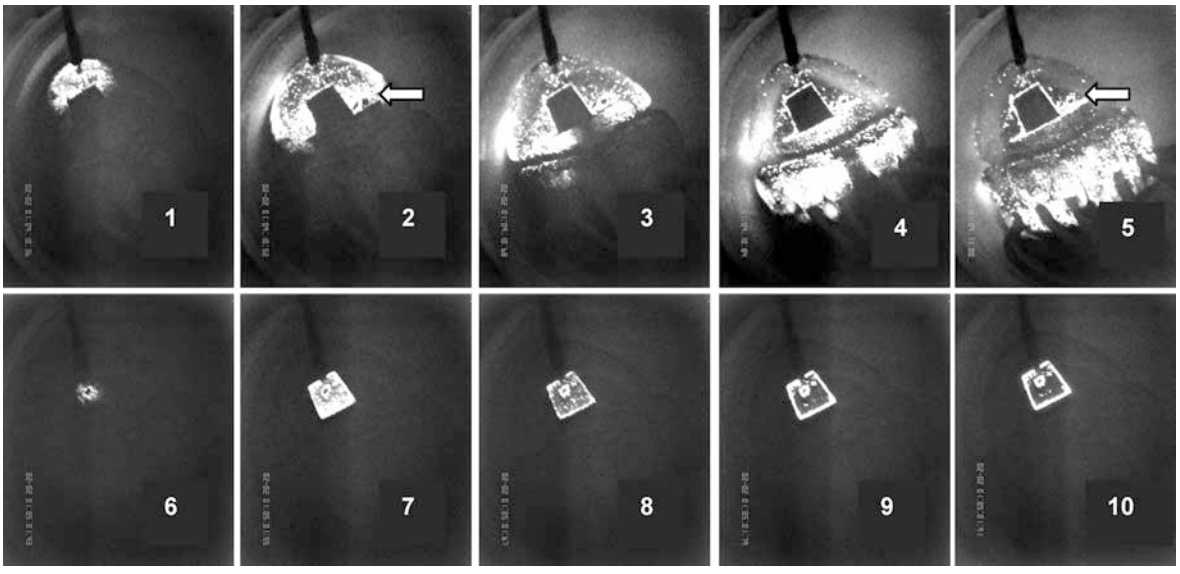


Fig. 17 Restricted propagation of an electrically stimulated luminous wave following surface incisions on the dome of a *P. periphylla*. Frames *1–5*: propagation of luminescence from the dome apex is blocked by incisions forming a square with an elongated base (arrow) (frame intervals 160 ms). The wave propagates round the obstruction, which leaves a temporary dark shadow below it. Frames *6–10*: stimulation within the square does not spread beyond it (frame intervals 400 ms). In both situations the incisions (and electrode puncture) continue to scintillate after the wave has passed

waves of luminescence may propagate around the coronal groove, the lappets or the tentacle bases in different specimens and occasionally even in opposite directions. Some specimens of *Periphylla periphylla* produced a luminous wave largely confined to the coronal groove and in others the waves induced late in the stimulus sequence could be confined to either the dome or the lappets. Clearly the coronal groove provides a potential barrier to conduction, which becomes increasingly marked in the course of long periods of stimulation. The lack of luminescence in *Atolla* spp. from within the area bounded by the coronal groove (the equivalent of the dome in *P. periphylla*) is simply due to an absence of luminous cells, not conduction failure, because stimulation within the area results in typical propagated responses outside it. This is a major difference in the characteristics of the bioluminescence in the two genera, for the dome of *P. periphylla* is uniformly luminous (as is that of *Paraphyllina intermedia* and *Nausithoe rubra*). Although stimulation within the (non-luminous) central region of *Atolla wyvillei* initiates luminescence elsewhere, stimulation of the tentacles has no such effect. The sensory network involved in tentacle responses is clearly divorced from the effector system of luminous responses. This is in marked contrast to the hydrozoan medusa *Solmissus incisa* in which luminescence spreads down the length of the tentacles (Herring and Widder, unpublished observations).

Secretory bioluminescence occurs in both *Atolla parva* and *P. periphylla*, but its appearance is quite different. *A. parva* is the only species of *Atolla* from which we have repeatedly observed secretion. The material appeared from just one or two sites, forming a discrete thread of steady luminescence, and dispersed gradually in the turbulence within the container. The lack of synchrony between the dark and the illuminated images of the specimens prevented us from establishing the precise source of the luminescence. The secretion from *P. periphylla* is very different; it consists of brightly scintillating particles and clearly emanates from the lappet margins, though the animal has to swim in order

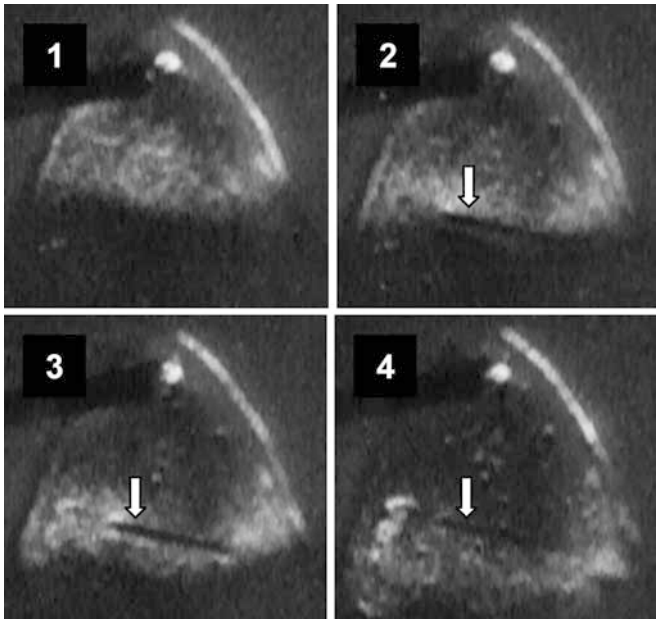


Fig. 18 Light inhibition: a portion of the dome epithelium of a *P. periphylla* was illuminated through a vertical slit (see text) and the animal later stimulated electrically. The passage of the luminous wave from the bright stimulus point at the top is unaffected (there is no shadow as there is in Fig. 17), but the region under the illuminated slit (*arrow*) remains dark (frame intervals 40 ms)

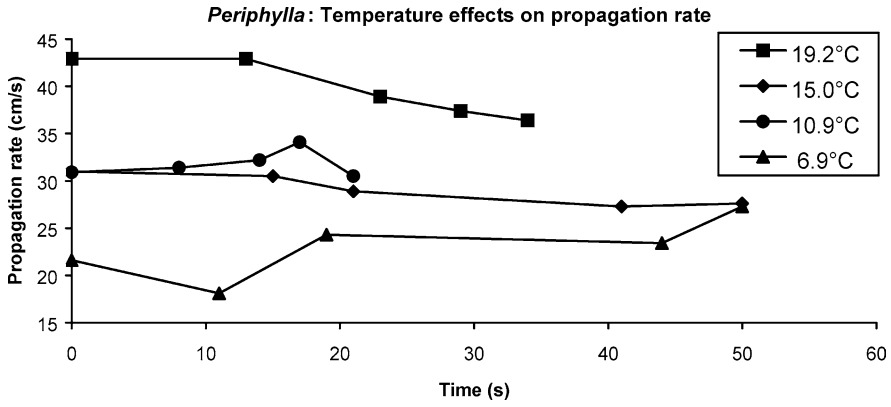


Fig. 19 Temperature effects on the propagation rates of the first five stimuli over four separate quadrants of the same specimen of *P.periphylla*

to shed the particles (unlike the *Atolla parva* material which will spread from a static animal). The individual particles flash persistently for several minutes at unchanged intensity but the frequency of flashing declines rapidly. The mechanism of scintillation is not known, but we speculate that an individual flash represents the output of one of the granules visible in electron micrographs of the rod-like organelles in the photocytes (Flood et al. 1997). It is likely that these rod-like structures represent a common subcellular luminous organelle present in all parts of the epithelium. The long-term in situ scintillation produced by mechanical damage (e.g. a cut) appears identical to that produced at the lappet margins in a stationary animal, and epithelial abrasion readily produces isolated scintillating fragments, akin to particles normally shed from the lappets. Flashing of the luminous organelles is normally brief and initiated only by the wave of stimulation that spreads from the stimulus site. Exposure in a cut or other mechanical damage does not immediately cause luminescence but appears to release the organelles from the inhibition that normally occurs after the stimulus has passed, thus initiating prolonged spontaneous flashing. The fact that during a long series of stimuli there is a gradual increase in background scintillation suggests that under these conditions, too, the intracellular control, like the neural control, gradually breaks down.

Secretory bioluminescence, sometimes particulate, is known from several other cnidarians as well as ctenophores (e.g. *Euplokamis* sp. and a number of other ctenophores; Widder et al. 1992a, 1999; Widder and Herring, unpublished observations), but scintillating material is restricted to *Periphylla periphylla* and *Periphyllopsis braueri*. Both secretory and intracellular bioluminescence have similar emission spectra, with λ_{max} at about 465–470 nm (Haddock and Case 1999). These animals have no visual (image-forming) capability and the role of the bioluminescent responses can only be to affect the behaviour of other, visual, species. It is clearly not a lure, because it is initiated only by contact stimulation. We presume its role is defensive, aimed either at potential predators or to reduce the risk of damage from accidental collisions (Morin 1983). Both secretory and flashing bioluminescence are used for defensive purposes in other taxa, but the complexity of the propagated responses in these medusae is unusual. We do not know whether that complexity has any particular relevance or whether it is simply a function of the widespread distribution of photocytes and the diffuse spread of the stimulus. In the absence of any real knowledge of the potential targets/predators at which the responses are aimed we cannot even be certain that the targets are capable of discriminating the spatial detail present in the responses. The bioluminescent "frenzies" that may take place during the later stages of stimulation (perhaps as a result of potentiation) have a parallel in the pennatulid anthozoans (Morin 1976). Buck (1973) described similar complex and long-lasting responses in the sea-pansy *Renilla reniformis*, but in this animal the responses were produced by conduction between individuals (polyps) and not within one individual. Musik (1978) reported an analogous bioluminescent response in another anthozoan, the gorgonian *Lepidisis olapa*. Multiple bioluminescent waves arising from a single stimulus are also features of some colonial hydrozoans (e.g. *Obelia geniculata*), siphonophores (e.g. *Hippopodius hippopodius*) and ctenophores (e.g. *Mnemiopsis leidyi*) (Morin 1974; Bassot et al. 1978). We assume that the repetitive and mobile nature of the responses we have observed reinforces their effect on a predator or on any other visual animal the scyphozoan may encounter. Other examples of complex frenzies, in which a single stimulus initiates propagated responses from several locations, include the ctenophore *Beroe* sp. (unpublished data) and the holothurian *Pannychia moseleyi* (Herring 1995).

That such similar responses can be obtained from both anthozoans and scyphozoans probably reflects the similar nerve net conduction systems involved (Anderson 1985; Josephson 1974). Hydrozoans have additional epithelial conduction systems; stimuli that originate in the nerve net induce responses, including bioluminescence (Bassot et al. 1978), which are further spread by epithelial conduction, both within the tissues of a single individual and between individuals in a colony (Spencer 1974; Anderson 1980; Mackie 1976). The propagation of a luminous wave over a hydrozoan medusa (Mackie 1991) is visually very similar to those that we have observed. Scyphozoans are generally believed to lack

epithelial conduction and responses are spread by a diffuse nerve net and a network of giant fibres (Passano and Passano 1971; Passano 1973, 1982, 1988; Arai 1997). However, the possibility of epithelial conduction in species of *Atolla* and *Periphylla periphylla* cannot be entirely ruled out. Both mechanisms can lead to conduction rates in the range we have observed (Satterlie et al. 1980; Passano 1982) and in both cases a superficial cut would block conduction. There is some pharmacological evidence to support epithelial conduction, in that Mallefet and De Bremaeker (1999) found that KCl-induced luminescence of tissue samples from *P. periphylla* was reversibly prevented by the gap-junction blockers octanol, heptanol and low pH sodium acetate (although gap junctions have not been reported in scyphozoans). Nerve net conduction is supported by the effects of Mg^{++} application and by the fact that the topical application of octanol to whole animals did not block conduction of a luminous wave (Herring et al. 1997). Intracellular recordings will be necessary to determine the issue unequivocally (G.O. Mackie and P.A.V. Anderson, personal communications).

The irreversible qualitative blocking of bioluminescence by local exposure to high light intensity, together with the lack of any effect on stimulus conduction, indicates that the effect of light acts on the effector system alone, either at the neuroeffector junction or by direct damage to the bioluminescence chemistry in the form of the photoprotein or the coelenterazine (the bound luciferin) (Anctil and Shimomura 1984). Photic inhibition of luminescence in pennatulids is very similar in its visible effect and has been postulated to act at the neuroeffector junction (Satterlie et al. 1980).

The bioluminescent system of *P. periphylla* (and probably of other scyphozoans) is coelenterazine-based (Shimomura and Flood 1998; Shimomura et al. 2001). It has been generally assumed that in cnidarians the coelenterazine is synthesised de novo, but Haddock et al. (2001) have recently demonstrated that this is not necessarily the case. They found that when individuals of the hydrozoan *Aequorea victoria* were cultured on a non-luminous diet they gradually lost their bioluminescent capability, which could only be restored by supplying coelenterazine by direct injection or in the diet. Young medusae of the genera *Bythotiara* and *Cunina* reared on non-luminous *Artemia* also failed to luminesce. This raises the possibility that the bioluminescence of species of *Atolla, Periphylla* and other deep-sea medusae may also be dependent on a dietary source of coelenterazine. There is no doubt that much of their diet consists of bioluminescent prey but it seems to us more likely that they synthesise their own coelenterazine to support the intense and enduring bioluminescence that we have observed.

The distribution of bioluminescence in these genera is predominantly exumbrellar (aboral). We have seen some subumbrellar surface luminescence and the ovaries of both genera are luminous when homogenised (Herring 1990; Shimomura et al. 2001). Other luminous scyphozoans include species of the semaeostomes *Pelagia*, *Poralia* and *Phacellophora*, (Herring 1990; Haddock and Case 1999; Herring and Widder, unpublished observations). *Poralia* glows locally on contact and releases luminous slime and glowing particles. The particles do not scintillate and could represent sloughed epithelial material. We have no details of the *Phacellophora* luminescence. *Pelagia noctiluca* has responses that are extensive and very like those of *Atolla* spp. and *P. periphylla*. Our recent observations have confirmed the luminous waves previously described (Dahlgren 1916; Herring 1990) and demonstrated that the waves also propagate up and down the long manubrium of this species. We have recorded luminous waves circling rapidly and continuously over the exumbrellar and umbrellar surfaces of the animal and flickering up and down the manubrium, as well as brighter, steady sources and luminous slime from the point of mechanical contact on the dome. In general, the responses were closely akin to those of *P. periphylla* but without the scintillating particles. Dahlgren (1916) also described luminescence of the tentacles in *Pelagia* species but we have not seen this in our specimens.

Clearly there is a wide variety of luminous expression in the Scyphozoa, but we cannot yet assess whether the different responses have particular ecological roles (e.g. defence against a predator or a warning against accidental contact). We believe, however, that the physiological variety in the bioluminescence indicates an equivalent behavioural variety in the animals' in situ responses.

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