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SPECTRAL COMPOSITION OF THE LIGHT OF POLYNOID WORMS

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(Text-figs. 1–14)

The spectral composition of the light of only one marine animal is known, viz. the ostracod *Cypridina hilgendorfii* (see Coblentz & Hughes, 1926; Eymers & van Schouwenburg, 1937). It is desirable to have spectral energy curves for the light of other animals in order to relate these to the spectral sensitivity of photoreceptors, and to calculate total radiant energy in the visible range. To further these ends I have measured the spectral energy distribution of the light of some polynoid worms.

The luminescence of polynoids originates in the elytra or scales covering the dorsal surface. The photocytes form a single epithelial layer on the lower surface of the centre of the elytrum, and the light shines through the elytrum, which is clear and non-pigmented over the luminescent tissue. The light appears emerald-green in colour, and presumably has an emission peak somewhere between 510 and 530 mμ. In the normal response the light is emitted in brief discontinuous flashes, some 0·1 sec. in duration (Nicol, 1953, 1957*a*). Repeated or strong shocks sometimes produce a prolonged glow, in which the luminescence tends to become exhausted. Separate flashes vary greatly in intensity, owing to facilitation and fatigue (Nicol, 1954). The problem, then, is to determine the spectral composition of brief flashes of weak green light, varying greatly in intensity. The procedure adopted is described below.

MATERIAL AND METHODS

Four species of polynoid worms were used, viz. *Lagisca extenuata*, *Gattyana cirrosa*, *Polynoë scolopendrina* and *Harmothoë longisetis*. Elytra were removed from a worm under $MgCl_2$-narcosis, and washed in sea water. An isolated elytrum was mounted in a glass and Perspex moist chamber over a pair of electrodes, and the chamber was sealed with a glass coverslip. This arrangement allowed the light to be detected from above and below the scale. Below the chamber lay an 11-stage photomultiplier (E.M.I. no. 6260), with a 45 mm end-window; above, a 14-stage photomultiplier (E.M.I. no. 6685), with a 9 mm end-window. The two photomultipliers were connected to separate cathode-ray oscilloscopes, and photographic records of the luminescent responses were made on moving paper. The arrangement is illustrated in Fig. 1.

The lower photomultiplier was used to provide an index of flash intensity. A coloured filter was placed between the specimen and the upper photomultiplier, which was used to measure the light transmitted by a certain spectral band. The coloured filters were Ilford's spectrum set, nos. 601 to 608, covering the visible spectrum; curves are given in *Ilford colour filters*, published by Ilford Ltd. In some instances a neutral filter (Chance's neutral glass ON 31 or 32) was placed over the lower photomultiplier to prevent saturation from very bright flashes. This arrangement allowed very bright responses to be recorded by the lower photomultiplier, without altering

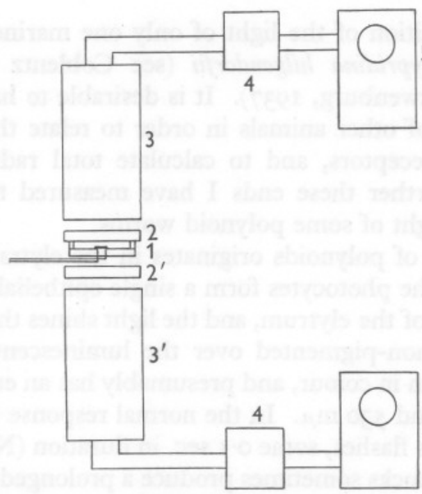


Fig. 1. Diagram of apparatus. 1, moist chamber containing elytrum and electrodes; 2, coloured spectral filter; 2′, neutral filter; 3, photomultiplier 6685; 3′, photomultiplier 6260; 4, d.c. amplifiers; 5, cathode-ray oscilloscopes.

voltage on the tube, in those instances when measurements were being made with coloured filters 606–608 in front of the upper photomultiplier. I determined the transmission of my set of filters with a spectrophotometer (Unicam SP 500).

Both photomultipliers had Cs–Sb photocathodes, with high sensitivity in the violet, and very low sensitivity in the red. The spectral sensitivity of photomultiplier no. 6685 was determined by the National Physical Laboratory. Combined data for photomultiplier no. 6685 and coloured filters are given in Table 1.

The mains voltage was maintained constant for all equipment by a voltage stabilizer. Both photomultipliers were run off the same power-pack, which held steady voltage, as determined by periodic checks.

Repeat determinations, made with a constant light source, showed that measurements could be made on the oscilloscope screen with an accuracy of ±2%.

A series of observations was made with the several coloured filters on each elytrum. If the elytrum continued to respond with sufficient intensity, it was possible to do a run with all filters on a single elytrum. From two to eight runs were made with all filters for the elytra of each species. Since the elytra flash repetitively to stimulation, each photographic record reveals a series of flashes (10 or more), a selected number of which was measured. The conclusions are based on about 150 photographic records and an analysis of some 1000 flashes.

Room temperatures during the measurements varied from 18° to 19° C.

TABLE 1. CERTAIN CHARACTERISTICS OF ILFORD SPECTRUM FILTERS AND E.M.I. PHOTOMULTIPLIER NO. 6685

Ilford filters	Filter range (mμ)	Maximal transmission λ	Maximal transmission %	Representative wavelength (mμ)	Area of curve $S_\lambda T_\lambda$*
Violet 601	400–485	440	16·8	433	230·0
Blue 602	440–495	465	7·1	465	48·0
Blue-green 603	470–525	490	13·5	490	81·0
Green 604	500–540	515	7·8	514	32·1
Yellow-green 605	525–570	545	6·7	548	24·43
Yellow 606	560–610	575	7	573	11·0
Orange 607	575–700	600	18·2	595	23·26
Red 608	610–700	700	81	661	5·6

* *S*, sensitivity of photomultiplier. *T*, transmission of filter.

OBSERVATIONS

ESTIMATION OF ACCURACY, USING A KNOWN LIGHT SOURCE

In order to evaluate the accuracy of the method, light from a known source was measured by means of the Ilford spectrum filters and photomultiplier no. 6685. The light was provided by a substandard lamp (colour temperature 2360° K), and was passed through a yellow-green filter (Chance OGR 2). Measurements of the response were made on the oscilloscope-face for each of the spectral filters nos. 601–608. To reduce intensities, a Chance neutral density glass (no. ON 31) was used in conjunction with Ilford filters nos. 603–607. Relative transmission values of the green filter OGR 2 were determined with a spectrophotometer (Unicam SP 500). The curves in Fig. 2 show the actual transmission of filter OGR 2 (curve *A*), and the calculated spectral composition ($J_\lambda T_\lambda$) of the light reaching the photomultiplier (curve *B*). The circles in Fig. 2 are the first estimations of relative spectral energy, made with spectral filters, photomultiplier and oscilloscope.

Most of the filters have rather broad transmission bands, and the sensitivity of the photomultiplier changes greatly over the visual spectrum. The values for the combination $S_\lambda T_\lambda$ are based on the assumption of an equal energy spectrum. Light having a pronounced spectral peak will differ from an equal energy spectrum in its effects at various wavelengths according to the characteristics of each factor in the combination $E_\lambda S_\lambda T_\lambda$. To predict the results, an approximate energy curve was drawn from the points in Fig. 2, and energy levels ($E_{\lambda A}$) from this curve were used to calculate a series of values for $\int E_{\lambda A} S_\lambda T_\lambda$. These values were then used to correct the results, in the manner outlined in the next section. The final corrected estimations of relative spectral composition, based on direct measurements, are represented by the circles in Fig. 3, which also gives the calculated spectral composition of artificial light ($\mathcal{J}_\lambda T_\lambda$). Values for $\mathcal{J}_\lambda$ were taken from Skogland (1929). There is reasonably good agreement between predicted and measured values. These measurements, of course, were made under optimal conditions (steady light intensity and absence of background noise).

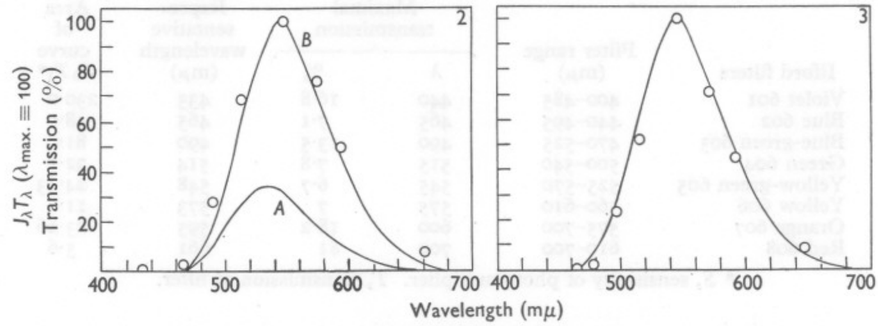


Fig. 2. Curve A: transmission (%) of a green filter. Curve B: calculated relative spectral energy of light from a tungsten lamp (2360° K), transmitted through a green filter (Chance OGR 2) ($\mathcal{J}_\lambda T_\lambda$). Circles are first measurements of relative spectral energy made by means of coloured filters and photomultiplier no. 6685.

Fig. 3. Curve as in Fig. 2B (calculated relative spectral energy of artificial light—source 2360° K plus Chance OGR 2). Circles are corrected measurements of relative spectral energy.

MEASUREMENTS OF THE LIGHT FROM POLYNOID ELYTRA

First approximate results obtained by measuring the light emission of four species of polynoids are given in Table 2, and the data are presented graphically in Figs. 4–7. Some representative photographic records are shown in Fig. 8. The data for an experiment on one animal (*Polynoë*) are given in greater detail in Table 3, to illustrate the calculations involved. Measurements for all four species of polynoids are pooled together to give the emission curve of Fig. 9. The measurements derived from the use of filters 602, 606, 607 and 608 are less reliable than the others, owing to high density of the filters (602, 606) and low sensitivity of the photomultiplier at long wavelengths (orange and red). The electrical response to red light which reached the photocathode was very small, and the results in the red region of the spectrum can be in error by a factor of 2. With the denser filters the transmitted light was equal to, or below, visual threshold. The main source of error lies in evaluating response height through the background noise at high amplification. This was

TABLE 2. FIRST APPROXIMATIONS FOR SPECTRAL ENERGY OF THE LIGHT OF POLYNOIDS

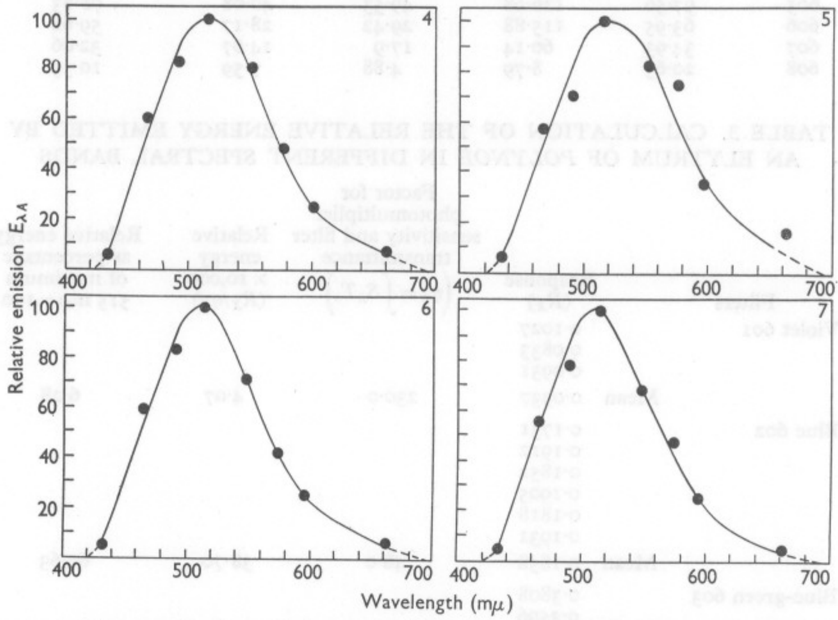
Filters	Averages of readings. *Lagisca*	*Gattyana*	*Harmothoë*	*Polynoë*	Means, four species ($\lambda_{max.}$ as 100)
601	6·63	9·43	3·61	3·62	5·85
602	71·17	87·63	41·37	32·88	58·53
603	98·77	109·96	58·24	45·60	78·50
604	118·91	151·36	69·57	58·32	100
605	97·56	126·68	49·53	40·08	78·83
606	63·95	115·88	29·42	28·17	59·62
607	34·93	60·14	17·9	14·67	32·06
608	20·65	8·79	4·88	7·59	10·53

TABLE 3. CALCULATION OF THE RELATIVE ENERGY EMITTED BY AN ELYTRUM OF *POLYNOË* IN DIFFERENT SPECTRAL BANDS

Filters		Response (R_X)	Factor for photomultiplier sensitivity and filter transmittance $\left(\eta_X = \int S_\lambda T_\lambda\right)$	Relative energy × 10,000 (R_X/η_X)	Relative energy as percentage of maximum 515 mμ = 100
Violet 601		0·1027			
		0·0833			
		0·0951			
	Mean	0·0937	230·0	4·07	6·28
Blue 602		0·1731			
		0·1912			
		0·1852			
		0·1905			
		0·1818			
		0·1931			
	Mean	0·1858	48·0	38·70	60·63
Blue-green 603		0·3808			
		0·3506			
		0·3818			
		0·3759			
		0·4100			
	Mean	0·3798	81·0	46·89	72·33
Green 604		0·2126			
		0·2136			
		0·2217			
		0·2068			
		0·1955			
		0·1982			
	Mean	0·2081	32·1	64·83	100
Yellow-green 605		0·1191			
		0·1040			
		0·1201			
		0·1002			
	Mean	0·1109	24·43	45·41	70·04
Yellow 606		0·0401			
		0·0344			
	Mean	0·0377	11·0	34·27	52·86
Orange 607		0·0367			
		0·0386			
	Mean	0·0376	23·26	16·17	24·94
Red 608		0·0356			
		0·0300			
	Mean	0·0328	5·6	5·86	9·04

particularly difficult in the weak responses obtained with the yellow, orange and red filters. Each point on the curves represents the mean of some thirty measurements.

Because of the broad transmission of the filters, the first approximate results for relative spectral emission were corrected as follows. The mean



Figs. 4–7. Curves showing relative spectral energy of light emitted by four species of polynoids, viz. *Lagisca* (Fig. 4), *Gattyana* (Fig. 5), *Harmothoë* (Fig. 6), and *Polynoë* (Fig. 7).

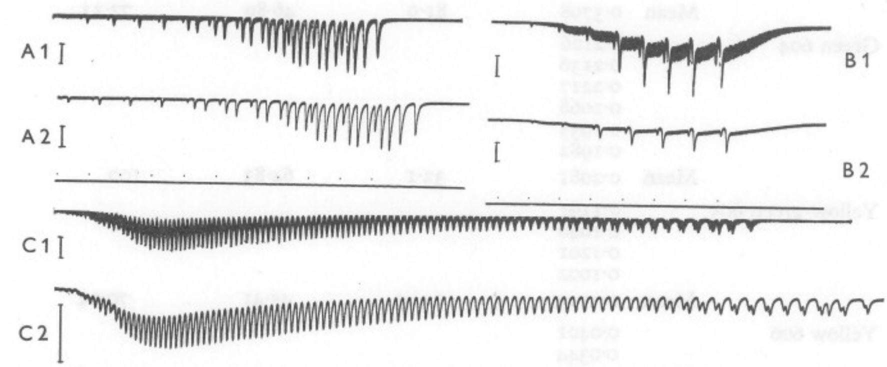


Fig. 8. Oscillograph records of the luminescent responses of *Harmothoë longisetis*, selected as examples. Records as follows: A 1, upper photomultiplier + blue filter 602; A 2, lower photomultiplier; B 1, upper photomultiplier + blue-green filter 603; B 2, lower photomultiplier; C 1, upper photomultiplier + yellow filter 606; C 2, lower photomultiplier. Relative amplifications shown by vertical lines. Electrical stimuli on lower lines of A 2 and B 2.

spectral energy curve of Fig. 9 was used to provide approximate values for radiant flux ($E_{\lambda A}$). With these values, curves were plotted for $E_{\lambda A} S_\lambda T_\lambda$ against λ (Fig. 11). Ratios were then obtained for each filter

$$\rho_1 = \frac{\int E_{\lambda A} S_\lambda T_{\lambda X}}{\int E_{\lambda A} S_\lambda T_{\lambda 604}},$$

which were used to predict the experimental results. Similar ratios were determined for measured responses

$$\rho_2 = \frac{R_X}{R_{604}}.$$

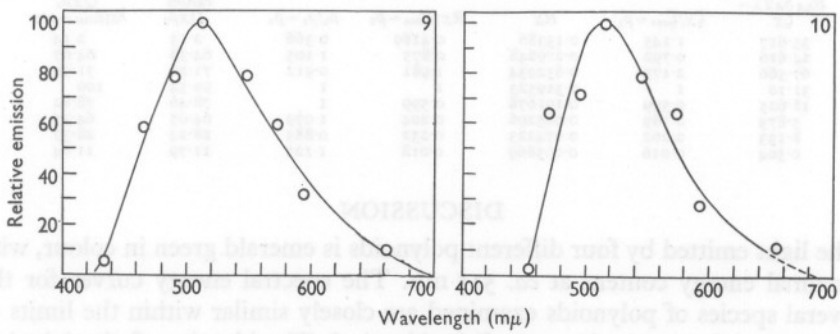


Fig. 9. Composite curve showing relative spectral energy of polynoid light (mean of determinations of four species, viz. *Lagisca*, *Polynoë*, *Harmothoë* and *Gattyana*). First approximate values.

Fig. 10. Composite curve showing relative spectral energy of polynoid light. Corrected results.

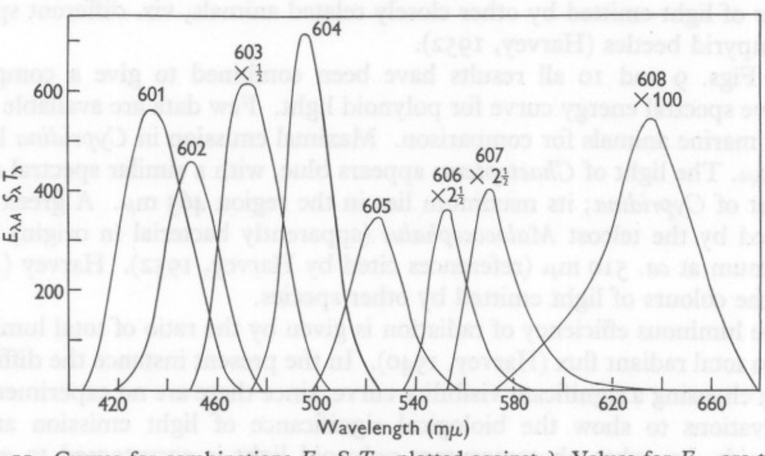


Fig. 11. Curves for combinations $E_{\lambda A} S_\lambda T_\lambda$ plotted against λ. Values for $E_{\lambda A}$ are taken from the curve in Fig. 9.

The ratios $\rho_3 = \rho_2/\rho_1$ were then used to correct the points on the spectral curve. New values for representative mean wavelengths were calculated from the centre of gravity of curves $E_{\lambda A} S_\lambda T_{\lambda X}$. The procedure is illustrated in more detail in another paper dealing with the light of *Chaetopterus* (Nicol, 1957*b*). The calculations are assembled in Table 4, and the corrected results are plotted against λ in Fig. 10.

The relative spectral energy curves (Figs. 4–7, 9, 10) are asymmetrical, with peaks at *ca.* 515 mμ (510–520 mμ). Most of the light emitted is in the blue-green, and there is very little in the violet and red.

TABLE 4. CALCULATION OF RELATIVE SPECTRAL COMPOSITION OF THE LIGHT OF POLYNOIDS

Filter	$E_{\lambda A} S_\lambda T_\lambda = \zeta_X$	$\zeta_X/\zeta_{604} = \rho_1$	R_X	$R_X/R_{604} = \rho_2$	$\rho_2/\rho_1 = \rho_3$	Corrected values $Q_X\rho_3$	$Q_X\rho_3$ $\lambda_{515 m\mu} = 100$	Mean λ (from curves $E_{\lambda A} S_\lambda T_\lambda$) (m$\mu$)
601	35·617	1·145	0·13386	0·4189	0·366	2·13	2·14	455
602	24·616	0·792	0·279648	0·875	1·105	64·38	64·67	470
603	67·566	2·173	0·632934	1·981	0·912	71·26	71·59	494
604	31·10	1	0·319523	1	1	99·54	100	515
605	18·625	0·599	0·191678	0·599	1	78·46	78·82	545
606	5·873	0·189	0·065296	0·204	1·079	64·05	64·35	572
607	8·133	0·262	0·074223	0·232	0·885	28·24	28·37	592
608	0·504	0·016	0·005869	0·018	1·125	11·79	11·84	653

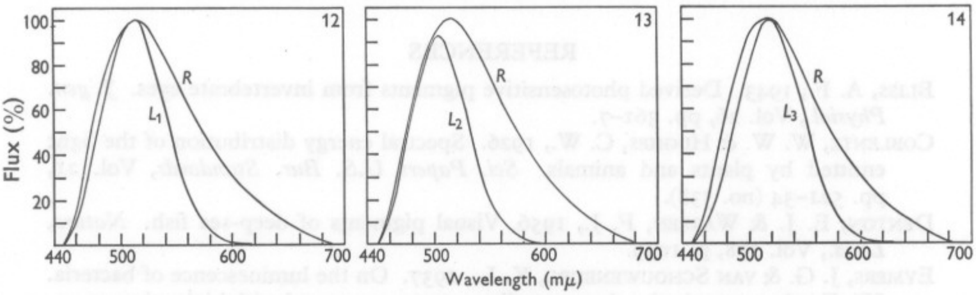
DISCUSSION

The light emitted by four different polynoids is emerald green in colour, with maximal energy content at *ca.* 515 mμ. The spectral energy curves for the several species of polynoids examined are closely similar within the limits of experimental error, and may well be identical. The identity of physiological mechanisms controlling luminescence in polynoids suggests that the same identity may exist in biochemical mechanisms. However, this argument cannot be pressed very far in view of the known differences existing in the colour of light emitted by other closely related animals, viz. different species of lampyrid beetles (Harvey, 1952).

In Figs. 9 and 10 all results have been combined to give a composite relative spectral energy curve for polynoid light. Few data are available from other marine animals for comparison. Maximal emission in *Cypridina* lies at 480 mμ. The light of *Chaetopterus* appears blue, with a similar spectral range to that of *Cypridina*; its maximum lies in the region 465 mμ. A green light emitted by the teleost *Malacocephalus* (apparently bacterial in origin) has a maximum at *ca.* 510 mμ (references cited by Harvey, 1952). Harvey (1955) lists the colours of light emitted by other species.

The luminous efficiency of radiation is given by the ratio of total luminous flux to total radiant flux (Harvey, 1940). In the present instance the difficulty lies in choosing a significant visibility curve, since there are no experiments or observations to show the biological significance of light emission among polynoids, i.e. what photoreceptors polynoid light is accustomed to excite. As a first attempt I have determined the luminous efficiency for human scotopic vision (from C.I.E. values for relative efficiency of radiation (scotopic vision)). The relevant curves are given in Fig. 12. Luminous efficiency for polynoid light is 61%. This means that the worms appear 39% less bright than they would be if all the energy were concentrated at that wavelength (505 mμ) for which the dark-adapted human eye is most sensitive.

Visibility curves for various invertebrates and lower vertebrates have maxima ranging from about 490 to 540 mμ (e.g. *Eledone*, 490 mμ; *Limulus*, 520 mμ; *Lepomis*, 540 mμ) (Graham & Hartline, 1935; Grundfest, 1932; Bliss, 1943). Vertebrate rhodopsins, occurring in marine coastal and surface



Figs. 12–14. Luminous efficiency of light emitted by polynoids. Curves for radiant flux (R) of polynoid light, and luminous flux based on human scotopic vision, $\lambda_{max.}$ at 505 mμ (L_1) (Fig. 12); luminous flux based on a theoretical visual curve with maximum at 490 mμ (L_2) (Fig. 13); and luminous flux based on a theoretical visual curve with maximum at 520 mμ (L_3) (Fig. 14).

fish, show absorption maxima around 500 mμ; porphyropsins, found in wrasse, trout, etc., have maxima around 520 mμ; deep-sea teleosts have visual pigments with absorption maxima around 480 mμ (Wald, 1952, 1953; Granit, 1955; Denton & Warren, 1956). The emission spectrum of polynoid light is fairly broad. Calculations based upon two generalized visibility curves, similar in shape to those for human scotopic vision, but having maxima at 490 and 520 mμ, give luminous efficiencies of 51 and 66% respectively (Figs. 13, 14). A narrow spectral emission curve, like that for *Cypridina* with its maximum at 480 mμ, will show much lower luminous efficiencies for visibility curves having maxima at longer wavelengths.

I wish to thank Mr F. A. J. Armstrong for help in technique, and Dr E. J. Denton for advice concerning measurements and calculations of light intensity. Part of the apparatus used in this investigation was purchased with a grant from the Royal Society.

SUMMARY

The spectral composition of the light of four species of polynoid worms has been measured, viz. *Harmothoë longisetis*, *Gattyana cirrosa*, *Polynoë scolopendrina* and *Lagisca extenuata*. The method involved the use of coloured spectral filters and two multiplier phototubes. The spectral emission curves of the four species are similar. They are asymmetrical in shape, prolonged towards longer wavelengths, with maxima at about 515 mμ (510–520 mμ). Values for luminous efficiency are calculated (ratio of luminous flux to radiant flux); for human scotopic vision ($\lambda_{max.}$ at 505 mμ), efficiency is 61 %.

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