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Observations on bioluminescence in some deep-water anthozoans

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Abstract

The luminous responses to electrical stimulation of isolated polyps of 4 deep-water anthozoans are described. All show facilitatory responses and summation at stimulus frequencies $> 2\ s^{-1}$. The responses of the gorgonian *Acanella arbuscula* comprise a slow summation of weak individual flashes. It is suggested that there is no fundamental difference between deep and shallow species, nor between the responses of scyphozoans, such as *Pelagia* and *Atolla*, and anthozoans, such as those described here. Both facilitated and decremental responses can be obtained from each of the 2 groups and the complexity of *in vivo* responses may be as much a reflection of selective pressures on the neural pathways as on the bioluminescent systems themselves.

Introduction

Many pelagic coelenterates, including scyphozoans (*Atolla*, *Pelagia*), hydromedusae (*Aequorea*, *Euphysa*) and siphonophores (*Nanomia*, *Hippopodius*), have spectacular and complex luminescent abilities (e.g. Widder *et al.*, 1989). These accord with the substantial body of observational and experimental data on the bioluminescence of coastal and neritic anthozoans, in which the recent experimental work has focussed on some Pennatulacea of the families Pennatulidae (*Pennatula*, *Ptilosarcus*), Renillidae (*Renilla*), Veretillidae (*Veretillum*) and Virgulariidae (*Virgularia*, *Stylatula*) (for references see Morin (1974) and Bilbaut (1975a, 1975b)). Pennatulaceans are to be found in both neritic and deep-sea environments and there are certain species (e.g., *Funiculina quadrangularis*) which occur over a wide range of depths (Grasshoff, 1981). The physiological characteristics of luminescence in shallow-water species may be determined by the selective pressures of that environment in particular. Those acting on deep-sea species may be different in emphasis and therefore induce different observed characteristics. Knowledge of deep-sea species is fragmentary and generally anecdotal: this report describes more detailed experimental studies of 4 such species, and notes luminescence in 3 others, with the objective of establishing whether any fundamental differences exist between deep and shallow species.

Material and methods

Animals were obtained in 1982 on RRS *Challenger* cruise 3/82 in the Porcupine Seabight area at depths between 230 and 2500 m. Station data are given in Table 1. Animals were sorted on deck into cooled seawater and taken into an experimental darkroom for observations of luminescence in intact animals. Electrical stimulation of isolated polyps was carried out 10–15 min after

Table 1. Station data for the species examined.

Station position	Depth (m)	Species
59° 09′ N 13° 21′ W	540–460	*F. quadrangularis*
51° 51′ N 13° 20′ W	820–760	*K. stelliferum*
		P. aculeata
49° 52′ N 12° 57′ W	2500–2455	*D. gracile*
49° 51′ N 12° 23′ W	2010–1940	*A. arbuscula*
		U. huxleyi
49° 33′ N 12° 12′ W	1265–1225	*A. arbuscula*
		K. stelliferum
		A. grandiflorum
49° 33′ N 11° 52′ W	1050–980	*A. arbuscula*
		U. huxleyi
		K. stelliferum
49° 30′ N 11° 17′ W	290–230	*F. quadrangularis*

excision using square wave pulses delivered from a Grass S9 stimulator through platinum electrodes. Light emission was detected with an EMI 6097S photomultiplier and recorded on an ultraviolet oscillograph (S.E. Laboratories Ltd). All experiments were carried out at room temperature (16 °C ± 2 °C).

Potential fluorescence of the photocytes, such as might be anticipated if energy transfer proteins are present, was investigated using a hand-held long-wave (365 nm) ultraviolet light (Hanovia Ltd).

Results

Acanella arbuscula *(Johnston). Gorgonacea: family Isididae*

This is a much branched species in which a blue luminescence is produced by both the stem and polyps when handled. No obvious fluorescence was visible and previous measurements of the emission spectrum (Herring, 1983) have indicated an emission maximum at about 485 nm with a substantial short-wavelength shoulder of about 410–415 nm. Individual polyps responded only very feebly to low frequency stimulation (1–2 s^{-1}) with a gradually increasing glow within which responses to the individual stimuli could be identified. A transient inhibition of the decay intensity occasionally preceded the response to the same stimulus (Fig. 1A). Short stimuli (5 ms) did not induce luminescence and 50 ms pulses of 50 V were required to elicit any response. Higher frequencies (5–10 s^{-1}) induced similar summation of responses with a very slow post-stimulus decay (Fig. 1B–D). Responses to individual stimuli were sometimes recognizable at rates of 5 s^{-1}.

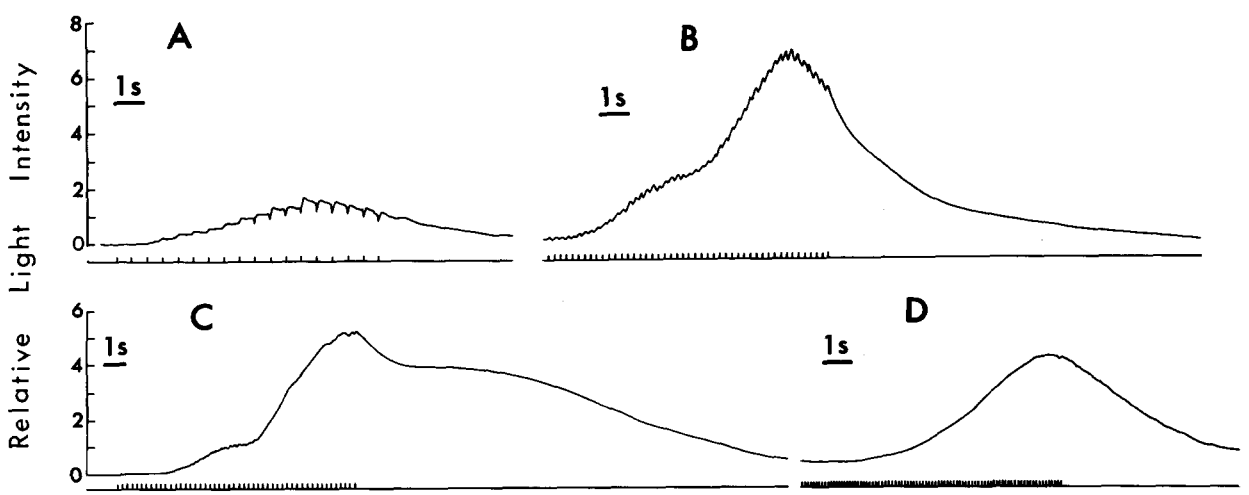

Fig. 1. Luminous responses of polyps of *A. arbuscula* to 50 ms 50 V stimuli at frequencies of (A) 2 s^{-1} (B & C) 5 s^{-1} and (D) 10 s^{-1}. All responses show a slow summation and prolonged decay period.

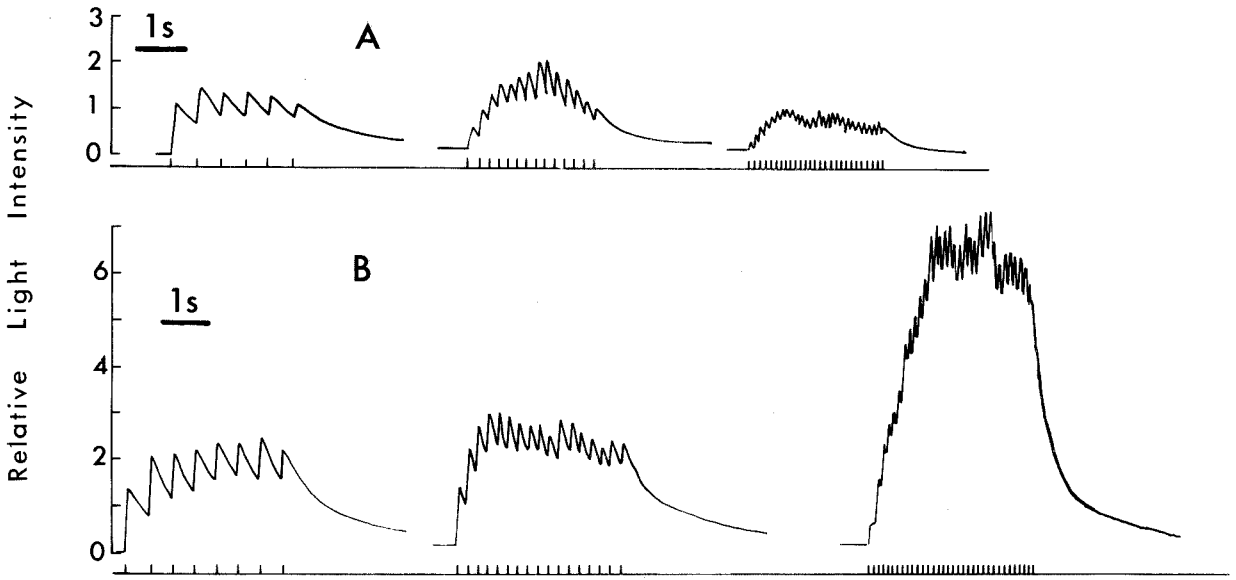

Fig. 2. Responses of 2 stem sections (A and B) of one colony of *Umbellula huxleyi* to 50 ms 50 V stimuli at frequencies of 2, 5, and 10 s^{-1}. No rest periods were given between stimulus trains.

Umbellula huxleyi *(Köllicker). Pennatulacea: family Umbellulidae*

Four specimens of this species were obtained and in each case the luminescence elicited by handling was remarkable for the fact that the major length of the stalk emitted a bright green light, whereas the distal tip and base of the polyps emitted a clear blue light (Herring, 1983). Similar spectral differences have been reported by Widder *et al.* (1983) in *Umbellula magniflora*. Only the basal region of the polyps was significantly luminous. A luminous mucus appeared to be produced on the surface of the stalk when handled, and rinsing the animals in seawater produced copious luminescence from the stalk. Segments of the main (green-emitting) stalk were stimulated electrically and responded with slowly decaying summated responses at all frequencies above 1 s^{-1}, though individual stimulus responses could still be identified at 10 s^{-1} (Fig. 2A, B). The rate of decay of the first few flashes tended to be rather slower than that of subsequent ones. Despite the remarkable colour differences on the stalk there was no visible fluorescence in either region, and there is therefore no evidence for differential energy transfer systems in different parts of the colony.

Funiculina quadrangularis *(Pallas). Pennatulacea: family Funiculinidae*

Each polyp emitted a bright blue-green luminescence from 8 areas arranged symmetrically around its base, very similar to the arrangement in *Stylatula* (Morin, 1974). Each site was weakly fluorescent though its colour was not distinguishable. Individual zooids flash repeatedly in response to a single stimulus, provided they are still attached to the colony. Herdman (1913) noted how the stem also produces a flickering luminescence. Isolated polyps produce only single responses to each stimulus (Fig. 3). Responses to short (10 ms) stimuli are brief flashes with marked facilitation in some cases while others show decremental intensity changes. Long pulses (50 or 100 ms) produce longer flashes with indications of multiple elements within the flash envelope. Summation may occur at stimulus frequencies as low as 1–2 s^{-1} with long pulses but

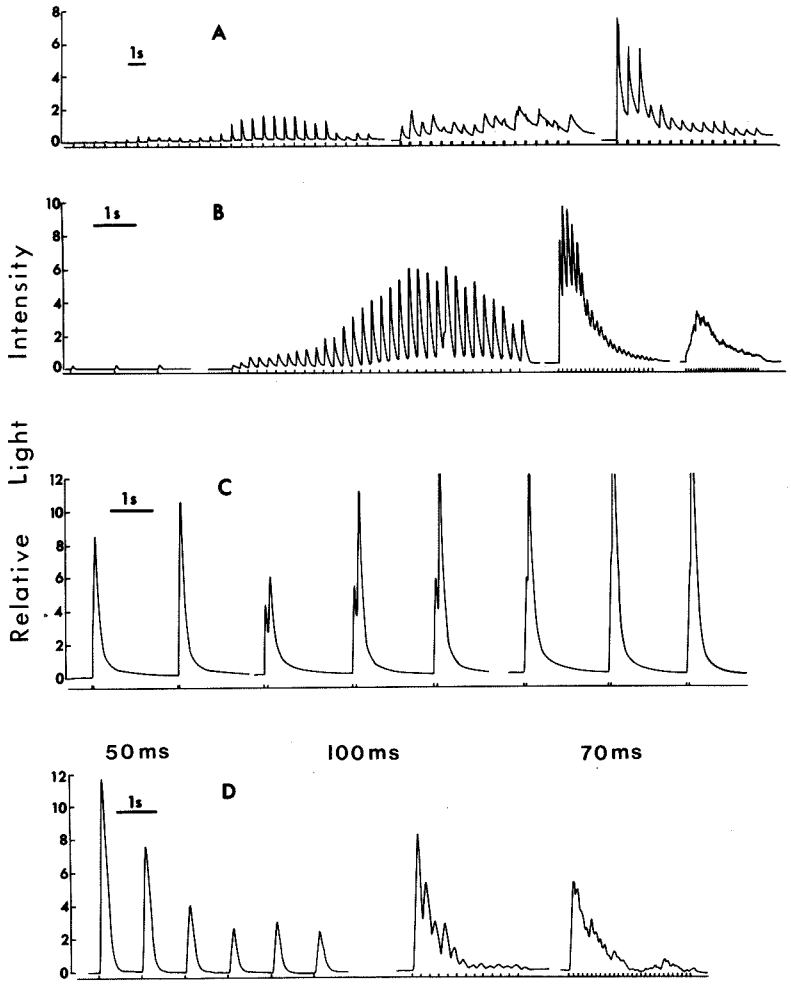

Fig. 3. Responses of excised polyps of *F. quadrangularis*. A. Responses to 2 s^{-1} trains of stimuli of 10 ms 20 V, 100 ms 20 V and 100 ms 40 V respectively. The longer stimuli produce longer flashes. B. Stimuli of 5 ms 30 V at frequencies of 1, 5, 10 and 20 s^{-1}. C. Responses to paired 5 ms 30 V stimuli at intervals of 50, 100, 70 ms respectively. D. Decremental responses to 5 ms 30 V stimuli at frequencies of 1, 5 and 10 s^{-1}. No rest periods were given between stimulus trains.

with shorter ones (5 ms) it may only be marked above 5 s^{-1} (Fig. 3A, B, D). The rise time for short flashes is 50–70 ms, with a similar 50% decay time and a latency of 30–40 ms. Twin 5 ms pulses with a delay of 100 ms produce clearly separable flashes; at 70 ms delay the 2 elements are still recognizable; while at 50 ms the response to the first pulse is only just distinguishable (Fig. 3C).

Distichoptilum gracile *Verrill. Pennatulacea: family Protoptilidae*

The colonies of this species are long and narrow, the polyps emitting a continuous clear green luminescence without additional stimulation. The luminescence of individual polyps waxed and waned in intensity imparting a slow overall flickering to the colony as a whole. The luminescence intensity was greatly increased by the mechanical stimulation of handling with forceps but did not spread significantly up or down the

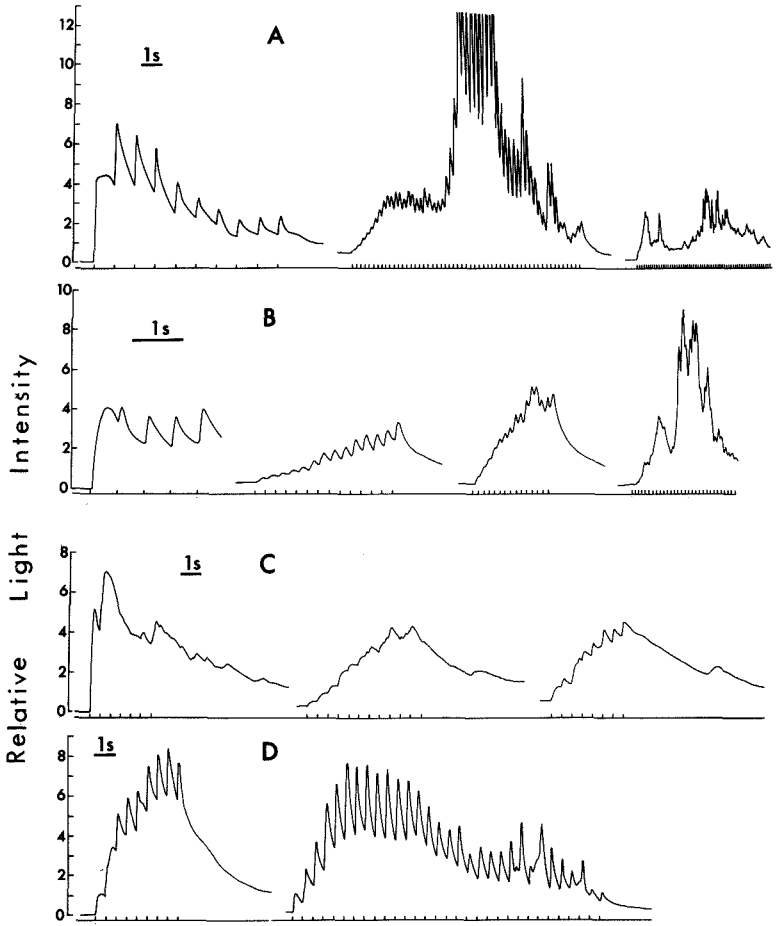

Fig. 4. Responses of excised single polyps of *D. gracile*. A, B. Responses of two polyps to increasing stimulus frequencyâ; 50 ms 50 V stimuli at 1, 5 and 10 s^{-1} (A) and 2, 5, 10 and 15 s^{-1} (B). C, D. Responses of a single polyp to increasing stimulus duration; 50 V stimuli at 2 s^{-1}, durations 5, 10, 20 (C), 50 and 100 ms (D) respectively.

stem and affected only those polyps immediately adjacent to the stimulus. Each polyp had a pair of green fluorescent spots in the axil of the polyp and stem but these were not the sole sources of luminescence. Individual polyps responded to electrical stimulation with slowly decaying flashes, showing summation even at frequencies of 1 s^{-1} though individual responses could be distinguished at up to 15 s^{-1} (Fig. 4A–D). The first flash in response to a train of pulses often had very slow decay kinetics (Fig. 4A, B, D) with a rapid initial rise in intensity and a more prolonged afterglow. Individual flash latencies were 20–50 ms.

Anthoptilum grandiflorum *Verrill,* Kophobelemnon stelliferum *(O. F. Müller) and* Pennatula aculeata *Koren & Danielssen*

Luminescence was observed from specimens of each of these species, but no experimental studies were undertaken.

Discussion

The response characteristics reported here suggest that deep and shallow water anthozoans have fundamentally similar characteristics. The variability of the flash kinetics of excised polyps

of *Distichoptilum* is more likely to be the consequence of excision than normal *in situ* variation. The slow kinetics of some first flashes in *Distichoptilum* polyps (Fig. 4) has also been noted in *Pennatula phosphorea* siphonozooids (Nicol, 1958). The latencies in the latter species (20–50 ms) were similar to those measured in *Distichoptilum* and *Funiculina*. Bilbaut (1975b) observed prolonged glows from the siphonozooids of colonies of *Veretillum cynomorium* that were not fully turgid, whereas autozooids of fully turgid specimens emitted rhythmic flashes. Studies of isolated autozooids (Bilbaut, 1975a) provided evidence for a conduction system round the tentacular crown and for a local centre driving the rhythmic flashes, which were obtained as an after-discharge in response to stimulus frequencies greater than $5\ s^{-1}$. No rhythmic after-discharge was observed in the isolated polyps of either *Distichoptilum* or *Funiculina* in the present study.

The responses of the stem segments of *U. huxleyi* are fundamentally similar to those of the other pennatulaceans, with a slow individual flash decay and a latency of 30–40 ms.

The gorgonian *Acanella arbuscula* is rather different in that all responses involved a slow summation of weak individual flashes terminating in a prolonged glow. The responses of intact colonies to mechanical stimulation were similar, with slowly increasing glows and long decay times. The experimental data seem an accurate reflection of the normal response. Observations on luminescence in gorgonians are very limited and there are no other experimental data on their luminescence characteristics. The most detailed account is that of Musik (1978) on *Lepidisis olapa*, from 400–500 m, who described how mechanical stimulation *in situ* produced multiple waves of light travelling up and down the colony and criss-crossing uninterruptedly. Clearly the *in situ* capabilities of some gorgonians are more dramatic and complex than those observed in *Acanella*. In particular the rapid passage of a wave of luminescence implies much shorter flashes in *Lepidisis* than those observed in *Acanella* colonies or isolated polyps.

Funiculina quadrangularis has a depth range extending from the sublittoral to the abyssal and there is no reason to believe that its responses vary across this range. Luminescence probably serves the same defensive purpose (Morin, 1974) in both deep and shallow specimens. The role of luminescence in obligate deep-water species of sessile cnidarians is probably similar and the physiological similarities reflect this. The relationship between sessile and pelagic species is less clear. Pelagic luminescent cnidarians occur at all depths and exhibit a great variety of luminescent responses, from secretions to multiple propagated responses. There is no single character, or suite of characters, which is fundamentally pelagic in terms of luminescence. This is emphasised by the fact that typical facilitated flash responses can be obtained from *Pelagia*, *Atolla* and other scyphozoans (P.J. Herring, unpubl.). The limited evidence available suggests a basic similarity in the luminescent responses of cnidarians in general, reflecting similar selection pressures in deep and shallow habitats, in large and small species and in sessile and pelagic habits. If bioluminescence is a non-selective defence to a range of different potential predators or undesirable settlers (Morin, 1974) then similar responses are to be expected across the phylum. The physiological characteristics of the responses will reflect the selective pressures on the neural systems rather than specifically on the bioluminescence system.

Acknowledgements

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