*J. mar. biol. Ass. U.K.* (1958) 37, 33–41
Printed in Great Britain 

# LUMINESCENCE IN POLYNOIDS

## IV. MEASUREMENTS OF LIGHT INTENSITY

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(Text-figs. 1–3)

## INTRODUCTION

Many polynoid worms (family Aphroditidae) are luminescent. In recent papers, physiological and histological aspects of polynoid luminescence have been considered in some detail (Bonhomme, 1942; Nicol, 1953, 1954, 1957*a*, *b*). The light appears in the scales or elytra which cover the dorsal surface of the worm. The photocytes form a single epithelial layer on the lower surface of the elytrum. They are concentrated near the centre of the scale and, when the latter is pigmented, there is a clear area above the photogenic tissue through which the light escapes.

Luminescence in polynoids is under nervous control and is evoked by external stimulation. The elytra usually flash repetitively to a single stimulus, and the responses can be analysed by recording from single elytra. Flash duration ranges from 100 to 200 msec. There is much variation in flash intensity: initially, consecutive flashes become progressively brighter, owing to facilitation, but fatigue soon sets in and the flashes become fainter and finally die away.

The light of polynoids is emerald-green in colour. Spectral emission extends from about 450 to 680 m$\mu$, with a maximum at about 515 m$\mu$ (Nicol, 1957*c*). The spectral emission curve is shown in Fig. 1. This information has been utilized to calculate the intensity of polynoid light, in the manner now to be described.

## MATERIAL AND METHODS

The light of two species was measured, viz. *Lagisca extenuata* and *Acholoë astericola*. Elytra were removed from the animals under $MgCl_2$-narcosis, and were subsequently washed in sea water. A single elytrum was mounted in a Perspex chamber over a pair of silver electrodes (see Nicol, 1953). Flashing was induced by stimulating with electric shocks: these were square wave pulses, about 2 msec in duration, and up to 10 V in intensity.

The light was detected by a photomultiplier (E.M.I. type no. 6685), the spectral sensitivity of which had been determined by the National Physical Laboratory. The photocathode of the photomultiplier is 9 mm in diameter. Sensitivity is maximal in the violet, and falls off steadily at long wavelengths, above 500 mμ (Fig. 2, curve A). The elytrum was positioned 2 cm beneath the photomultiplier, so that its upper surface lay beneath the centre of the photocathode, and was parallel to the face of the latter. The light-emitting region of the elytrum is less than 1 $mm^2$ in area, and can be regarded as a point-source.

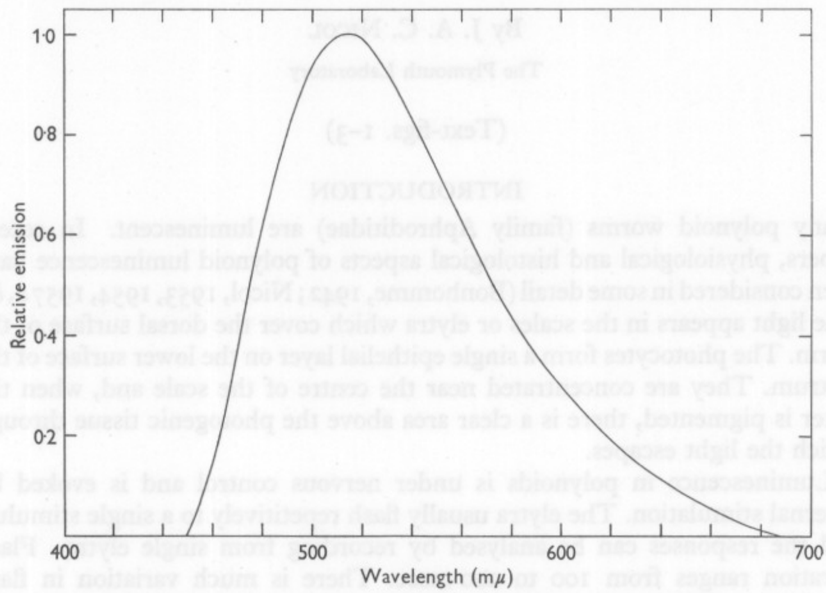


Fig. 1. Relative spectral emission curve of polynoid light (from Nicol, 1957*c*).

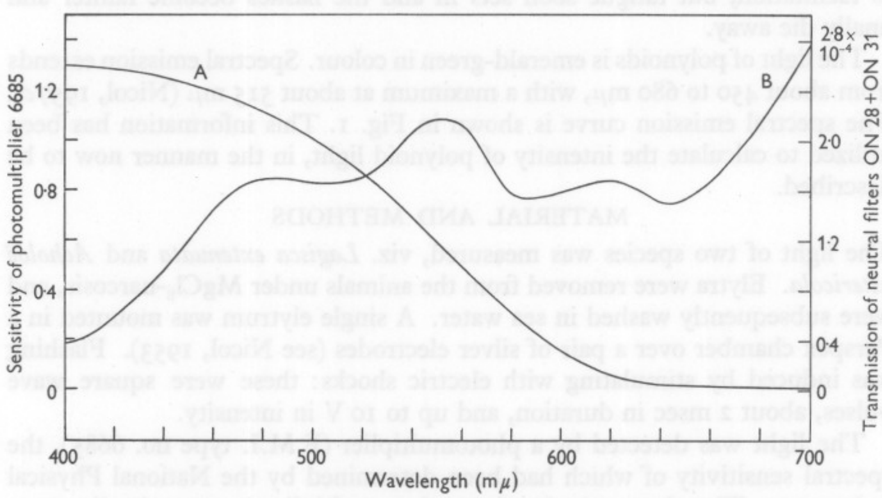


Fig. 2. Curve A: relative spectral sensitivity of photomultiplier type E.M.I. no. 6685. Curve B: combined transmission of two neutral glass filters (Chance ON 28 + ON 31).

The photomultiplier was connected to a cathode-ray oscilloscope, and photographic records of the responses were made on moving paper.

Measurements were made at ambient temperatures of 18–19° C.

## CALIBRATION OF THE PHOTOMULTIPLIER

The sensitivity of the apparatus, that is of photomultiplier 6685 plus amplifier and C.R.O., was determined against a known light source. This was a substandard (tungsten) lamp, of colour temperature 2360° K, and energy output of 24·15 candelas. The lamp was calibrated by the National Physical Laboratory. It was placed on an optical bench 2 m from the face of the photomultiplier. Intensity was reduced by two neutral glass filters (Chance ON28 + ON31), the transmission characteristics of which were measured in a spectrophotometer (Unicam SP500) (see curve B, Fig. 2).

The flux of light from the substandard lamp at 2 m, falling on the surface of the photomultiplier perpendicular to the direction of the light beam, is

$$\int_{400}^{700} pK\mathcal{J}_\lambda V_\lambda \, d\lambda = pK\int_{400}^{700} \mathcal{J}_\lambda V_\lambda \, d\lambda = I/4\times 10^4 \text{ lumens/cm}^2.$$

$I$ is the intensity of the substandard lamp in candelas, $p$ is a quantity for the particular experimental set-up, and $K$ is the luminous efficiency of radiation, which is 682 lumens/W at 555 mμ (Walsh, 1953):

$$p = \frac{I}{4\times 10^4} \times \frac{1}{682\int_{400}^{700} \mathcal{J}_\lambda V_\lambda \, d\lambda} \text{ W/cm}^2/\text{m}\mu.$$

Let the light from the substandard lamp at 2 m, passing through the neutral filters, give a deflexion on the oscilloscope $= D_L$. Let $T_\lambda$ be the combined transmission of the neutral filters, $S_\lambda$ be the spectral sensitivity of the photomultiplier, and $q$ be a constant such that $q/S_\lambda = \text{W/cm}^2$ of a given wavelength ($\lambda$) required to produce unit deflexion. Then

$$D_L = \int_{400}^{700} \frac{p\mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda}{q} = \frac{p}{q}\int_{400}^{700} \mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda$$

and

$$q = \frac{p}{D_L}\int_{400}^{700} \mathcal{J}_\lambda S_\lambda T_\lambda \, d\lambda \text{ W/cm}^2.$$

Values for $V_\lambda$ were taken from the C.I.E. table for photopic vision (International Relative Luminous Efficiency of Radiation for Photopic Vision, table VI in Keitz, 1955). Values for $\mathcal{J}_\lambda$ were obtained from Skogland, 1929, for a lamp of colour temperature 2360° ($\mathcal{J} = 1$ at $\lambda = 590$ mμ).

To determine $p$ and $q$, the following values were used. Intensity, $I$, of the substandard lamp is 24·15 candelas. With set amplification on the C.R.O.

(30 V/mm), and known voltage on the photomultiplier (1400 V), the deflexion produced by the light is 22·02 mm.

From this quantity, an estimate was made of the deflexion which would be produced at higher amplification, viz. 10 V/mm on the C.R.O., and photomultiplier voltage of 1600 V. This estimated deflexion was 227·36 mm.

$$\int_{400}^{700} J_\lambda V_\lambda = 83{\cdot}52,$$

and $$p = \frac{24{\cdot}15}{4 \times 10^4} \times \frac{1}{682 \times 83{\cdot}52} = 1{\cdot}0599 \times 10^{-8}\ \mathrm{W/cm^2/m\mu}.$$

$$\int_{400}^{700} J_\lambda S_\lambda T_\lambda \, d\lambda = 105{\cdot}1 \times 10^{-4}$$

and $$q = \frac{1{\cdot}0599 \times 10^{-8}}{227{\cdot}36} \times 105{\cdot}1 \times 10^{-4} = 0{\cdot}49 \times 10^{-12}\ \mathrm{W/cm^2}.$$

In order to make periodical checks on the sensitivity of the apparatus, an alternative light source was employed. This consisted of a stilbene phosphor irradiated by $^{60}$Co (called, for brevity, Co source). The phosphor emitted a very faint blue light. Random emission by the phosphor gave a very broad beam-trace, which was smoothed out by putting a 0·1 $\mu$F condenser across the input of the oscilloscope. The Co source was set 2 cm from the face of the photomultiplier, and the oscilloscope deflexion was photographed.

## MEASUREMENTS OF THE INTENSITY OF POLYNOID LIGHT

Records were obtained of the flashes of eight elytra of *Lagisca*, and of six elytra of *Acholoë*. Some examples are shown in Fig. 3. Various amplifications were used, and the measured deflexions were calculated on the basis of instrument-sensitivity occurring at a C.R.O. setting of 10 V/mm and 1600 V on the phototube. These calculated deflexions, $D_s$, for elytra at 2 cm distance, are shown in columns 2 of Tables 1 and 2. Column 3 shows calculated deflexions at a distance of 1 m, according to the inverse square law.

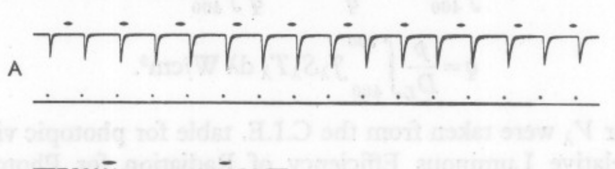


Fig. 3. Oscillograph records of the flashing of polynoid elytra. A, *Acholoë*, B, *Lagisca*. Time signal, above, 1/sec. Electrical stimuli on lower line.

The flashes of polynoids are much briefer than 1 sec. In order to calculate the energy of one flash, the response curve for a flash was averaged over 1 sec in terms of a maximal deflexion lasting 1 sec. These calculations gave factors, A, for response curves of: 0·02 for *Lagisca*, and 0·084 for *Acholoë*. The deflexions ($D_s$) for the responses of *Lagisca* and *Acholoë*, averaged over 1 sec, are shown in columns 4 of Tables 1 and 2 ($D = D_s A$).

TABLE 1. CALCULATION OF THE INTENSITY OF LIGHT EMITTED BY AN ELYTRUM OF *LAGISCA*

| Elytrum and record | Maximal deflexion $D_s$ at 2 cm (mm) | Calculated deflexion $D_s$ at 1 m (mm) | $D\ (=A^* \times D_s)$ at 1 m | Energy at 515 mμ in 1 flash, μJ/cm² at 1 m ($\times 10^{-12}$) | Total energy in 1 flash, μJ/cm² at 1 m ($\times 10^{-10}$) |
|---|---|---|---|---|---|
| 2 A | 0·6 | 0·00024 | 0·0000048 | 0·03129 | 0·03309 |
| B | 0·9 | 0·00036 | 0·0000072 | 0·046944 | 0·04964 |
| C | 0·3 | 0·00012 | 0·0000024 | 0·015648 | 0·01655 |
| D | 0·55 | 0·00022 | 0·0000044 | 0·028688 | 0·03034 |
| 3 A | 4·1 | 0·00164 | 0·0000328 | 0·213856 | 0·22615 |
| B | 2·5 | 0·00100 | 0·0000200 | 0·13040 | 0·13790 |
| C | 16·5 | 0·00660 | 0·0001320 | 0·860640 | 0·91013 |
| 4 A | 0·9 | 0·00036 | 0·0000072 | 0·046944 | 0·04964 |
| B | 4·5 | 0·00180 | 0·0000360 | 0·234720 | 0·24822 |
| C | 52·5 | 0·02100 | 0·0004200 | 2·73840 | 2·89586 |
| 5 A | 6 | 0·00240 | 0·0000480 | 0·31296 | 0·33096 |
| B | 3·9 | 0·00156 | 0·0000312 | 0·203424 | 0·21512 |
| 6 A | 3 | 0·00120 | 0·0000240 | 0·15648 | 0·16548 |
| B | 66 | 0·02640 | 0·0005280 | 3·44256 | 3·64051 |
| 7 A | 10·8 | 0·00432 | 0·0000864 | 0·56332 | 0·59572 |
| B | 43·2 | 0·01728 | 0·0003456 | 2·253312 | 2·38286 |
| 8 | 6·48 | 0·002592 | 0·0000518 | 0·337997 | 0·35743 |
| 9 A | 8·64 | 0·003456 | 0·0000691 | 0·450662 | 0·47658 |
| B | 1·44 | 0·000576 | 0·0000115 | 0·075110 | 0·07943 |

$$* \ A = \frac{\text{area of response curve for a single flash averaged over 1 sec}}{\text{maximal response} \times 1 \text{ sec}}.$$

TABLE 2. CALCULATION OF THE INTENSITY OF LIGHT EMITTED BY AN ELYTRUM OF *ACHOLOË*

| Elytrum and record | Maximal deflexion $D_s$ at 2 cm (mm) | Calculated deflexion $D_s$ at 1 m ($\times 10^{-4}$) (mm) | $D\ (=A^* \times D_s$ at 1 m) ($\times 10^{-4}$) | Energy at 515 mμ in 1 flash, μJ/cm² at 1 m ($\times 10^{-10}$) | Total energy in 1 flash, μJ/cm² at 1 m ($\times 10^{-8}$) |
|---|---|---|---|---|---|
| 1 A | 1·824 | 7·296 | 0·6129 | 0·39961 | 0·42259 |
| B | 8·683 | 34·732 | 2·9175 | 1·90221 | 2·01159 |
| 2 | 0·917 | 3·668 | 0·3081 | 0·20089 | 0·21244 |
| 5 A | 1·643 | 6·572 | 0·5520 | 0·35994 | 0·38064 |
| B | 7·126 | 28·504 | 2·3943 | 1·56108 | 1·65084 |
| 8 | 1·376 | 5·504 | 0·4624 | 0·30148 | 0·31882 |
| 9 A | 1·099 | 4·396 | 0·3693 | 0·24078 | 0·25462 |
| B | 1·985 | 7·940 | 0·6670 | 0·43488 | 0·45989 |

* See Table 1 for definition.

A relative spectral emission curve for polynoid light is shown in Fig. 1, in which relative energy, $E_\lambda$, is plotted against $\lambda$ (Nicol, 1957c). This curve can be put on an absolute basis in terms of a quantity $r$ such that $E_\lambda r$ gives Watts/cm² of receptor surface/m$\mu$ under the experimental conditions specified.

$$D=\int \frac{rE_\lambda S_\lambda}{q}\,d\lambda,\quad D=\frac{r}{q}\int E_\lambda S_\lambda\,d\lambda.$$

$$r=\frac{Dq}{\int E_\lambda S_\lambda\,d\lambda}\ \mathrm{W/cm^2/m\mu},\quad \int_{400}^{700} E_\lambda S_\lambda\,d\lambda=75\cdot 1,$$

$$r=D\times\frac{0\cdot 49\times 10^{-12}}{75\cdot 1}=D\times 0\cdot 652\times 10^{-14}\ \mathrm{W/cm^2/m\mu}.$$

The energy at 515 m$\mu$ in 1 flash is equal to

$$r=D\times 0\cdot 652\times 10^{-14}\ \mathrm{W/cm^2/m\mu}\ \text{at 1 m}.$$

The total energy in 1 flash is equal to

$$r\int_{400}^{700} E_\lambda\,d\lambda=D\times 0\cdot 652\times 10^{-14}\int_{400}^{700} E_\lambda\,d\lambda\ \mathrm{W/cm^2}\ \text{at 1 m}.$$

From integration of the curve in Fig. 1,

$$E_\lambda\,d\lambda=105\cdot 75.$$

Values for energy in 1 flash at 515 m$\mu$, and for total energy in 1 flash, are shown in columns 5 and 6 of Tables 1 and 2. For *Lagisca* the total energy in a flash ranges from $0\cdot 017\times 10^{-10}$ to $3\cdot 641\times 10^{-10}$ $\mu$J/cm² at 1 m. For *Acholoë*, the total energy in a flash ranges from $0\cdot 212\times 10^{-8}$ to $2\cdot 012\times 10^{-8}$ $\mu$ J/cm² at 1 m.

These values were determined by measuring that light which was emitted in a cone having its axis perpendicular to the upper surface of the elytrum, and possessing a solid angle $w=0\cdot 16$ sterad.

The data and calculations of Tables 1 and 2 pertain to a single flash. Polynoid elytra usually flash repetitively, so that there may be 1 or many flashes in a given second; at fast rates there is often some degree of summation. A calculation for *Lagisca* gives the following results:

(*a*) Total energy in a single brief flash (maximal flash in a series of flashes): $0\cdot 182\times 10^{-9}$ $\mu$J/cm² at 1 m.

(*b*) Total flux in a series of 13 flashes occurring in 1 sec (maximal flash intensity as in (*a*)): $2\cdot 600\times 10^{-9}$ $\mu$W/cm² at 1 m.

## INTENSITY OF LIGHT EMITTED PER PHOTOCYTE

The photocytes lie in a single cellular layer. This arrangement permits an estimation of the light emitted per photocyte. Elytra were sectioned (at 7 $\mu$), and the total number of photocytes counted. In elytrum no. 7 of *Lagisca* there were 1250 photocytes. Minimal and maximal values for the light emitted by a photocyte in 1 flash are: minimum, $0{\cdot}048 \times 10^{-12}$ $\mu$J/cm² at 1 m; maximum, $0{\cdot}191 \times 10^{-12}$ $\mu$J/cm² at 1 m.

## COMMENT

Mean values for the intensity of light emitted in a single flash by an elytrum of *Lagisca* are $1{\cdot}8 \times 10^{-6}$ $\mu$J/cm² at 1 cm, and $1{\cdot}8 \times 10^{-10}$ $\mu$J/cm² at 1 m. Corresponding values for the flash of an elytrum of *Acholoë* are $1{\cdot}11 \times 10^{-4}$ $\mu$J/cm² at 1 cm and $1{\cdot}11 \times 10^{-8}$ $\mu$J/cm² at 1 m. These estimates are for air-paths.

Some estimations are available for the radiant flux emitted by other marine species. The luminescence of a ctenophore *Mnemiopsis leidyi*, 35 mm in diameter, was $0{\cdot}5 \times 10^{-4}$ to $>0{\cdot}75 \times 10^{-4}$ $\mu$W/cm² at 50 cm (recalculated as $0{\cdot}125 \times 10^{-4}$ to $>0{\cdot}187 \times 10^{-4}$ $\mu$W/cm² at 1 m in air) (Clarke & Backus, 1956). The light of *Euphausia pacifica* has a mean intensity of $1{\cdot}8 \times 10^{-3}$ $\mu$W/cm² at 1 cm. An adult *Euphausia pacifica* is about 20 mm long and has 10 light-emitting organs (photophores). The light intensity of *Pyrosoma atlantica* ranges from $8 \times 10^{-3}$ to $4 \times 10^{-2}$ $\mu$W/cm² at 1 cm. A colony 10 cm long emitted light of intensity $2{\cdot}5 \times 10^{-2}$ $\mu$W/cm² at 1 cm (Kampa & Boden, 1957).

The flashes of polynoids are shorter than 1 sec. If the light of these worms remained constant during 1 sec at maximal flash intensity, then the radiant flux for an elytrum of *Lagisca* and *Acholoë*, respectively, would be: $0{\cdot}9 \times 10^{-4}$ and $1{\cdot}31 \times 10^{-3}$ $\mu$W/cm² at 1 cm (in air). These values for radiant flux of polynoid elytra are of about the same order as that for *Euphausia*. Ctenophores and Pyrosomas are much bigger animals, with correspondingly larger light-emitting surfaces.

The light emitted in 1 flash by a single photocyte of *Lagisca* is $0{\cdot}12 \times 10^{-12}$ $\mu$J/cm² receptor surface at 1 m. For a flash of 1 sec duration, the estimated radiant flux would be $1{\cdot}3 \times 10^{-12}$ $\mu$W/cm² receptor surface at 1 m. Harvey (1925) has estimated the total radiant flux emitted by a single bacterium of *Bacillus phosphorescens* to be $4{\cdot}95 \times 10^{-10}$ $\mu$W at $\lambda = 510$ m$\mu$ (the wavelength of maximal emission). Over the entire emission spectrum $\left(\text{i.e.} \int_{400}^{700} E_\lambda \, d\lambda\right)$, the radiant flux emitted by a single bacterium is about $0{\cdot}4 \times 10^{-12}$ $\mu$W/cm² receptor surface at 1 m. I have recently estimated the energy in a single brief flash from a cell of *Noctiluca miliaris* to be about $1{\cdot}1 \times 10^{-12}$ $\mu$J/cm² receptor surface at 1 m (in air) (unpublished research). This is about ten times greater than the light output of a photocyte of *Lagisca*.

The threshold of the dark-adapted human eye for a flash having a duration $<0\cdot1$ sec is about 100 quanta at 510 m$\mu$ in a test field of 10′ (Graham & Margaria, 1935; Hecht, Shlaer, & Pirenne, 1942; Pirenne & Denton, 1952). When fully exposed, the human pupil has an area, mean $S = 0\cdot5$ cm². Now, 1 quantum at 510 m$\mu = 4 \times 10^{-12}$ erg. For a just detectable flash, the human eye needs 100 quanta $= 4 \times 10^{-10}$ erg to fall in the pupil, area $S$.

The mean value for energy in a flash of *Lagisca* is $1\cdot8 \times 10^{-10}$ $\mu$J/cm² receptor surface at 1 m, or $1\cdot8 \times 10^{-9}$ erg/cm² receptor surface at 1 m.

At $x$ m, the equivalent energy, if all were concentrated at 510 m$\mu$, in a polynoid flash

$$= \frac{1\cdot1 \times 10^{-9}}{x^2} \text{ erg/cm}^2 \text{ receptor surface}$$

$$= \frac{1\cdot1 \times 10^{-9}}{x^2} S \text{ erg/mean pupil area.}$$

The distance in air at which an average flash of *Lagisca* can be seen, by the dark-adapted human eye, is given by

$$4 \times 10^{-10} \text{ erg} = \frac{1\cdot1 \times 10^{-9}}{x^2} S \text{ erg/mean pupil area,}$$

$$x^2 = \frac{1\cdot1 \times 10^{-9} \times 0\cdot5}{4 \times 10^{-10}} \text{ metres squared,}$$

$$x = 1\cdot2 \text{ m.}$$

I am indebted to Dr E. J. Denton for guidance in the calculations of light intensity. Part of the apparatus was purchased with grants from the Royal Society. The $^{60}$Co-stilbene light source was devised and loaned by Dr G. N. Harding of the U.K. Atomic Energy Authority, to whom I am grateful.

## SUMMARY

The light energy emitted in a flash by single elytra of two polynoid worms has been measured, viz. *Acholoë astericola* and *Lagisca extenuata*. Maximal emission occurs at 515 m$\mu$. Mean values for light intensity per flash from 1 elytrum are: *Lagisca*, $1\cdot8 \times 10^{-10}$ $\mu$J/cm² receptor surface at 1 m; *Acholoë*, $1\cdot11 \times 10^{-8}$ $\mu$J/cm² receptor surface at 1 m. The light emitted in 1 flash by a single photocyte of *Lagisca* is $0\cdot12 \times 10^{-12}$ $\mu$J/cm² receptor surface at 1 m. It is estimated that the light from 1 elytrum of *Lagisca* could be seen by the dark-adapted human eye at 1·2 m in air.

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