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ORIGINAL ARTICLE

The spatial pattern of bioluminescent flashes in the polychaete *Eusyllis blomstrandi* (Annelida)

S. A. Zörner · A. Fischer

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Abstract Each of the trunk segments of the polychaete *Eusyllis blomstrandi* is equipped with paired epidermal luminescent domains. They luminesce upon mechanical or electrical stimulation. Light emission can be rapidly turned on and off, appears intracellular and is highly coordinated among the trunk segments. Luminescent light is typically emitted in series of flashes. Light emission in a flash starts locally in a group of segments and recruits adjacent segments at a rate as fast as ≤1 ms/segment. The collapse of light emission at the end of a flash is almost simultaneous in all of the segments involved. In the intact worm, the luminescent reaction usually involves only a posterior group of segments. Facilitation becomes manifest as the consecutive flashes in a series increase in brightness and duration and recruit additional anterior segments that were not active in earlier flashes. The flash series stops abruptly instead of decreasing asymptotically in brightness. In posterior fragments, all the segments participate in flashing luminescence, indicating the loss of an inhibitory effect exerted by the anterior end in the case of whole animals. Posterior fragments survive and are still capable of luminescence weeks after fragmentation although they do not regenerate a head. Immediately upon fragmentation of the worm, the posterior fragment luminesces continuously for some seconds while the anterior part quickly stops light emission. This suggests a decoy and/or a predator-alerting function of prolonged, strong luminescence by the moribund posterior fragment to the benefit of the survival of the anterior fragment.

Keywords Bioluminescence · *Eusyllis* (Polychaeta) · Segmental light organs · Facilitation · Predator alert

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S. A. Zörner · A. Fischer (✉)
Zoological Institute, University of Mainz,
55099 Mainz, Germany
e-mail: afischer@uni-mainz.de

Introduction

Many species of polychaetes display bioluminescence. Best-known are bioluminescent phenomena in scale worms (Aphroditoidea), in the parchment worms (Chaetopteridae), and in the syllids (Syllidae), some of which are known as "glowworms" (e.g. *Odontosyllis luminosa*, Gaston and Hall 2000) or "fireworms" (*Odontosyllis enopla*; Fischer and Fischer 1996). For a number of other polychaetes, like the holopelagic genus *Tomopteris*, bioluminescence has only been noted (Dales 1971).

Spontaneous light emission in polychaetes has only been reported from mature *Odontosyllis* species (Fischer and Fischer 1996; Gaston and Hall 2000) during their swarming excursions. Field observations of their sexual activity leaves little doubt that light emission by the *Odontosyllis* female enables the male to locate and meet its sexual partner. In other polychaetes as well as in immature *Odontosyllis* (Fischer and Fischer 1996), bioluminescent activity seems to occur as a response to mechanical irritation, like touching the worm or mechanically shocking the substrate, and, in *Chaetopterus* (Martin and Anctil 1984), to electrical stimulation of the ventral nerve cord or of isolated parapodia. Light emission in these cases is interpreted

as an alarm response serving a deterrent or a decoy function (Morin 1983).

A variety of kinetics is observed in polychaete light emission. A luminescent glow (>2 s duration; Hastings and Morin 1991) emitted from secretions is found in *Chaetopterus* and in swarming female *O. enopla* (Fischer and Fischer 1996) and *O. luminosa* (Gaston and Hall 2000). Emission of serial light flashes, on the other hand, is ascribed to intracellular light production and has been long-known in the scales of scale worms, in swarming male and immature *Odontosyllis* (Fischer and Fischer 1996), and in other luminescent syllids like *Pionosyllis pulligera* (Bassot 1979) and *Eusyllis blomstrandi*.

Research on polychaete bioluminescence has so far focussed on behaviour and biorhythmicity in polynoid scale worms, *Chaetopterus*, and *Odontosyllis* (Syllidae), and, in *Chaetopterus*, on the role of the nervous system in eliciting light production. Among the polychaetes, detailed studies on histology and fine structure of light-emitting cells and organelles and on the temporal and spatial patterns of light emission only exist for the isolated scales of polynoids. These "scales" are specialized parapodial appendages which are readily shed by autotomy and are then easily handled in the laboratory. A considerable body of knowledge on structural conditions and physiological parameters has accumulated for this experimental material (Pavans De Ceccatty et al. 1977; Bilbaut and Bassot 1977; Bassot and Bilbaut 1977a, b; Bassot 1987).

In intact polychaetes, the temporal and spatial patterns of bioluminescent flashing, however, are still not precisely known. This is due to the technical problems posed by the low intensity of the luminescent light signals and by the rapidity of the changes in their intensity and spatial pattern. The only exception is a brief report on serial light flashes emitted by the syllid *P. pulligera* upon electrical stimulation (Bassot 1979). Recent technical progress now allows high-speed video imaging at extremely low light intensities, and modern data management enables easy processing of the raw material from video- and photomultiplier recording.

We have carried out the study of bioluminescence of the syllid polychaete *E. blomstrandi*, a species closely related to the genera *Odontosyllis* and *Pionosyllis* among the Eusyllinae. *E. blomstrandi* can easily be collected around the island of Helgoland in the North Sea. As in other Eusyllinae, its light is emitted from paired segmental spots. We have studied the spatial and temporal patterns of bioluminescence by recording the luminescent light and show that these patterns cannot be resolved by the unaided human eye. Here, we characterize the kinetics of bioluminescence in *Eusyllis* under several different conditions. The films from which most of the present images were taken are accessible on-line (Zörner and Fischer 2006).

Materials and methods

The animals

The polychaete *E. blomstrandi* occurs on the coasts of the Northern Atlantic. A dense population is found in the phytal on the coast of the island of Helgoland in the North Sea where the worms construct and inhabit silk tubes on the thalli of the red alga *Delesseria sanguinea*. Such thalli were collected at about 7 m depth by divers of the Biologische Anstalt Helgoland/AWI Bremerhaven and placed in containers while underwater. In the lab, the algal thalli were transferred into shallow dishes, covered with a new change of seawater and left without aeration. The worms then left the thalli, some immediately after transfer, and some during the following 24 h. They were then collected with a glass pipette and transferred into glass bowls or transparent plastic containers. Other polychaetes and nemerteans were sorted out under a dissecting microscope to keep them from attacking the *Eusyllis*.

Eusyllis blomstrandi can be transported and kept for months in transparent round flat-bottom plastic containers with a lid. To avoid intraspecific attacks and cannibalism, worms must not be kept too crowded: e.g. three worms in a 100-ml container filled with 15 ml seawater will do well. For transport, the worms are placed in such containers at least 1 day prior in order to permit tube construction overnight, providing them with a retreat during the buffetings of transport. *E. blomstrandi* must not be exposed to temperatures higher than 18°C except for brief periods of handling and experimentation. Sunlight or microscope illumination may quickly warm the container temperature beyond the upper threshold of tolerance. For maintaining *E. blomstrandi* in the lab for weeks or months, a dense suspension of living brine shrimp (*Artemia*) larvae can serve as food.

Video imaging: handling the worms

A single *Eusyllis* worm is placed in the centre of a circular cover slip 42 mm in diameter. Some drops of seawater are added to form a small pond (about 20 mm in diameter) in the centre of the cover slip. The cover slip is placed in a 100-mm plastic Petri dish on two plastic bar supports, and the Petri dish is stored in a wet chamber (a 20 cm × 20 cm × 6 cm refrigerator plastic container with a seawater-wetted filter paper on the bottom). The

worms are then left overnight in the dark where most of them will spin tubes for dwelling. In preparation for video imaging, such preparations are brought into a darkroom for at least 20 min and for the following are handled under dim red light or in the dark.

The cover slip with a worm in its tube is placed in a cell culture chamber (POC chamber system; H. Saur, Reutlingen, Germany). Two silicone gaskets (42 mm in diameter, 2 mm high) are placed on the cover slip and pressed against it by a stainless steel ring screw. The resulting trough (3.6 ml volume) is filled with seawater and left without cover. In the dim red light from a fibre optic illuminator with a red filter, the whole device is brought into position for video imaging on a rotary microscope stage. The same red light source is used to illuminate a white diffuse light reflector mounted below the microscope stage. This arrangement provides the minimum background illumination that is needed to identify the contours of the worm in the video films.

Light emission is stimulated in the dark either in a diffuse manner by shocking the cover slip with a metal rod or as a localized stimulus by touching the worm with a needle. Alternatively, luminescent light emission can be elicited by placing a worm between two field electrodes that are operated using square wave pulses at a direct voltage of ≥5 V. Autotomy is induced by pressure exerted with the curved cutting edge of a scalpel.

Video imaging: optics and cameras

The macro lens systems LUMINAR 40 ($f = 40$ mm) and LUMINAR 16 ($f = 16$ mm) (ZEISS) were used for video imaging in combination with a variety of extension tubes. For this study we used the HAMAMATSU video camera system C9100-02, a particularly light-sensitive camera with a maximum spectral sensitivity between wavelengths of 500 nm and about 700 nm.

At its full resolving power of 512 × 512 pixels, this camera yields 35.8 complete frames/s. However, when a binning rate of 4 × 4 pixels is applied, the camera chip is read more quickly such that as many as 118.2 frames are read per second, though at the expense of image resolution. The effective "shutter" interval is influenced by the storage process and is subject to some variation according to the nature of the image motifs. In our material, this interval varied between 8.46 and 12 ms and, in total, came very close to an average of 10 ms so that a "10-ms" interval was selected as the approximate "shutter" frequency in our video image series (Figs. 4, 8) and diagrams (Figs. 5, 6, 7, 9, 10); the actual time intervals took, however, 9.71 ms on average.

For each one of the recorded pixels of every video image, a numerical value on the half-tone scale of up to 14-bits is stored. For use in the graphs in Figs. 5, 6, 7, 9, and 10, the amount of locally emitted light was estimated from the video images and manually symbolized by three levels of shading: dim (heavy shading), medium, and full intensity (no shading).

Video imaging: calibrating the camera and the background illumination

In order to adjust the video camera to the intensity of the light flashes emitted by *Eusyllis*, some flashes of luminescence were recorded by a photomultiplier tube (PMT), type IP28 (BURLE Industries, Lancaster, PA, USA). Using the PMT, an LED light source (T-85 2684; MENTOR GmbH & Co. Präzisions-Bauteile KG, Erkrath, Germany) was then adjusted to emit a light intensity less than 30% of the luminescence brightness but well above noise. This was the case at an LED supply voltage of 1.7 V. This light signal with a spectral maximum at a wavelength of 473 nm was then used for calibrating the sensitivity of the video camera while the LED was in the focal plane.

Finally, the dim red background illumination was adjusted to a level allowing the recognition of the worm's body contour but down-regulated to such a low level that background light did not interfere with video imaging of the luminescent flashes.

Results

Luminescent sites

Luminescence in *E. blomstrandi* is confined to the trunk. In response to extremely strong stimulation, light can be produced in every trunk segment (Fig. 1). Luminescent light is emitted from a pair of patches in each of the trunk segments (Fig. 1). Each of these sources is a round patch of luminescent tissue of the dorsal epidermis surrounding the base of the dorsal cirrus (Fig. 2). Every such patch appears to be composed of a number of smaller light sources. Minor accessory light sources are also found elsewhere in the dorsal epidermis (Fig. 2) and ventrally in the parapodial bases. Light emission always remains confined to the epidermal domains and thus apparently does not originate in secreted material.

Eliciting and observing bioluminescent activity in *Eusyllis*

In the material included in the present study, light emission was induced by touching the worms with a metal tool (Fig. 2), by pulling on the wall of the silk

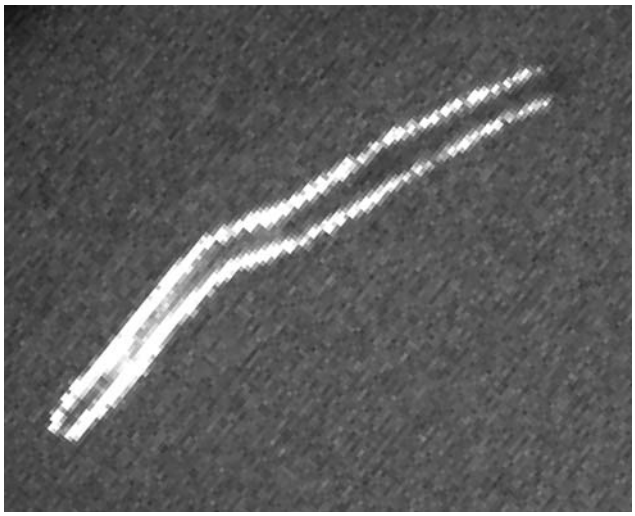

Fig. 1 *Eusyllis blomstrandi*. After extremely strong stimulation, all of the trunk segments may luminesce

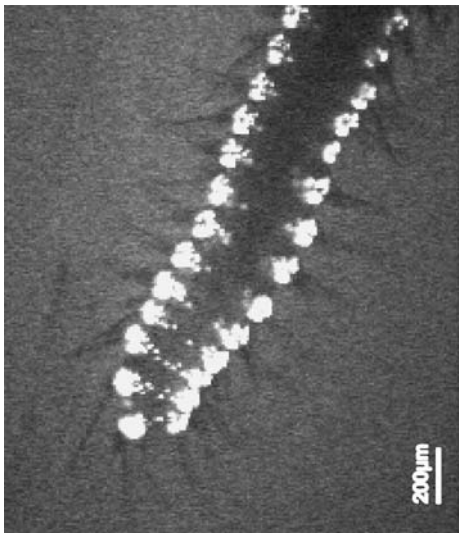

Fig. 2 Posterior end of a luminescing, intact *Eusyllis blomstrandi*. Note the elongate dorsal cirri (*dark*, filamentous contours projecting on either side of the trunk) originating in the middle of the segmental luminescent domains and the individual segmental patterns of small, accessory light sources in the vicinity of the main luminescent domains. In consecutive luminescent flashes, the same luminescent sites are involved. Stimulation by a touch with a needle

tubes that the worms construct and inhabit (Figs. 3, 7), or by single or repeated mechanical shocks to the substrate (Figs. 4, 5, 6, 10). Electrical stimulation was abandoned after it was found that considerable voltage ($\geq$5 V) is necessary to overcome the threshold of reaction and because many worms under such conditions autotomized right upon stimulation.

The usual luminescent display of *E. blomstrandi* is seen by the naked eye as a series of flashes. Most flashes appear to move along the trunk, but the course and

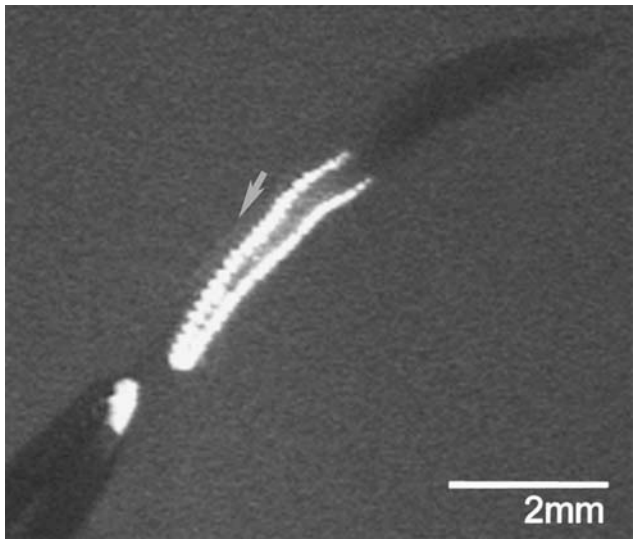

Fig. 3 Luminescence of *Eusyllis blomstrandi* elicited by using forceps (*lower left*; luminescent light is reflected at its tip) to pull on the silk tube (*arrow*) which the worm has constructed for dwelling

spatial pattern of the luminescent events in *Eusyllis* was only revealed through high-speed video records. In the present study, we have used video imaging at a frequency of up to 100 frames/s to obtain information about the sites of luminescence and the kinetics of light emission: changing brightness, propagation of luminescent activity (excitation) by recruitment of additional segmental light sources, and fading.

The individual light flash

We followed the emission of light in whole worms and in posterior trunk fragments. Posterior trunk fragments survive for weeks and remain functional with respect to luminescence. Their patterns of serial flashing appear less modulated than those in whole worms and therefore play a role in our characterization of the spatio-temporal pattern of light emission. The posterior ends that we have used for video imaging had fragmented the same day that the images were taken.

Upon mechanical stimulation, a first very faint luminescent reaction was observed after a brief delay. Luminescence may start as dim flashes, for example after a latent period of 165 ms in the case shown in Fig. 10. It may, alternatively, start as a flicker composed of asynchronous, spatially incoherent, faint pulses ($\leq$10 ms) of luminescent light from single luminous domains, and in one case this flicker began 116 ms after the worm was touched with a needle. Following this brief phase of spatially and temporally rather erratic luminescence, serial flashing sets in.

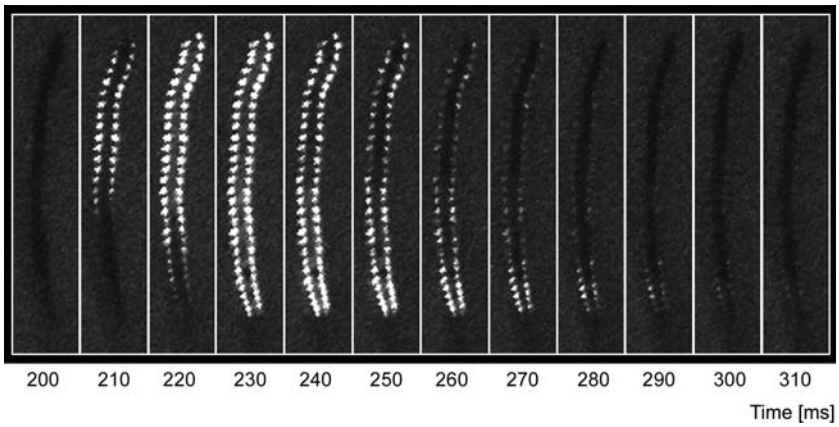

Fig. 4 *Eusyllis blomstrandi*. The pattern of luminescence in the course of a single flash [labelled (a) in Figs. 5, 6] in a fragment comprising the posterior 29 segments of a 68 segment-long worm. Luminescence starts in the anterior segments (nos. 39–49 on top in this figure) and ends in the most posterior segments of the fragment. The video images are taken at 10 ms intervals (see Sect. "Materials and methods"). Stimulation by mechanical shocks to the glass support

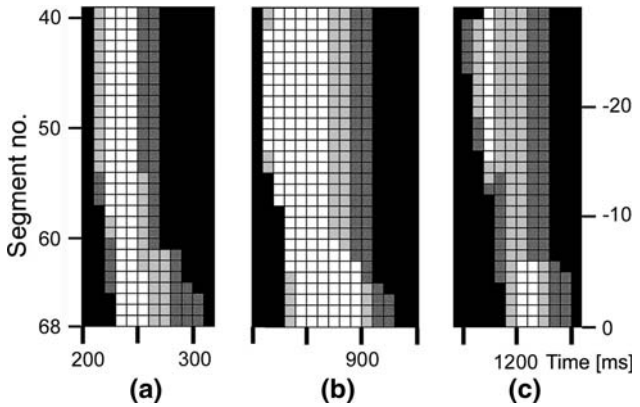

Fig. 5 *Eusyllis blomstrandi*. Schematic representation of the segmental distribution and intensity of light emission in an early (**a**), middle (**b**), and late flash (**c**) of a series in a posterior fragment identical with that shown in Figs. 4 and 6. A square unit represents one segment (*vertical axis*) and a time interval of 10 ms (*horizontal axis*). The *numbers* on the ordinate *left* and *right* of the graphs in this figure and in Figs. 6, 7, 9, and 10 symbolize the positions of the individual segments in the trunk in an anterior–posterior (*left*) and in a posterior–anterior (*right*) direction. A numbering from the posterior end towards the anterior is included because the light pulses may start from the posterior-most segment (Fig. 10), and because the total number of luminescing segments may increase in subsequent flashes towards the anterior, such that the posterior-most segment may become the point of reference. By estimating the light intensities seen in the original video images, like those in Figs. 4 and 8, three categories of emission intensities are symbolized in the graphs by plotting manually three grades of shading, from dim light (*heavy shade*) to full light intensity (*no shade*), as shown in **a** representing the original video images of Fig. 4

Figures 4 and 8 each show a sequence of video images taken at 10 ms intervals, illustrating the propagation and fading of luminescent excitation during a single flash in a posterior fragment and in a whole worm, respectively. We have processed some of the information in such images as shown by the diagrams in Fig. 5 where luminescence of each of the segments is plotted over time. A square unit represents the degree of luminescent activity of a single segment (vertical axis) in a 10-ms interval (horizontal axis). Left and right photocyte clusters of a segment are represented by a single square, as both of them with few exceptions share the same degree of light-emitting activity. Brightness of emitted light is expressed in these diagrams by three categories: (1) white squares—full light saturation in all those pixels that make up the image of one luminescent site; (2) light grey squares—the majority of pixels appear saturated; (3) dark grey squares—a minority or none of the pixels saturated. Segments and intervals in which the luminescent system was silent are represented by black squares.

A flash starts with the apparently simultaneous luminescent activity of a group of consecutive trunk segments (Figs. 4, 8). Depending upon the site along the trunk at which luminescent excitation starts, the luminescent activity expands either posteriorly (Figs. 4, 5, 6, late flashes in Figs. 7, 9b, c) or anteriorly (Fig. 10, late flashes) or simultaneously in both a posterior and an anterior direction (amphidromous propagation; Figs. 7, 8, 9a, 10, early flashes). In posterior fragments, all segments become involved in light emission (Figs. 4, 5, 6). As at the beginning of a flash, luminescence does not simultaneously terminate in all of the segments: light fading appears as a wave that runs along the series of segmental light sources. Such a sequential course of both the start and the fading of light emission along the chain of trunk segments is a general pattern observed in the light flashes produced by *Eusyllis*.

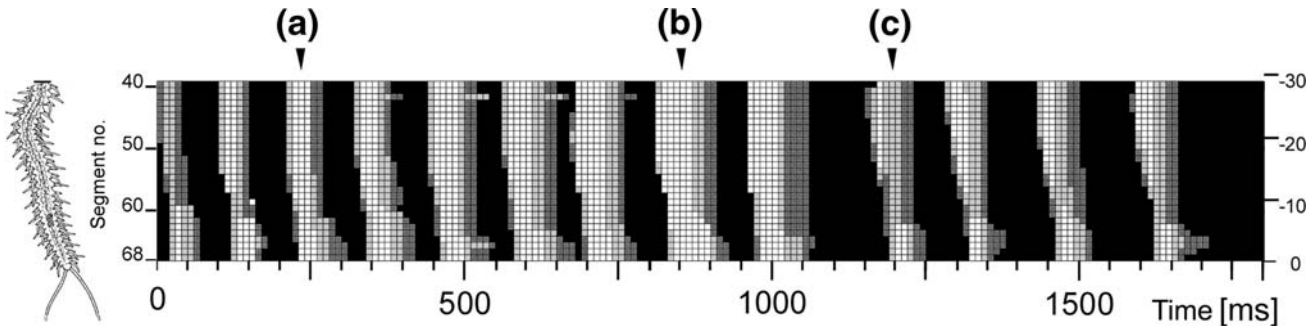

Fig. 6 A flash series emitted by a posterior fragment of *Eusyllis blomstrandi* following a series of mechanical shocks to the support (identical material as in Figs. 4, 5). The flash series was rhythmic but asynchronous to the shocks. The flashes labelled by **a**, **b**, and **c** are seen as close-ups in Fig. 5. *Coordinates* and *symbols* as in Fig. 5. *Left margin* Drawing of a posterior fragment of *E. blomstrandi*

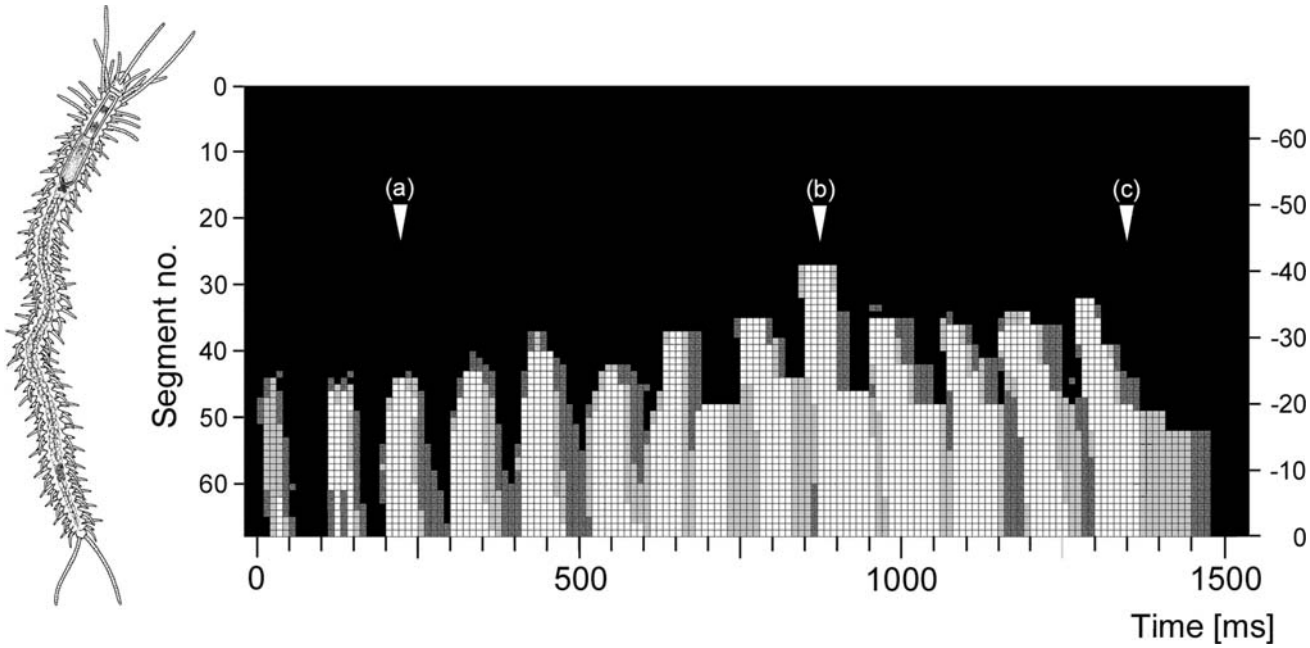

Fig. 7 Diagram showing a flash series in an intact *Eusyllis blomstrandi* with 68 trunk segments (see *drawing* at *left margin* of an *E. blomstrandi* by K. Rehbinder). Stimulation was by pulling on the silk tube (see Fig. 3). Light emission is restricted to the posterior segments, with a gradual recruitment of a number of more anterior segments. Flash (**a**) is illustrated by an original video in Fig. 8, and flashes **a**, **b**, and **c** are shown in more detail in Fig. 9. Same animal as in Fig. 3. *Coordinates* and *symbols* as in Fig. 5

The following detailed information can be gathered from analysis of the diagrams in Figs. 5, 6, 7, 9, and 10. Excitation, expressed as luminescent activity, quickly propagates by recruiting segment after segment with a delay of, in some cases, less than ≤1 ms/segment. In the example shown in Figs. 4, 5, and 6, luminescence propagates from the anterior towards the posterior end where the excitation arrives after 10–20 ms in the early flashes of a series and after 40 ms in the late bursts (Fig. 5a, c). Likewise, the flash fades over a period of about 20–40 ms. Faint light emission, or a low level of excitation, propagates as faint emission, and strong emission propagates as a strong emission. Thus, coordination of light emission among the segments also pertains to quantitative aspects of luminescence. In a single segment, light intensity increases during an early flash and then decreases. In later flashes, an individual segment typically reaches full light intensity during the initial 10 ms whereas fading usually takes 10–40 ms. As a combined effect of intersegmental propagation and intrasegmental modulation of light intensity, the light maximum may shift during a flash along the series of trunk segments (see below).

Flashes in a series

The typical flash series of *E. blomstrandi* is a rhythmic discharge, with a frequency of about 7 Hz and a total

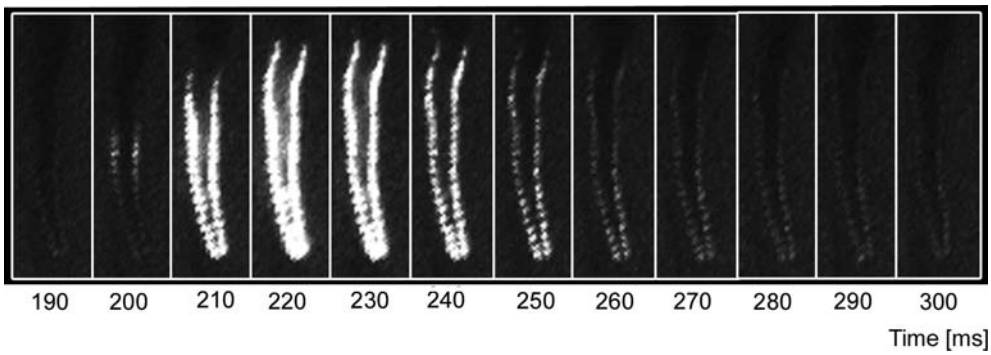

Fig. 8 Video images of a single flash out of the series emitted by an intact *Eusyllis blomstrandi* [flash (a) in Figs. 7, 9]

duration of about 1.5–2 s (Figs. 6, 7, 10). The rhythms of flashing and the quantitative aspects of light emission of *E. blomstrandi* are best studied with photomultiplier recordings and will be treated in a later paper. Here, we concentrate on the contribution of individual segments and on the intersegmental coordination of flashing in both posterior fragments and whole animals.

A flash series emitted by a posterior fragment is shown in Fig. 6, and flash series from intact worms in Figs. 7 and 10. It is evident that (1) flash frequency first accelerates and finally slows down in the course of a series, (2) both the light intensity and the duration of the flashes increase and may continue at a high level, (3) a flash series terminates with a full burst instead of flash peak heights diminishing continuously towards the base line, (4) flashing in a posterior fragment involves all of the segments, while in whole worms, with exceptions, only a posterior part of the trunk is emitting light, and (5) the site on the trunk where excitation starts in a flash may shift from flash to flash towards a terminal position, e.g. towards the posterior end, as in the case shown in Fig. 10.

The earliest flashes in a series may fail to reach full brightness in some or in all of the luminescing segments (shown in Fig. 10). Alternatively, flashes in a series may start with dim light, and switch to full intensity after a 10-ms interval, or in late flashes the segmental light sources may commence at full light intensity already in the first 10 ms interval. Early flashes last for a short period of time only in an individual segment (≤10–60 ms). In this case light production ceases completely before the next flash sets in. In later flashes, light emission may last longer in the individual segment, e.g. 100 ms in total (Figs. 5, 6) or more (Figs. 7, 9).

During a flash series, luminescent activity may increase to such a degree that light is produced during the whole individual flashing cycle such that the flashes tend to overlap. Flashing at such an elevated level of excitation

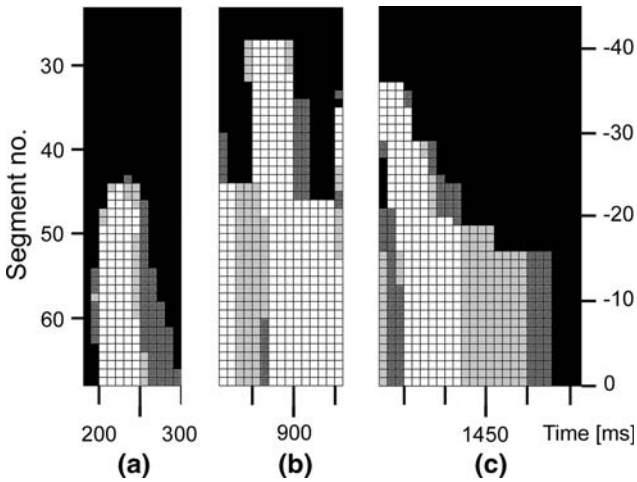

Fig. 9 Details of Fig. 7 (flash series in an intact *Eusyllis blomstrandi*). Note the increasing duration of the flashes, the recruitment of more anterior segments, the increasing duration of bright light emission per segment, the delay in the beginning of bright light emission existing between the more anterior and the posterior segments in (**b**) and (**c**), and the prolonged emission and dim light phase typical of the last flash in a series (**c**). Same *coordinates* and *symbols* as in Fig. 5. The non-luminescing anterior segments (see Fig. 7) are omitted from this graph

appears as a rhythmic increase and decrease of brightness superimposed upon permanent light emission at a lower level (Fig. 7). In extreme cases, e.g. immediately before and after fragmentation, all of the light sources luminesce continuously for seconds at full brightness, in whole animals as well as in posterior fragments (see below). Late flashes in a series typically persist in a state of weak luminescence for an extended period of time (60–80 ms). This is best seen in the last flash of the series in Fig. 7: A terminal posterior group of 16–18 segments including the last segment remain in a state of dim luminescence before their lights finally vanish synchronously.

Luminescent flashing in whole animals, other than in posterior fragments, is usually restricted to a posterior part of the trunk and always includes the terminal segments (Figs. 7, 8, 9, 10). During a flash series, the group

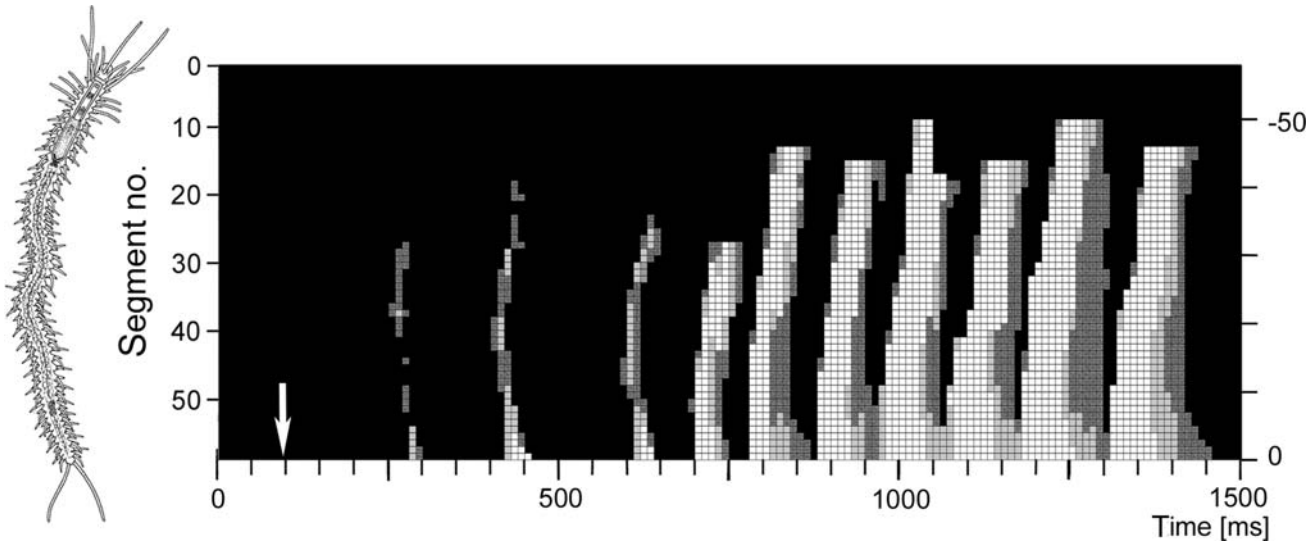

Fig. 10 A complete, brief flashing cycle emitted after a single mechanical shock (*arrow*) to the glass support carrying an intact *Eusyllis blomstrandi* of 58 segments trunk length. Note the amphidromous propagation of excitation in the early flashes accompanied by a shift of the starting points of the flashes towards the posterior end. In the later flashes, recruitment of luminescing segments runs from posterior towards anterior. Same animal as in Fig. 3. *Coordinates* and *symbols* as in Fig. 5

of segments involved in luminescence expands forward, recruiting flash by flash new segments, e.g. 18 in the case of Fig. 7, and 17 in the case of Fig. 10. Recruitment towards the anterior terminates somewhere in the middle or anterior part of the trunk. In late flashes, some of the anterior segments recruited before may stop luminescing again before the last flashes of the series set in. The borderline between the posterior luminescent and the anterior non-luminescent segments is always distinct, such that a fully luminescent segment adjoins a completely inactive one, even though this borderline during a flash series repeatedly jumps from one segment border to another.

Luminescent behaviour following fragmentation of the worm

Eusyllis blomstrandi, like all of the free-moving polychaetes, is often subject to loss of trunk fragments in its natural habitat. Unlike the posterior fragments, the anterior fragments can fully recover from the loss by regenerating the lost part. *E. blomstrandi* have been cut into two pieces or, by strong mechanical stimulation, were induced to commit autotomy in our experiments. Fragmentation is always linked with an outburst of strong luminescence.

Luminescent behaviour displays a striking asymmetry between anterior and posterior fragments (Fig. 11a, b). After autotomy or artificial fragmentation, the anterior fragment rapidly departs and abandons luminescence after emitting some more light pulses during a brief period of time (four flashes in the case shown in Fig. 11a, b emitted during a period of 806 ms). The posterior fragment, on the other hand, first glows for a few seconds extremely brightly and then continues luminescence in the flashing mode for a period far exceeding the duration of a regular flash series, in the case shown in Fig. 11a, b almost 6 s. The posterior fragments remain responsive to stimulation and capable of luminescent flashing for weeks despite their inevitable starvation.

Discussion

Analysis of the luminescent behaviour of the polychaete *E. blomstrandi*, using high-speed video imaging has revealed rhythmical flashing as the basic pattern of light emission. The kinetics of flashing is characterized by co-operative light emission from epidermal luminescent domains in a number of segments, by the capacity of the luminescent domains to modulate the intensity of the light emitted, and by coordination of the luminescence patterns among the segments in space and time. In the typical luminescent response, light emission is restricted to the posterior segments. A division of labour and risk is demonstrated by experimental fragmentation of the trunk when the posterior fragment continues to display particularly bright luminescence while the anterior fragment with the head and the feeding apparatus stops emitting light and rapidly moves away from the site of the damaging encounter. All these phenomena can be viewed in motion picture on-line in Zörner and Fischer (2006).

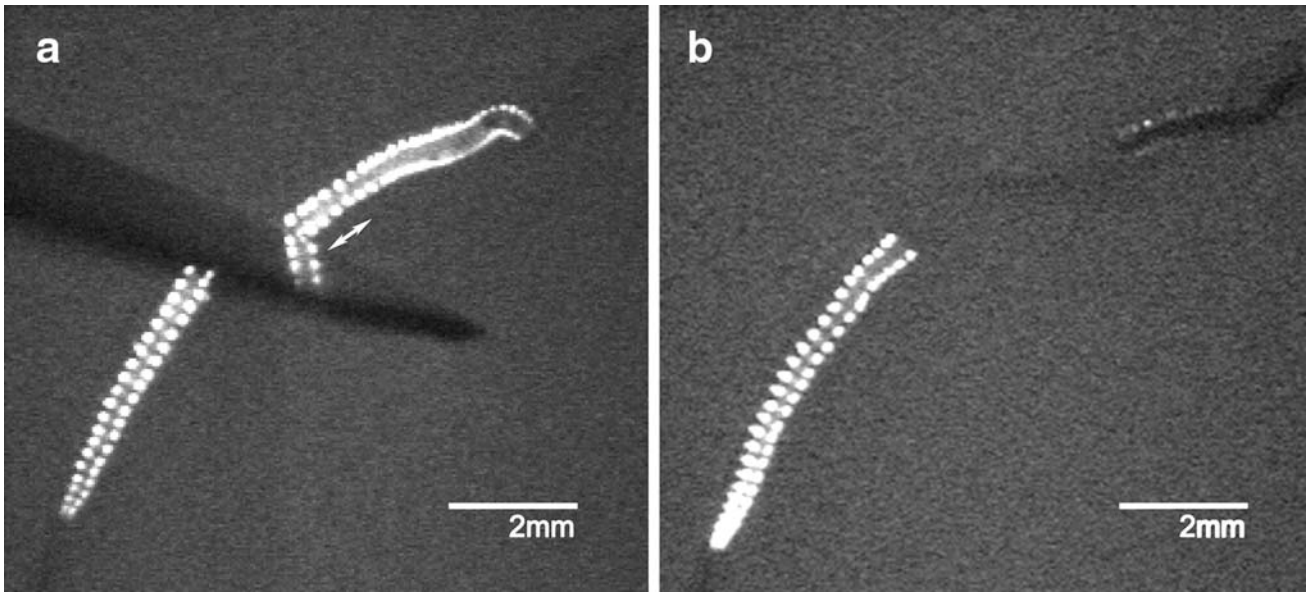

Fig. 11 **a** Under the squeezing pressure of a scalpel blade (*double arrow* light reflections on the blade) and 525 ms prior to autotomy a *Eusyllis blomstrandi* luminesces simultaneously in all of its trunk segments. **b** Luminescent behaviour of the anterior and posterior fragments of *E. blomstrandi*, same animal as in Fig. 11a, 1,158 ms later and 633 ms after fragmentation. The light emission has almost completely vanished in the anterior fragment (*top right*) while in the posterior fragment luminescence continues for 8.37 s after fragmentation, the initial 2.54 s of this period luminescing incessantly at full brightness

Every segment of *E. blomstrandi* is equipped with paired clusters of photocytes in the epidermis in the dorsal parapodial base and additional small luminescent units. Luminescence is always confined to these epithelial sites and thus appears to be strictly intracellular. Intermittent light emission might seem to result from repeated cycles of decay and might thus suggest the requirement for refuelling the light-emitting system after every flash. Such a fuelling problem, however, is obviously not the ultimate reason for the flashing pattern since *Eusyllis* can produce light continuously for several seconds following extreme stimulation. The flashing mode of light emission, therefore, must have another basis and is likely to be functionally relevant.

Light emission in the flashing mode in contrast with a continuous glow requires rapid intersegmental signalling and thus suggests a centralized neural coordination of luminescent activity. A number of observations support the existence of a central control of luminescence in *E. blomstrandi*: Both left and right luminescent domains in a segment start and terminate light emission synchronously. A particularly strong argument is the high speed at which excitation propagates and fades along the segmented trunk. Light emission may start in as many as 24 segments "at once," i.e. during the basic unit of time resolution possible with our equipment (10 ms), and light emission may cease in up to 30 segments during the same time span. From these data, a time interval of as little as $\leq$1 ms can be extrapolated as a minimum time required for transmitting or silencing of the excitation from segment to segment. Likewise, the modulation of luminescent brightness can propagate among the segments at the same high speed.

The kinetics of luminescence in *Eusyllis* also suggest the existence and activity of a pacemaker controlling the rhythmic course of flashing. Rhythmic light discharge can be triggered by a unique, arrhythmic stimulus, proving the capability of the worm for autonomous rhythmic flashing. The modulated rhythm of flashing does not appear to be influenced by the variable duration of the individual flashes: Even if the late flashes last longer and tend to overlap, the phase-length allotted to the single cycle of flashing does not extend correspondingly (Figs. 7, 10). The location of the pacemaker along the trunk appears variable: In Fig. 6 and in the late flashes of Fig. 7 the anterior-most luminescent segments light up first, suggesting that the pacemaker function resides in these anterior segments. In Fig. 10, the pacemaker function appears to move during the first five flashes from segments 37/38 in the first flash to the last, 58th segment in the fifth flash and to remain there until the end of the flash series. In the first part of the flash series shown in Fig. 7, the pacemaker function consolidates at the posterior end before the sense of pacemaking reverts in the second part of the series. The pacemaker function is even divisible: Immediately after fragmentation, the anterior fragment of *Eusyllis* still

continues rhythmic flashing for a few bursts (four in the case shown in Fig. 11a, b) before luminescence becomes silenced, and the posterior fragment, after emitting maximum light intensity continuously for some seconds, resumes a periodic flashing activity as well. Rhythmic flashing can also be organized in isolated scales and even in fragments of scales of polynoid polychaetes ("emissions autoentretenues"; Bilbaut and Bassot 1977). Thus, the rhythmicity of luminescent discharge in polynoids depends neither upon an intact segmental organization nor an intact central nervous system.

Two sorts of thresholds indicate a central, neural control of luminescence in *Eusyllis.* First, a threshold appears to exist for a flash event to take place. The last flash in a series is displayed at full or nearly full brightness by a substantial number of the segments before luminescence suddenly vanishes. This suggests that an integral threshold valid for the whole trunk or trunk fragment is no longer being crossed and that it has to be crossed for a flash to take place at all. On the contrary, in the polynoid scale system serial flashes emitted by isolated scales devoid of central nervous connections decrease continually and exponentially in amplitude with continuing stimulation until complete fading of responsiveness (Bilbaut and Bassot 1977). The most anterior of the luminescent segments may emit light at full brightness while the adjacent segment further to the anterior remains completely dark; nevertheless, that adjacent segment may be recruited for bright luminescence in the following flash. On the other hand, in posterior fragments all of the segments participate in light emission. This suggests the absence of a threshold in such fragments while in the intact animal the anterior end may exert a silencing effect, establishing a threshold in the anterior segments and thereby controlling the expansion of luminescence from the posterior to the anterior part of the trunk. The assumed existence of an inhibitory effect exerted by the anterior end is also supported by the rapid halt to which light emission is brought in the anterior fragment after the worm has been divided into two fragments.

Facilitation appears to take place in the course of a flash series. This can be concluded from three sorts of phenomena: Initially, the flashes appear weak, as most of the activated segments emit dim light only, whereas later the activated segments tend to start a flash emission at full light intensity. Second, the duration of light emission in a flash increases during a series. Third, the group of luminescent trunk segments initially, flash by flash, recruits more anterior segments. It is unknown whether facilitation takes place at the effector tissue or the neural level.

In the scale worm system, facilitation is brought about by recruitment of previously inactive cells of the scale epidermis as well as by intracellular recruitment and coupling of the photosomes, the luminescent organelles (Bassot 1987). Working with free-moving, un-anesthetized worms or worm fragments at low optical magnification, we could not yet ascertain a role played by intracellular coupling of intracellular organelles in modulating the intensity of segmental light emission in *Eusyllis*.

Fatigue, the phenomenon complementary to facilitation, occurs to a lesser degree than facilitation. It may become manifest as a decrease in the number of segments involved in luminescent discharge (Fig. 7) and/or a lowering of light intensity emitted from the individual segment (Fig. 6). In the case of isolated polynoid scales, fatigue can be attributed to the definitive exhaustion of photogenic material (Bassot 1987). In *E. blomstrandi*, however, the decrease of intensity that may be observed in the later course of a flash series cannot be explained by a shortage in the stock of photogenic material: Harsh treatment of the worm can without delay lead to extended periods of full strength luminescence and thus reveals the existence of large stocks of photogenic material that would otherwise go unnoticed. Thus, a different explanation must account for the fatigue phenomenon observed in *Eusyllis* luminescence.

We still have no information on the neural organization of bioluminescence control in syllid polychaetes. The existence of giant fibres in the ventral nerve cord is a common feature of the polychaetes which allows rapid communication among the segments and quick reflexes. Specific information on the structure and function of this system in relation to syllid bioluminescence, however, is lacking.

Our present paper on the kinetics of flashing luminescence in *E. blomstrandi* is the first on this subject, with the exception of a brief description of flashing bioluminescence in the closely related syllid *P. pulligera* (Bassot 1979). Polychaete bioluminescence has, however, been extensively studied in the very special case of polynoid scales that are easily shed upon irritation of the worm and can emit bright luminescence. The limited level of emitted luminescent light and the resulting technical problems in video imaging were overcome in this system by using artificially induced epifluorescent light, as the light-producing organelles in polynoids become fluorescent while luminescing. Detailed knowledge has been gathered in this system on the histological and organelle structure of the luminescent tissue and its nerve equipment as well as on many kinetic parameters of luminescent activity (Bassot 1966, 1979,

1987; Bassot and Bilbaut 1977a, b; Bilbaut and Bassot 1977; Pavans De Ceccatty et al. 1977; Herrera 1979; Hastings et al. 1987). After this impressive start of research on polychaete luminescence in the polynoid scale system, we now expect that working with the intact *Eusyllis*, possessing its complete neuronal circuitry and behavioural integrity, will expand our understanding of how luminescence in polychaetes is operated and controlled and will justify the establishment of a different experimental animal. Self-mutilation after electrical stimulation in *Eusyllis* indicates that mechanical instead of electrical release of luminescence is preferable for the study of typical luminescent patterns and raises the question whether the occurrence of "atypical" patterns of luminescent flashing in isolated scales (Bilbaut and Bassot 1977) might be artefacts due to electrical shocking. Moreover, the use of modern equipment gives us the possibility of using natural light for imaging of the luminescent discharges in *E. blomstrandi*, whereas equipment-induced fluorescent light had to be used for technical reasons in the imaging of polynoid scale luminescence (Bassot and Bilbaut 1977b).

The functional significance of luminescent behaviour in marine animals has for a long time intrigued scientists, and the numerous explanations (Morin 1983; Hastings and Morin 1991) still rest on little experimental evidence. In some cases, the plain observation is very suggestive of certain functions, as in the "Bermudian fireworm," *O. enopla*. The ripe females in this species give off a luminescent secretion which, according to field observations (Fischer and Fischer 1996), attracts ripe males. Morin's classification ascribes a deterrent function to bioluminescent flash signals and an attracting function to luminescent "glow" (continuous emission of $\geq$2 s).

In many trials, we have made the consistent observation that fragmentation of a *Eusyllis* is immediately followed by an excessive, long-lasting luminescent display of the posterior fragment and a rapid evanescence of light emission in the anterior fragment. Immediately following fragmentation, luminescence of the posterior fragment is a bright glow slowly changing into a flash series of unusually long duration, and light emission continues to be easily elicited by mechanical stimulation in posterior fragments as long as they survive. This observation suggests that the behaviour of the posterior fragment is designed to attract the attention of potential predators while the non-luminescent anterior fragment can meanwhile escape in the dark. This interpretation can be extended to the luminescence pattern in intact worms: Light emission is usually confined to the posterior part of the trunk and might distract the attention of a predator from the anterior end of the prey.

The luminescent behaviour of *E. blomstrandi* strongly suggests a beneficial effect: Only the anterior fragment is capable of regenerating the lost part and can survive as a potentially reproductive member of the population while posterior trunk fragments may survive for a while but are incapable of regenerating the lost head and feeding apparatus (personal observation; Boilly 1961). Bioluminescence of the posterior trunk or trunk fragment in an antagonistic encounter would serve the purpose of a decoy or an alerting signal. The discharge of luminescent light by autotomized scales of polynoids has been interpreted to have the same function (Herrera 1979). Since *E. blomstrandi* is readily available and we know its luminescent behaviour and how to handle the worms, we can now think of observations strictly demonstrating the validity of the suggested beneficial effect. Testing the validity of our hypothesis in an experimental predator–prey encounter would, however, require the knowledge of the potential predators in the littoral habitat of Helgoland.

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