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An annual cycle of flash induced luminescence in the euphausiid *Thysanoëssa raschii*

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Abstract

The euphausiid *Thysanoëssa raschii* (M. Sars) rarely luminesces spontaneously in the laboratory. The flash-stimulated luminescent response (FSLR) was therefore used to investigate seasonal changes in luminescent ability. Some observations were also made on the responsiveness of *Meganyctiphanes norvegica*. In the case of *T. raschii* both sexes showed maximum responsiveness from March through June and in some years to September. The strength of the tendency to respond was strongest in May and June. The FSLR delay was least during April, May and June. These results are interpreted in the light of the hypothesis that the form of the FSLR is the result of opposing inhibitory and excitatory components. It is tentatively concluded that luminescence is involved in behaviour related to spermatophore transference, which takes place from April to June.

Introduction

The complexity of the euphausiid luminescent system (see review by Mauchline and Fisher, 1969) makes it seem likely that luminescence is an important behavioural mechanism in these animals. Information on the function of luminescence is however scarce, partly because very few direct observations have been made of euphausiids luminescing under natural conditions in the sea. Underwater photometers have been used to make a number of observations of luminescence which may have been euphausiid in origin, but the evidence for this is scant and circumstantial (Boden and Kampa, 1957; Kampa and Boden, 1957, see also the review by Boden and Kampa, 1964). Using an underwater photometer in the Clyde sea area in places where the euphausiids *Meganyctiphanes norvegica* (M. Sars) and *Thysanoëssa raschii* (M. Sars) are known to be abundant, I have rarely recorded any euphausiid luminescence. It seems that the movements of any recording instrument lowered from the surface will frighten the animals away (Clarke and Hubbard, 1959).

Ivanov (1969) has noted the luminescence of surface swarms of krill *Euphausia superba* (Dana), and Mauchline (1960) has suggested that pre-spawning aggregations of *Meganyctiphanes norvegica* might be effected by the use of photophores. If euphausiid luminescent behaviour is related to swarming and breeding, then it seems possible that species such as *M. norvegica* and *Thysanoëssa raschii* which show seasonal changes in their swarming behaviour in the Clyde sea area (Mauchline, 1960, 1966) might show an annual pattern of changes in their luminescent behaviour.

Euphausiids such as *Meganyctiphanes norvegica* and *Thysanoessa raschii* (the two common species in the Firth of Clyde), when taken by tow-netting, are usually seen to be luminescing brightly on first examination. The animals are very active, and if placed in a tray will swim vigorously, sometimes spinning about their body axis, while the intensity of luminescence from the photophores waxes and wanes over periods of a few seconds. However, this activity and luminescence declines rapidly. By the time the animals are brought into the laboratory and even if they are kept in cold sea water in darkness, spontaneous luminescence occurs rarely. Hardy and Kay (1964) found a sharp decline in the spontaneous luminescent activity of *M. norvegica* within the first 10 h after capture. No spontaneous luminescence occurred later than 50 h after capture. *T. raschii* shows even less spontaneous luminescent activity in the laboratory. Most animals of both of these species die within a few days of capture even when kept at their usual environmental temperatures between 2° and 10 °C, and it seems safest to assume that laboratory animals are in an unhealthy state. All observations should therefore be interpreted with care, and it seems likely that the way in which animals are selected for observation or experiment may markedly effect the conclusions to be drawn. Mauchline (1960) found that *M. norvegica* from the Firth of Clyde were more likely to luminesce spontaneously in the laboratory during the period from November to January than from February to October. These observations were confirmed by David and Conover (1961) who found that *M. norvegica* from Cape Cod bay (USA) did not luminesce spontaneously except during the breeding season (December to February). However, Hardy and Kay (1964) found no evidence for any annual periodicity with Clyde animals

similarly, while MAUCHLINE (1960) found evidence for a nocturnal increase in spontaneous luminescence, HARDY and KAY (1964) did not.

Another of the problems involved in making observations of euphausiid spontaneous luminescence is that of freeing the animals from luminescent dinoflagellates, especially during the summer months when the latter are common. Dinoflagellates are liable to get caught in the feeding appendages, and washing them out is difficult without so much handling of the euphausiids that the chances of their later spontaneous luminescence are reduced. However, it is often possible to distinguish euphausiid and dinoflagellate luminescence by the time scale of the luminescent flashes recorded.

During 1966, 1967 and 1968 I made approximately weekly observations on groups of 2 to 10 *Thysanoëssa raschii* left overnight in a luminescence recording apparatus. Groups of *Meganyctiphanes norvegica* were observed less frequently. Definite spontaneous luminescence was produced on only a few occasions, although on most dates some or all of the animals would luminesce in response to stimulation. The months in which spontaneous luminescence occurred are shown in Table 1. "Faint" refers to luminescence which was either very dim or consisted of a few brief flashes only. Bright luminescence was noticeable and persistent. Whilst the number of these observations is too few to be significant, there is a little evidence for spontaneous luminescence being most common from February until June (13 out of a total of 17 records for both species). Most of the luminescence recorded occurred within 8 h of the commencement of observation.

It is clear that laboratory observations of spontaneous luminescence do not provide sufficient information for a study of luminescence. Recourse must be made to stimulated luminescence, and a number of ways of inducing luminescence artificially are known. Crude mechanical stimulation, such as squeezing the animals, is effective but harmful. KAY (1962, 1965) and DOYLE and KAY (1967) have described the effects of 5-HT (5-Hydroxy-tryptamine) and LSD (Lysergic acid diethylamide) in stimulating or facilitating the production of luminescence when present in sea water containing *Meganyctiphanes norvegica* and *Thysanoëssa raschii*. MAUCHLINE (1960) found that individuals of *M. norvegica* that had been kept in the light luminesced on transfer to darkness. He found a seasonal variation in the proportion of animals tested that showed this "light/dark" reaction. HARDY and KAY (1964) and KAY (1965, 1966) used an electronic photoflash as a more discrete stimulus, and made use of the reaction to study the nature of the relationship between the stimulus and the bioluminescent response. The effect of temperature upon various parameters of this "flash-

Table 1. *Laboratory observations of spontaneous luminescence in two euphausiids*

Thysanoëssa raschii			*Meganyctiphanes norvegica*	
1966	August	(faint)		
	December	(faint)		
1967			February	(faint)
	March	(faint)		
	April	(bright)		
	April	(bright)		
	May	(faint)		
	June	(faint)	June	(bright)
			June	(bright)
	September	(faint)		
			October	(faint)
1968			February	(faint)
			February	(faint)
			February	(faint)
	May	(bright)		
	May	(faint)		
Total observations bright luminescence			3	2
Total observations faint luminescence			7	5
Total number all observations			84	36
Period of observations: from			20/6/66	28/11/66
	to		9/7/68	28/ 8/68

stimulated luminescent response" (FSLR) has also been described (TETT, 1969). This work, and other information upon the physiology of euphausiid luminescence, is summarized by MAUCHLINE and FISHER (1969).

Since the flash stimulus appears to have no harmful effects upon the animals (KAY, 1965; MAUCHLINE and FISHER, 1969), it was used to investigate the readiness to luminesce of samples of *Thysanoessa raschii* brought into the laboratory from several areas of the Firth of Clyde between June 1966 and August 1968. Some *Meganctyiphanes norvegica* were also tested. Observations showed that there was a yearly cycle of responsiveness to flash stimulation.

Methods

The material was obtained from hauls with a 1 m diameter stramin net off Garroch Head, Isle of Bute, and Glen Sannox, Isle of Arran, at depths of 50 to 100 m. The sea areas concerned are the same as those from which MAUCHLINE (1960, 1966), KAY (1962, 1965, 1966), HARDY and KAY (1964), and DOYLE and KAY (1967) obtained their material. Individuals of *Thysanoëssa raschii* and *Megancytiphanes norvegica* were picked out, washed by immersion in filtered sea water and placed in jars containing 0.8 to 2 l of filtered sea water. The jars were stored in a darkened refrigerated cupboard at temperatures between 4° and 10 °C Tests were not made earlier than 3 h after placing the jars in darkness, nor later than 30 h after the capture of the animals. Jars were transferred to the experimental apparatus in dim red light, and the temperature of the sea water in them was measured before and after testing for a luminescent response. Each animal was given three flashes from a Mecablitz electronic flash, intensity 120 Joules, duration 1 msec, with intervals of 20 min between successive flashes. Fig. 1 shows a typical response. The nominal distance from the flashgun to the animal was 10 cm, but since the euphausiids were usually swimming around inside the jar it was not possible to calculate the energy entering the eye of the animal. Variation in the stimulus intensity was, however, supposed to be random.

Luminescence was recording using RCA 5819 and EMI 9524 B photomultipliers, both of which have S-11 spectral response (peak at 440 nm). After amplification, the photomultiplier output was displayed on a pen recorder capable of response up to 50 Hz. The distance of the euphausiids from the photomultiplier cathode varied from 10 to 30 cm, and the gain of the photomultiplier and associated amplifiers was adjusted so that the threshold of detection of luminescence corresponded to a light intensity of about 2×10^{-8} μW/cm^2 at the photocathode. The adjustment was made using light of wavelength 486 nm, which is close to the peak wavelength of euphausiid luminescence (BODEN and KAMPA, 1959).

Animals were preserved after testing, and observations later made of size, sex and the occurrence of spermatophores on females. Size was measured as the length of the cephalothorax from the rear border of the eye socket to the notch at the hinder end of the carapace, using a micrometer eyepiece in a low power binocular microscope. In the case of *Thysanoëssa raschii*, total length is about 3.9 times carapace length (MAUCHLINE, personal communication).

A series of standard hauls with a 1 m stramin net was made off Garroch Head between February 1967 and August 1968, in order to obtain a less selected sample from the natural population of *Thysanoëssa raschii*. When numbers caught were large, a proportion was taken at random using a simple splitting device. Observations of sex, spermatophore occurrence on females, and carapace size measurements, were made as described above.

Results

Results of the analysis of *Thysanoëssa raschii* in standard stramin net hauls are shown in Table 2. The number caught per standard haul — if interpreted with caution — gives an idea of the animal's abundance at the sampling station at different times of the year. Table 2 shows the extent of two important periods in the euphausiid's life history. The first is the reproductive period. Spermatophores were present on females during April, May and June. Observations on the size and sex of animals that were tested for luminescent ability (Table 3) also show the presence of spermatophores on females from April through June. In 1967 very few spermatophores were found on females before the middle of April; later in the month a majority of females had spermatophores affixed. A similar date for the commencement of spermatophore attachment may be presumed in 1968. Except in 1966 females with attached spermatophores were not found in July. The female breeding season may thus be taken as extending from mid-April until the end of June. This agrees with MAUCHLINE (1966) who also found that the ejaculatory ducts of the males contained spermatophores from February until May or June.

Egg laying must take place from April onwards, for the larvae require about two months to develop from egg to adolescent (MAUCHLINE, 1966) and the first adolescents appeared in stramin tow-nettings in late June or early July. Because of the difficulty of distinguishing adolescent males from adolescent females, it seems possible that the low proportion of males cited in Table 2 for the summer and autumn months may be explained as a result of misidentification of many males as females. Larger animals are easier to sex, and changes in the sex ratio at other times of the year may be explained by the effects of differential mortality. The percentage of males in the samples was highest just before the commencement of

Table 2. *Thysanoëssa raschii. Analysis of Garroch Head samples*

Date of sample	Total caught, standard haul	♂♂ Median size[a]	♂♂ Range[b]	♂♂ % of all animals	♀♀ Median size[a]	♀♀ Range[b]	♀♀ % with spermatophores
15 + 20 February 1967	10 + 38	3.3	2.7—4.4	29	3.6	2.7—4.9	0
15 March 1967	252	3.4	2.7—4.4	49	3.6	3.0—4.3	0
11 April 1967	1,584	3.7	3.0—4.5	59	4.1	3.0—5.5	2
20 April 1967	287	3.9	3.4—4.4	37	4.8	4.2—5.6	93
10 May 1967	524	4.3	3.8—4.9	34	5.1	4.6—6.6	63
7 June 1967	106	4.5	4.2—5.0	15	5.2	4.6—6.5	86
15 August 1967	3,674	3.9	2.4—5.1	5	3.3	2.4—5.3	0
2 November 1967	97	3.9	3.6—4.1	4	3.9	2.8—4.4	0
29 November 1967	33	4.0	3.4—4.6	15	3.7	2.6—5.2	0
18 December 1967	524	3.6	2.7—4.3	45	3.6	2.7—4.4	0
16 January 1968	179	3.5	2.7—4.2	46	3.5	2.7—4.6	0
6 February 1968	43	3.4	3.0—4.2	34	3.3	2.7—4.0	0
28 March 1968	7	3.5	2.8—4.0	57	4.0	3.8—4.8	0
18 April 1968	1,070	4.0	3.5—4.6	59	5.0	3.8—5.6	68
28 May 1968	130	4.2	3.6—5.5	28	5.5	4.6—7.0	80
27 June 1968	412	3.6	2.1—5.4	15	2.7	2.2—4.2	0
14 August 1968	10,064	4.1	3.4—4.6	14	3.6	2.3—4.6	0

[a] Median cephalothorax size in mm.
[b] Range of cephalothorax size which includes 95% of individuals measured (mm).

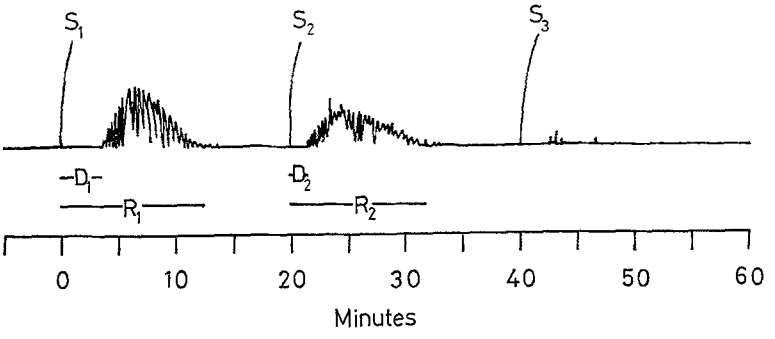

Fig. 1. *Thysanoëssa raschii*. Typical responses to flash stimulation. The diagrammatic representation shows pen recorder tracing of photomultiplier output. $S_1 - S_3$ successive flash stimulations; D_1 and D_2 response delay periods (D_2 shorter than D_1). R_1 and R_2 total duration of the response. The response to S_3 is weak and, therefore, a D_3 and R_3 would not be measured

spermatophore transference. After the transfer it would seem that male mortality was considerably higher than that of females at the same time. Later during the summer there is a high mortality rate amongst all the adults. Only a few survive to breed in the following year (MAUCHLINE, 1966).

The second period in the euphausiids' lives which is of interest here is the main period of adult growth. This takes place between mid-March and mid-June in each year. After this the median size decreases as most of the adults disappear and adolescents constitute the majority of the sample.

The typical response of a single *Thysanoëssa raschii* to a series of flash stimulations is shown in Fig. 1. Following the stimulus (at S) there is a delay period (D) of from 10 to 300 sec before the commencement of luminescence. The period of luminescence lasts for another 10 to 600 sec. The total duration of the response is indicated by R. D_1, D_2, R_1, R_2 refer to the response delays and durations following successive stimulations. Both R and D are dependent upon temperature — increasing temperature results in a shorter delay period and a shorter response duration.

Responsiveness was scored as follows. A unit value was given to marked responses such as those shown following S_1 and S_2 in Fig. 1. A weak response such as that shown following S_3 was given a value of $^1/_2$. Hence Fig. 1 shows a total of $2^1/_2$ responses to 3 stimulations. Since the frequency of response does not appear to be significantly temperature-dependent below 19 °C, it was not necessary to correct the observed values of responsiveness for the effects of temperature.

During the course of each month between June 1966 and August 1968 inclusive, a number of batches of *Thysanoëssa raschii* were brought into the laboratory, and the responsiveness of individual euphausiids tested by three flash stimulations. The results are shown in Table 3. For example, the responsiveness of 65% given for June 1966 represents a total of 37 responses (calculated as described above) by 19 animals to a total of 57 stimulations. In Fig. 2 the response percentage is plotted separately for males and females. During most of the period studied there was reasonable agreement between the changes in responsiveness of the sexes — the significance of the differences will be considered below. Table 3 and Fig. 2 show a cycle of responsiveness which is more or less annual. Responsiveness was highest between March and September in 1967 and between March and August (when experiment ceased) in 1968. In 1966 a high percentage response was recorded in June, but the percentage had declined markedly by August. Low responsiveness was observed between August 1966 and February 1967, and between October 1967 and February 1968, although in each year there was a lesser increase in responsiveness during November and December.

The major increase in responsiveness, during March, April and May, took place at the same time as the main adult growth period and the period of spermatophore development in males and their transferrence to females. This is shown in Fig. 2, where these periods are indicated.

Table 3. *Thysanoëssa raschii. Percentage responding to flash stimulus. Each individual given 3 stimulations. For details consult text*

Year	Month	Response (%)	Details of individuals tested			
			Number	♂ (%)	With spermatophores ♀ (%)	Mean cephalothorax size (mm)
1966	June	65	19	20	33	5.7
	July	37	23	26	13	5.3
	August	5	63	28	0	5.1
	September	8	21	39	0	4.1
	October	14	44	21	0	4.8
	November	36	30	30	0	4.0
	December	37	14	40	0	4.6
1967	January	25	42	56	0	3.9
	February	17	70	59	0	3.8
	March	69	22	20	0	4.1
	April	48	60	32 (before 15th)	0 (after 15th) 71	4.3
	May	69	29	39	77	4.6
	June	50	18	19	54	5.1
	July	72	13	31	0	5.5
	August	52	19	35	0	5.1
	September	49	37	20	0	4.2
	October	18	31	14	0	4.2
	November	27	16	44	0	3.7
	December	20	16	44	0	3.6
1968	January	11	28	48	0	3.9
	February	4	25	42	0	4.0
	March	45	11	64	0	3.7
	April	46	26	48	67	4.5
	May	70	65	34	70	5.0
	June	76	34	24	42	5.3
	July	80	13	62	0	4.9
	August	67	33	24	0	4.9

Table 3 also gives details of the animals tested. In general, these agree with the statistics derived from the analysis of routine samples (Table 2), although the mean sizes of tested animals were a little larger throughout most of the year. During the summer, however, there were marked differences between tested animals and those in the routine samples. The mean size of tested animals was considerably higher than the median (approximately equal to the mean) size of sampled animals, and the percentage of males recorded was higher. These differences are the result of selecting larger animals for testing. It seems possible therefore that the high responsiveness observed in July and

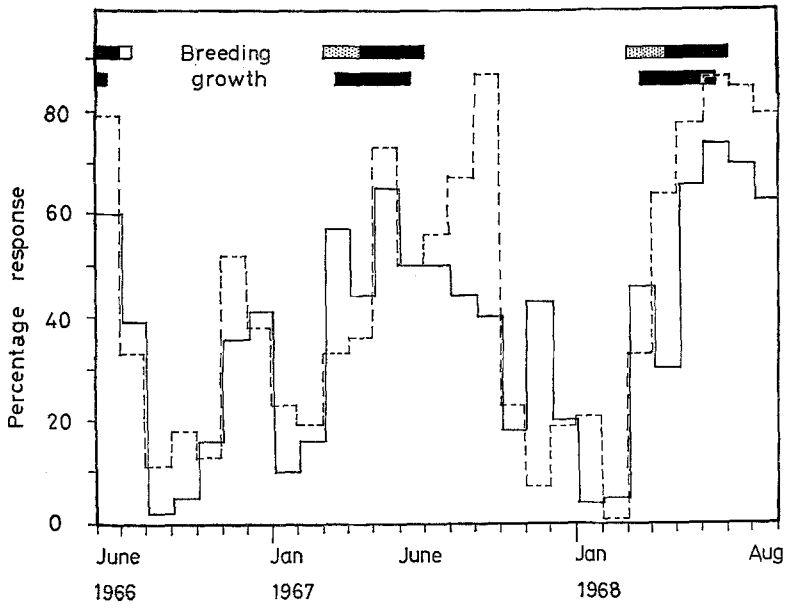

Fig. 2. *Thysanoëssa raschii*. Sex differences in percentage response. Males: dashed line. Females: continuous line. Breeding period: dotted part of bar indicates occurrence of males with spermatophores (MAUCHLINE, 1966); solid black part indicates occurrence of spermatophores on females. In 1966, spermatophores were found on females during the first half of July. Growth period: period of maximum adult growth (see Table 2)

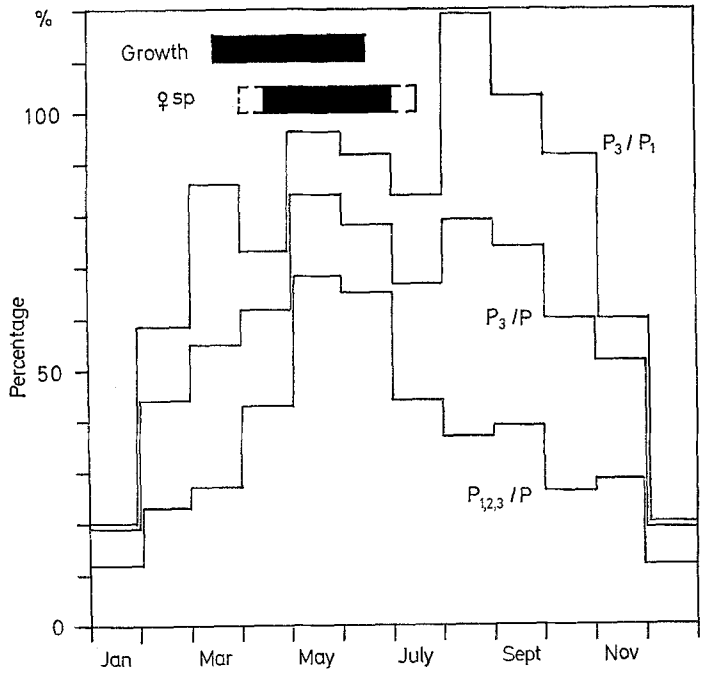

Fig. 3. *Thysanoëssa raschii*. Response to successive stimulations. Calculation of the percentages $P_{1,2,3}/P$, P_3/P and P_3/P_1 is explained in the text. Values for same months in different years averaged together. Growth and breeding periods (females with spermatophores) shown by black bars

August 1967 and 1968, and in September 1967, would not be reflected in random samples drawn from the natural population, which would contain a higher proportion of adolescents and young adults at this time. Experiments with adolescents showed that luminescence was more difficult to elicit and was fainter.

Analysis of male and female responsiveness (Fig. 2) shows that males tended to respond more frequently than females. Thus, the proportion of males responding was greater in 17 out of 27 months, and more than 10% greater in 12 of these months. The proportion of females responding was only markedly higher in March 1967 and 1968 and in November 1967. Another point of particular significance is that male percentage response increased in all 3 months March, April and May, both in 1967 and in 1968. Female percentage response increased in March (outstripping that of the males) but decreased in April before increasing again in May of both years.

The strength of the tendency to respond was investigated in two additional ways. The first consisted of comparing the number of animals that responded to particular stimulations with the numbers that responded at all. This is shown in Fig. 3, where values for the same months of different years are averaged together. Only data for animals that gave at least one clear response during the course of three stimulations were considered. The following percentages were calculated: P_1/P; P_3/P; $P_{1,2,3}/P$ and P_3/P_1. P gives the numbers of animals responding to one or more stimulations. P_1 and P_3 give the numbers that responded to S_1 and S_3 respectively, and $P_{1,2,3}$ gives the numbers of animals which responded to all three stimulations.

P_1/P varied from 58 to 100% but showed no clear seasonal behaviour and so is not shown in Fig. 3. This might be expected if P and P_1 shared the same seasonal pattern. P_3/P, $P_{1,2,3}/P$ and P_3/P_1 did show an annual cycle, which can be seen in Fig. 3. There was an increase in the percentages from February until May. Highest values of P_3/P_1 were obtained in May, June, August, September and October; of P_3/P in May, June, August and September, and of $P_{1,2,3}/P$ in May and June. Lowest values of all percentages were recorded in December and January.

$P_{1,2,3}/P$ is clearly a measure of the euphausid's potential responsiveness. Interpretation of P_3/P and of P_3/P_1 is more complicated, since some animals responded to S_3 without prior response to S_1. If the characteristic form of the FSLR is the result of opposing inhibitory and excitatory tendencies (TETT, 1969), then P_3/P and P_3/P_1 may measure the extent to which the inhibitory tendency decreases more rapidly, with successive stimulations, than does the excitatory tendency.

It may be concluded that Fig. 3 does demonstrate an annual cycle in the strength of responsiveness. Not only did more animals responded, but they responded to more successive stimulations, during the periods when the percentages in the figure were highest.

It was not possible to measure the intensity of the FSLR since there was no way of knowing how far away from the photomultiplier or in what orientation an animal was luminescing (it seems likely that they swam rapidly around their jar whilst revolving about a head-telson axis). The second means of investigating the strength of the tendency to respond consisted of measuring response delay (D) and duration (R). Individual values of D and R were logarithmically

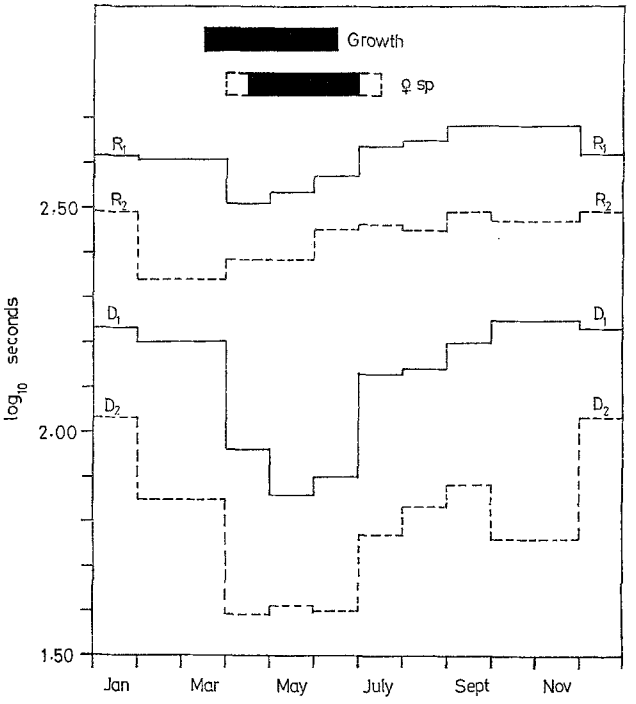

Fig. 4. *Thysanoëssa raschii*. Response delays and durations. Log_{10} response delay (D) and duration (R) values, in seconds, corrected to 8 °C. Values for same months in different years averaged together. Subscripts refer to successive stimulations. Growth and breeding periods (females with spermatophores) shown by black bars

transformed and corrected to a temperature of 8 °C using the regression equations of TETT (1969). Fig. 4 shows mean values for D_1, D_2, R_1 and R_2, all values for the same month in different years being taken together. It was also necessary to group together February and March, and October and November, in order to obtain sufficient values for satisfactory analysis. Values for D_3 and R_3 are not given — their behaviour was similar to that of D_2 and R_2. Standard errors varied, but typically were about 0.10 for delay periods and slightly less for duration periods. Hence only the changes in D values are significant, although R values also seem to display an annual cycle.

Table 4. *Meganyctiphanes norvegica. Percentage responding to flash stimulus. Each individual given 3 stimulations. For details consult text*

Year	Month	Response (%)	Details of individuals tested	
			Number	Mean cepalothorax size (mm)
1966	June, July	63	13	8.8
	August, September	24	24	8.0
	October, November	43	21	6.3
1966/67	December, January	40	15	6.3
	February, March	64	7	6.7
	April, May	57	19	6.9
	June, July	77	21	8.2
	August, September	75	7	7.3
	October, November	51	19	6.0
1967/68	December, January	42	13	5.8
	February, March	61	9	7.5
	April, May	73	20	8.0
	June, July	86	19	8.1
	August	62	9	7.9

It seems likely that D measures the duration and/or strength of the inhibitory period which is the immediate result of confronting the euphausiid with a shock stimulus such as a bright flash. This inhibitory tendency seems therefore to be least in April, May and June, when D_1 and D_2 are minimal. The changes in R_1 and R_2 (supposedly measures of the strength/duration of the excitatory tendency which also results from the stimulus) are less, and if significant could be interpreted in several different ways. One of these is that luminescence that commences relatively sooner after the stimulus (owing to a shorter delay period) may also terminate sooner, although the excitatory tendency may on the average remain constant throughout the year.

A number of experiments were also carried out involving the larger species of Clyde euphausiid, *Meganyctiphanes norvegica*. Table 4 shows the results of the tests, which were carried out in the same way as for *Thysanoëssa raschii*. There is some evidence of an annual cycle. A high percentage response was recorded from February until July, and also in August (1968) and August/September (1967). It is interesting that the results for 1966 parallel those for *T. raschii*, responsiveness decreasing in August. It is possible that an instrumental failure was responsible, perhaps a decrease in the discharge energy of the electronic flash and hence a reduction in stimulus intensity. Alternatively, environmental conditions may have been responsible for both species behaving in the same way. In both 1966/67 and 1967/68, responsiveness was lowest from October until January. The life history of *M. norvegica* is similar to that of *T. raschii* except that it generally lives longer and breeds earlier in the year. Spermatophore transferrence takes place in January and February, egg laying in March and April (MAUCHLINE, 1960).

Discussion

It is clear that an annual cycle is present in most of the FSLR parameters of *Thysanoëssa raschii*. Both sexes show maximum responsiveness from March through June and possibly to September. As measured by the number of successive responses produced, the strength of the tendency to respond is strongest in May and June. The FSLR delay (D) is least during April, May and June. The major question to be discussed is this: does this seasonal cycle in the FSLR reflect seasonal changes in the euphausiids' luminescent behaviour in the sea? Alternatively, do the changes in the FSLR merely indicate changes in the animals' physiology, and thus in their condition in the laboratory, at different times of the year?

The major part of the information available on seasonal changes in the physiological state of *Thysanoëssa raschii* is indirect and is provided by observations on the growth and breeding periods. It seems likely that the animals will be in the best condition in the sea and in the laboratory during the period of growth in the spring when food should be abundant. The effects of breeding, however, may be to weaken their condition in the laboratory. For example, there is evidence of an increased rate of male mortality in the sea after spermatophore transfer (Table 2). MAUCHLINE (1960) suggests that there is a similar increase in mortality in the case of male *Meganyctiphanes norvegica*.

The physiological condition of *Thysanoëssa raschii* at different times of the year might be investigated through changes in chemical composition, or in respiratory or metabolic rate, or in survival time in the laboratory. The only relevant work is that of FISHER (1962) and FISHER et al. (1954, 1955) who showed that stored lipids reached minimum levels during the period January to March.

In the case of the other alternative, which supposes a relation between responsiveness to flash stimulation in the laboratory and the euphausiids' luminescent behaviour in the sea, it is probable that the latter is also related to the breeding period. Alas for the possibility of distinction of the two alternatives, the breeding period and the growth period (Table 2; Figs. 2, 3 and 4) overlap substantially. Insufficient animals were tested in the crucial periods in March and late June to show what happened to the FSLR parameters during these periods. Hence any conclusions on the relation of the seasonal changes in the FSLR to the animals' luminescent behaviour in the sea must be reached on the basis of indirect evidence.

Discussion of the relation between the FSLR and the animals' natural luminescent behaviour must take into account the nature of the flash-stimulated luminescent response. The following hypothesis is based upon KAY (1965, 1966), TETT (1969) and some unpublished observations. I consider the characteristic form of the FSLR to result from two opposing effects of the flash stimulus (Fig. 5). The first component of the response (I) is inhibitory. It is probably nervously mediated, and its effect is rapid. It seems likely that in the sea, the euphausiid's immediate response to a shock stimulus (such as the attack of a predator) is to inhibit light production, if luminescing, and to perform some sort of escape reaction. In the laboratory the bright flash seems to act as a similar shock, resulting in the animals darting rapidly about their jars. If they are luminescing already, as the result of a prior flash stimulus, the flash causes immediate inhibition of light production. The physiological condition of laboratory animals must be considered to be depressed, and the second effect of the flash stimulus is to produce a general increase in central nervous excitement. This is the excitatory component of the response (E). It is general rather than particular, and may be nervously or hormonally mediated. KAY (1966) suggests the latter. When the inhibitory component falls in intensity, the effect of the excitatory component is to cause luminescence. The production of light then continues until the excitatory component fades away. The duration of the response delay period (D) may be taken as an indication of the strength and/or duration of the inhibitory component. The duration of the response (R) may be taken as an indication of the duration of the excitatory component.

If animals were not in a depressed physiological state in the laboratory, they would probably luminesce spontaneously in proportion to the extent of their natural luminescence in the sea. The responsiveness of the animals to flash stimulation may therefore be an indication either (a) of their readiness to luminesce in the sea, or, (b) of the degree of their depression in the laboratory.

The results of the observations upon seasonal changes in the FSLR of *Thysanoëssa raschii* and *Meganyctiphanes norvegica* may now be interpreted in the light of this hypothesis although the results must be qualified by the fact that from July until September, experimental *T. raschii* were different from random (tow-net) samples of the natural population at Garroch Head.

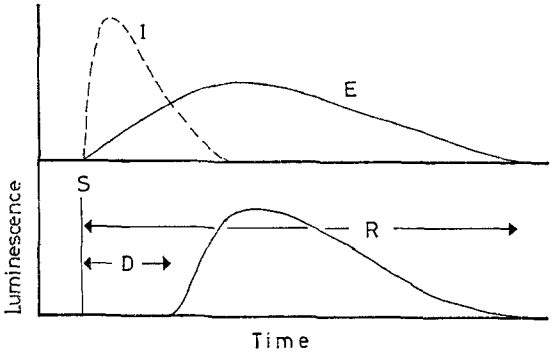

Fig. 5. Hypothesis of the effects of the flash stimulus. *I* inhibitory component, *E* excitatory component; their interaction results in the production of luminescence after a delay (*D*)

(1) There is a little evidence to support the hypothesis of a relationship between spontaneous luminescence in the laboratory and responsiveness to flash stimulation. Taking results from *Thysanoëssa raschii* and *Meganyctiphanes norvegica* together 13 out of 17 records of spontaneous luminescence were made between February (for *M. norvegica*), March (for *T. raschii*) and June (Table 1). The only records of bright spontaneous luminescence were made in April and May (*T. raschii*) and June (*M. norvegica*) (compare with Tables 3 and 4 and Figs. 2, 3 and 4). Whilst HARDY and KAY (1964) concluded that spontaneous flashing did occur outside the breeding season (which is from January to April), MAUCHLINE (1960) found that *M. norvegica* rarely luminesced spontaneously in the laboratory outside the months of November to January, and that the highest proportion of "light/dark" reactions was obtained from January until July. In the case of *M. norvegica*, HARDY and KAY (1964) concluded that the main determinant of spontaneous flashing in the laboratory was the length of time in captivity, most luminescence occurring during the first 10 h.

(2) Table 3 shows that *Thysanoëssa raschii* individuals displayed maximum responsiveness from March through June and to August or September, and minimum responsiveness from October until February. The main period of adult growth commences in mid-March, and spermatophores first appear on females in mid-April. The only part of the reproductive cycle which occurs before the period of maximum responsiveness is in February when males have spermatophores in their ejaculatory ducts.

MACDONALD (1928) states that *Thysanoëssa raschii* has a second breeding period in the Clyde, from mid-August to mid-September. MAUCHLINE (1966) found no evidence for this, nor did I find any spermatophores upon females during these months. However, I made no examination of male ducts, and it is interesting that the high percentage response figures observed in September 1967 (Fig. 2) were due to males rather than females.

There is also the secondary increase in responsiveness in the months of November and December. This could perhaps be explained in relation to the breeding cycle, but MAUCHLINE (1966) noted that there were some indications of an increase in feeding intensity between October and December.

(3) MAUCHLINE (1960) found no sexual dimorphism in "light/dark" reactiveness in the case of *Meganyctiphanes norvegica*. In the case of *Thysanoëssa raschii*, Fig. 2 shows that males were generally more responsive than females. This situation was only markedly reversed in March 1967 and 1968 and in November 1967. Between February and March of both years there was a rapid increase in the proportion of females responding This proportion declined in April, while the proportion of responding males continued to increase. Spermatophore transfer commences in April, so that this decrease in the proportion of responding females might be related to breeding behaviour. With what result, is not clear. The alternative hypothesis is that egg-laying, which commences in April, might weaken the females so that a lower proportion respond to a flash stimulus in the laboratory. If this were the case, it is difficult to see why the proportion of females responding should rise so markedly in May (both years) whilst egg-laying continued.

(4) Fig. 3 indicates that responsiveness to successive stimuli was higher in May and June, and in some respects in August, September and October, than at other times of the year. This may be because laboratory animals were in better condition, or because the inhibitory component of the FSLR was either less strong or else decreased more quickly with successive stimulations.

(5) In general D_2 is less than D_1 (TETT, 1969; see also Fig. 4). This figure also shows that the response delay is least in April, May and June. Hence the inhibitory component of the FSLR is presumably minimal at this time. It seems probable that the response duration (R) remains fairly constant through the year. There is also some indication that D_2 decreases more than D_1 from winter to spring, confirming one of the suppositions of (4) above. The fact that D changes considerably more than R indicates that it is not the general physiological condition of the animal which is totally responsible, but that more specific behavioural changes also play a part. This leads to the hypothesis that the delay period is reduced during April, May and June, the period of spermatophore transfer, because the central inhibition of luminescence is terminated more quickly. This implies that the animals luminesce more readily in the sea.

The balance of the evidence seems to imply a relationship between the FSLR, the euphausiid's luminescent behaviour in the sea, and the period when spermatophore transfer takes place.

In the case of *Meganyctiphanes norvegica*, MAUCHLINE (1960) also concluded that there was a relation between luminescing and breeding. However, he found that "immediately spermatophore transfer begins in January, most of the population seem to stop luminescing spontaneously... there seems to be no sexual dimorphism of luminescence in *M. norvegica*". MAUCHLINE concluded that "during the summer the adults are dispersed throughout the Clyde Sea in areas of more than 130 m depth until the (late) autumn when shoaling takes place. It would seem, therefore, that luminescence functions to bring about this shoaling of the population and thus to facilitate mating. During the other months of the year its sole function seems to be as a defence mechanism as they will only luminesce if stimulated mechanically or chemically".

In the case of *Thysanoëssa raschii*, aggregation takes place in the winter months (MAUCHLINE, 1966). During this time responsiveness is at its lowest. It is only after aggregation should have taken place that responsiveness increases.

These two conclusions could be explained in several ways. There could be a genuine species difference. MAUCHLINE (personal communication) has commented that the Arran Deep (his animals) and Garroch Head populations of *Meganyctiphanes norvegica* appear to display different luminescent behaviour. However, while *M. norvegica* females with spermatophores attached are not found at Garroch Head, *Thysanoëssa raschii* does appear to breed there. Samples with the largest number of adults were taken at Garroch Head in April 1967 and 1968 (Table 2; the large numbers in August of both years are due to adolescents). Hence concentration of individuals from the shallow water areas of the Clyde does take place at Garroch Head, reaching a peak in the spring.

The hypothesis, that *Thysanoëssa raschii* luminescence is related to spermatophore transfer, could be tested by a detailed examination of the response parameters of both male and female, during the months of February through July, in relation to the occurrence

of spermatophores in males and on females, and to survival time in the laboratory. Although responsiveness might be correlated with survival time, a significant correlation would be expected between responsiveness and spermatophore transfer and a significant negative correlation between response delay and spermatophore transfer. If both response delay and duration were similarly correlated with survival time, it would be evidence against the interpretation here placed on the seasonal cycle in the FSLR.

Summary

1. The euphausiids *Thysanoëssa raschii* and *Meganyctiphanes norvegica* rarely luminesce spontaneously in the laboratory. Between 1966 and 1968 only 17 records of spontaneous luminescence were made in total for these species. 13 of these records were obtained between February and June.

2. The flash-stimulated luminescent response (FSLR) was therefore used to investigate changes in luminescent ability between June 1966 and August 1968.

3. Individuals of *T. raschii* displayed maximum responsiveness from May through June and to August (1968) or September (1967). Minimum responsiveness occurred from October to February, with a secondary increase in November and December of each year.

4. Individuals of *M. norvegica* displayed maximum responsiveness from February through July and to August/September (1967, 1968). Minimum responsiveness occurred from October to January.

5. Males of *T. raschii* were generally more responsive than females. This situation was only markedly reversed in March 1967 and 1968 and in November 1967. The proportion of tested females that responded increased in March of each year, fell in April and increased again in May. The proportion of males responding increased from February through May each year.

6. *T. raschii* responsiveness to successive stimulations was higher in May and June, and in some respects in August, September and October, than at other times in the year.

7. Mean values of *T. raschii* FSLR delay were minimal in April, May and June. Response duration values also showed a decrease at this time, but this was not statistically significant.

8. Observations on sampled and tested animals showed that spermatophores were found on females of the Garroch Head population of *T. raschii* from mid-April until the end of June, and that the main period of adult growth took place between mid-March and mid-June in each year.

9. On the basis of the hypothesis that the form of the FSLR results from opposing inhibitory and excitatory components, it was tentatively concluded that seasonal changes in the FSLR could be interpreted as being at least partly related to changes in the animals luminescent behaviour in the sea. The seasonal changes recorded in the FSLR implied that luminescence was related to breeding behaviour and in particular to spermatophore transference.

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Literature cited

BODEN, B. P. and E. M. KAMPA: Records of bioluminescence in the ocean. Pacif. Sci. **11**, 229—235 (1957).

— — Spectral composition of the luminescence of the euphausiid *Thysanoëssa raschii*. Nature, Lond. **184**, 1321 (1959).

— — Planktonic bioluminescence. Oceanogr. Mar. Biol. Ann. Rev. **2**, 341—371 (1964).

CLARKE, G. L. and C. J. HUBBARD: Quantitative records of the luminescent flashing of oceanic animals at great depths. Limnol. Oceanogr. **4**, 163—180 (1959).

DAVID, C. N. and R. J. CONOVER: Preliminary investigations on the physiology and ecology of luminescence in the copepod *Metridia lucens*. Biol. Bull. mar. biol. Lab., Woods Hole **121**, 92—107 (1961).

DOYLE, J. D. and R. H. KAY: Some studies on the bioluminescence of the euphausiid *Meganyctiphanes norvegica* and *Thysanoëssa raschii*. J. mar. biol. Ass. U.K. **47**, 555—563 (1967).

FISHER, L. R.: The total lipid material in some species of marine zooplankton. Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer **153**, 129—136 (1962).

FISHER, L. R., S. K. KON and S. Y. THOMPSON: Vitamin A and carotenoids in some marine invertebrates. II. Studies of seasonal variations in some marine crustacea. J. mar. biol. Ass. U.K. **33**, 589—612 (1954).

— — — Vitamin A and carotenoids in some marine invertebrates. III. Euphausiacea. J. mar. biol. Ass. U.K. **34**, 81—100 (1955).

HARDY, A. C. and R. H. KAY: Experimental studies of plankton luminescence. J. mar. biol. Ass. U.K. **44**, 435—484 (1964).

IVANOV, B. G.: On the luminescence of the Antarctic krill (*Euphausia superba*). Okeanologia **9**, 505—506 (1969).

KAMPA, E. M. and B. P. BODEN: Light generation in a sonic scattering layer. Deep Sea Res. **4**, 73—92 (1957).

KAY, R. H.: Bioluminescence of the euphausiid crustacean *Meganyctiphanes norvegica*: the influence of 5-hydroxytryptamine. J. Physiol., Lond. **165**, 63P—64P (1962).

— Light-stimulated and light-inhibited bioluminescence of the euphausiid *Meganyctiphanes norvegica* (G. O. SARS). Proc. R. Soc., B **162**, 365—386 (1965).

— The inhibition of optically stimulated bioluminescence in *Meganyctiphanes norvegica*. *In:* Some Contemporary Studies in Marine Science, pp 421—427. Ed. by H. BARNES. London: George Allen & Unwin Ltd. 1966.

MACDONALD, R. M.: The life history of *Thysanoëssa raschii*. J. mar. biol. Ass. U.K. **15**, 57—79 (1928).

MAUCHLINE, J.: The biology of the euphausiid crustacean. *Meganyctiphanes norvegica* (M. SARS). Proc. R. Soc. Edinb. B **67**, 141—179 (1960).

— The biology of *Thysanoëssa raschii* (M. SARS), with a comparison of its diet with that of *Meganyctiphanes norvegica* (M. SARS). *In:* Some Contemporary Studies in Marine Science, pp 493—510. Ed. by H. BARNES. London: George Allen & Unwin Ltd. 1966.

— and L. R. FISHER: The biology of euphausiids. Adv. mar. Biol. **7**, 1—454 (1969).

TETT, P. B.: The effects of temperature upon the flash-stimulated luminescence of the euphausiid *Thysanoëssa raschii*. J. mar. biol. Ass. U.K. **49**, 245—258 (1969).

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