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Journal

Journal of Experimental Marine Biology and Ecology, 136(1)

ISSN

0022-0981

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Publication Date

1990-03-01

DOI

10.1016/0022-0981(90)90097-v

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Peer reviewed

JEMBE 01378

Recovery and stimulation of copepod bioluminescence

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(Received 5 July 1989; revision received 31 October 1989; accepted 30 November 1989)

Abstract: The depletion of bioluminescence that occurs during net collection and handling, and its subsequent recovery, was investigated in three species of oceanic calanoid copepods, *Pleuromamma xiphius* (Giesbrecht), *Lucicutia flavicornis* (Claus), and *Gaussia princeps* (T. Scott). Only 14–38% of total bioluminescence was present 1 h after collection. Recovery of light emission occurred at an exponential rate which did not vary with species. Full recovery of bioluminescence occurred within 1 day, and was due to increases in both the quantum emission of individual flashes and the number of flashes contributing to the response. Total bioluminescence was related to species size, as was the time to full recovery. Once fully recovered, *P. xiphius* produced three types of flashes in response to mechanical stimulation. These flash types differed in response kinetics and quantum emission. The rate of mechanical stimulation affected the rate at which flashes were produced and not the total amount of bioluminescence. Therefore, stimulus strength and duration will influence the nature of the bioluminescent response.

Key words: Bioluminescence; Copepod; Flash; Quantum emission; Recovery; Total stimutable light

INTRODUCTION

Models under development for predicting spatial and temporal patterns of bioluminescence in the upper ocean are based on estimates of bioluminescence potential (Wroblewski, 1986), the total amount of stimulated emission by luminescent organisms. These estimates can be obtained in two ways. One approach is based on in situ bathyphotometer measurements of stimulated bioluminescence (Swift et al., 1985a; Ondercin & Fuechsel, 1986; Case et al., 1987) referenced to laboratory biological calibrations. A second approach involves determining the species contributions to the bioluminescence field. This is done by combining species abundance, obtained from plankton net collections, with laboratory measurements of total mechanically stimulated light (TMSL) from identified phyto- and zooplankton sources (Swift et al., 1985b; Batchelder & Swift, 1988, 1989) to estimate the bioluminescence potential. Estimates of bioluminescence potential can then be incorporated into “photon budgets”, for photosynthetically active radiation, describing the bio-optical properties of the water column (Marra, 1985; Smith et al., 1989).

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Biowatt Contribution 37.

Dinoflagellates are the only group of luminescent phytoplankton (Herring, 1987); some of the factors regulating light emission in the autotrophic dinoflagellates are well characterized. They possess a circadian rhythm in both spontaneous and stimulated bioluminescence (e.g., Sweeney & Hastings, 1957; Biggley et al., 1969; Krasnow et al., 1981). TMSL values for laboratory cultured organisms vary with cell size within species (Swift & Meunier, 1976; Widder & Case, 1981), prior photophase irradiance (Sweeney, 1981), and scotophase irradiance which causes photoinhibition (Esias et al., 1973; Hamman et al., 1981; Filimonov & Sadvovskaya, 1986). Shipboard studies using freshly collected cells reveal complex depth-dependent patterns in TMSL (Swift et al., 1981).

Conversely, factors affecting the bioluminescence of heterotrophic dinoflagellates and zooplankton, including copepods are poorly understood. Although photoinhibition of luminescence occurs in coelenterates (Harvey, 1952; Nicol, 1955) and heterotrophic dinoflagellates (Tyulkova & Filimonov, 1981), there is no evidence for the presence of a circadian rhythm or light inhibition of bioluminescence in most copepod taxa (David & Conover, 1961; Hardy & Kay, 1964; Barnes & Case, 1972; Evstigneev & Bitjukov, 1986; Neidhardt, 1989). Interspecific differences in levels of copepod bioluminescence are not explained by organism size, although there is an intraspecific ontogenetic increase in light emission (Evstigneev, 1983; Lapota et al., 1988a; Batchelder & Swift, 1989). Coelenterate-type luciferin has been identified in several copepod genera (McCapra & Hart, 1980; Campbell & Herring, cited in Herring, 1988), and, in at least one instance among crustaceans, is required in the diet (Frank et al., 1984). Feeding enhances the bioluminescence of *Metridia lucens* after a period of 3 wk (David & Conover, 1961), although no such effect occurs for *Gaussia princeps* (Barnes & Case, 1972). While the occurrence of a seasonal cycle in copepod luminescence has not been investigated, seasonal rhythms in euphausiid luminescence (Tett, 1969, 1972) may be related to reproductive cycles.

The magnitude of copepod bioluminescence is reduced following their collection in nets (Clarke et al., 1962; Evstigneev, 1983; pers. obs.). Recovery after depletion, due to collection or laboratory testing, occurs in < 1 day (David & Conover, 1961; Clarke et al., 1962; Barnes & Case, 1972), although it is not clear whether bioluminescence recovers through increases in flash quantum emission and/or flash number. Due in part to depletion of bioluminescence during collection and handling, copepods exhibit variable TMSL values prior to full recovery.

The calanoid copepod *Pleuromamma xiphius* (Giesbrecht), a common member of the plankton of the Sargasso Sea (Deevey, 1971), migrates at night from midwater daytime depths to shallower epipelagic depths (Roehr & Moore, 1965). Bioluminescence in *P. xiphius* originates from glands located on the body (Clarke et al., 1962; Bannister & Herring, 1989) and is readily elicited by electrical stimulation (Clarke et al., 1962; Latz et al., 1987a). However, since bathyphotometer measurements of in situ bioluminescence involve mechanical excitation, the kinetics and quantum emission of mechanically stimulated flashes is of interest in evaluating bathyphotometer performance. The present study describes the single mechanically induced flash of *P. xiphius*,

and investigates the depletion of bioluminescence during collection and handling and subsequent recovery with time. Once bioluminescence has recovered, the rate of stimulation has little effect on total quantum emission (TMSL) but strongly influences the rate at which bioluminescence is expressed. Similar patterns are present in two additional copepod species, *Lucicutia flavicornis* (Claus) and *Gaussia princeps* (T. Scott).

MATERIALS AND METHODS

Three species of calanoid copepods were examined. Adult specimens of *P. xiphias* (body length 5 mm) and *L. flavicornis* (body length 1.5 mm) were collected during cruises to the western Sargasso Sea in August 1986, May 1987, and August 1987. Specimens were collected at night with 1-m diameter plankton nets of 335- μ m mesh towed obliquely for 10 min between 80 and 100 m depth. Most net tows involved closing the net prior to retrieval. Specimens were sorted, individually placed in vials containing 5 ml filtered seawater, and dark acclimated until testing for ≥ 16 h (unless otherwise stated). Even though organisms were not fed during this time, short-term starvation should have no effect on bioluminescence emission (David & Conover, 1961; Barnes & Case, 1972; Batchelder & Swift, 1989).

Adult specimens of *G. princeps* (body length 10 mm) were collected during September 1986 and 1987 from the San Clemente Basin, off the coast of California ($\approx 32^\circ$ N, 117° W). Collection was performed with a 10-m² opening-closing Tucker trawl (Childress et al., 1977), towed between 400 and 800 m depth. Specimens were sorted and maintained in chilled seawater (6 °C), until testing.

Mechanically stimulated bioluminescence of single, identified organisms was measured in a calibrated detection apparatus consisting of an integrating sphere

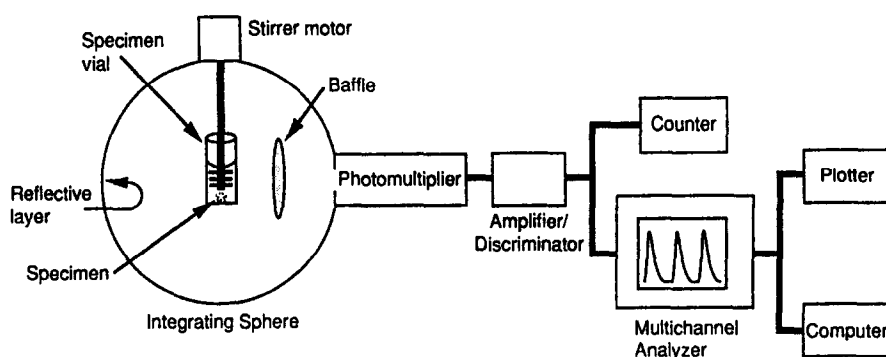


Fig. 1. Schematic diagram of detection apparatus. Vial containing a specimen is positioned within an integrating sphere and stirred at constant rpm. Baffle assures that only reflected bioluminescence reaches the photomultiplier (PMT) detector. PMT anode current is processed through a low-gain amplifier, and a discriminator, which acts as a voltage-to-pulse converter; pulse frequency is proportional to intensity of emission. Pulses can be counted or time binned and displayed on a multichannel analyser (MCA). MCA record is stored on computer floppy diskette and later plotted.

collector coupled to a photon counting photomultiplier (Fig. 1). A vial containing a single specimen in 5 ml filtered seawater was placed in the center of the 10-in diameter integrating sphere (Labsphere). Bioluminescence was detected with an RCA model 8850 photon counting photomultiplier baffled so that only reflected light was measured. The photomultiplier signal was processed through an amplifier/discriminator (Pacific Instruments model AD6) which produced NIM fast pulses which were counted by a Norland model 5400 multichannel analyser (MCA) operating with either a 10- or 50-ms channel dwell time. MCA sweeps of 40 or 200 s duration were stored in digital form on diskette, and displays were expanded up to 25 times in the horizontal (time) scale during later analysis.

The spectral responsivity of the collector/detector system was determined with an NBS referenced multifilter calibration source (Optronics Laboratories model 310) so that all measurements of quantum emission are corrected for the spectral emission of bioluminescence (Latz et al., 1987a). The calibrated spectral responsivity of the system was $\approx 2 \times 10^3$ photons $\cdot$ NIM pulse $^{-1}$. In order to avoid coincidence counting by the AD6 amplifier/discriminator, a neutral density filter with 1% transmission at 490 nm (Ditric Optics) attenuated the bioluminescence signal from *P. xiphias* and *G. princeps*. Since even the maximum intensity of 2×10^{12} photons $\cdot$ s $^{-1}$ encountered in the present study (equivalent to 1×10^7 pulses $\cdot$ s $^{-1}$) was less than the maximum limit of resolution of the system (which occurs at 5×10^7 pulses $\cdot$ s $^{-1}$), coincidence counting was not encountered.

Bioluminescence was mechanically stimulated by stirring the contents of the vial with a vertical shaft and three attached cross tines, which entered the top of the integrating sphere through a 1-in diameter light-proof port. The stirring shaft design minimized physical damage to specimens, which were observed intact and swimming after testing. The shaft was attached to a 1/50-HP DC motor which was operated in two modes. For measurements of TMSL the motor was continuously operated at a constant speed of 2000 rpm. Single flashes were routinely elicited by operating the motor for very brief periods of < 1 s in duration. This pulsed method has been successfully used to stimulate single flashes not only from copepods but also from dinoflagellates, radiolaria and other plankton (unpubl. data).

Bioluminescence of *G. princeps* was electrically stimulated by two stainless steel screen meshes lightly contacting the animal and connected to a Grass S48 stimulator. Total stimuable light was induced with monophasic electrical pulses of 5-ms duration and 50 V delivered at 2 Hz until exhaustion of luminescence. Individuals were also stimulated with single electrical pulses for determination of single flash kinetics during the recovery period.

All experiments were performed in the shipboard laboratory. The following single flash parameters were determined: rise time, decay time constant (based on E-folding decay time), 97% decay time, total duration, maximum intensity, and total quantum emission (refer to Latz et al., 1987a). E-folding time refers to the initial decay time from the maximum intensity to $1/e$ of the maximum intensity, and its inverse gives the rate

of exponential flash decay. In addition, records of total stimutable light (TSL or TMSL) were analysed for total photons emitted per individual ($\text{photons} \cdot \text{ind}^{-1}$), number of flashes, and total duration of light emission. %TSL or %TMSL is the percentage of TSL expressed in a single flash. Unless otherwise stated, $\bar{x}$ values $\pm$ SE of $\bar{x}$ are presented. Exponential and logarithmic regressions were calculated for TSL recovery data according to least squares analysis. Significance of statistical tests is based on the 5% probability level. Multiple comparisons are based on Fisher's protected least-significance difference (PLSD) (Winer, 1971).

RESULTS

INDIVIDUAL FLASHES OF *P. XIPHIAS*

From a total of 149 mechanically stimulated flashes from 23 specimens, stimulated by brief stirrer "pulses" at least 16 h after collection during August 1987, 94% of the flashes were represented by three flash types (Fig. 2): (1) a single fast flash, (2) a single

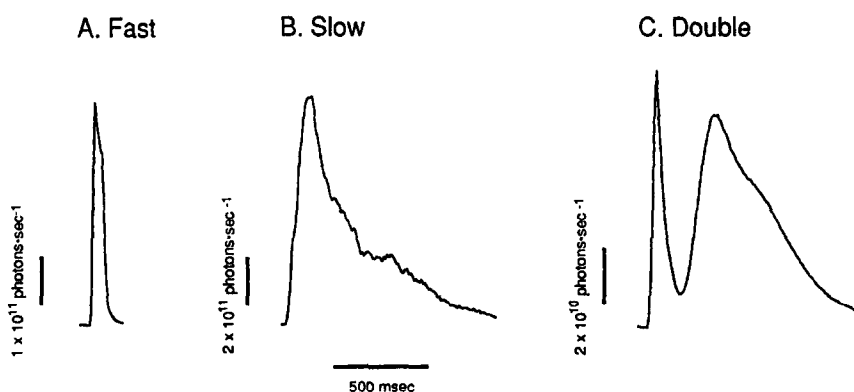


Fig. 2. Individual flash types of *P. xiphias*. Representative examples show intensity ($\text{photons} \cdot \text{s}^{-1}$) as a function of time (ms). (A) Single fast flash. (B) Single slow flash. (C) Double component flash, displaying both fast and slow components.

slow flash, and (3) the fast-slow double flash described by Latz et al. (1987a). The maximum intensity of single flashes averaged $1\text{--}3 \times 10^{11} \text{ photons} \cdot \text{s}^{-1}$; quantum emission ranged from 2 to $8 \times 10^{10} \text{ photons} \cdot \text{flash}^{-1}$, depending on flash type (Table I). Not all specimens produced all three flash types, and flash types could occur in any sequence during light emission.

Single fast flashes were the most common flash type observed. They were characterized by a rapid rise to maximum intensity in $< 65 \text{ ms}$, followed by a rapid decay of $\approx 300 \text{ ms}$. Flashes averaged $< 400 \text{ ms}$ in duration and each constituted $\approx 8\%$ of TMSL

TABLE I

Kinetics and quantum emission of individual flashes of *P. xiphioides* measured from a sample of 149 flashes. Three flash types represented 94% of observed flashes. $\bar{x}$ values with $SE \bar{x}$ are shown with range in parentheses. See text for description of flash parameters. *N* refers to number of flashes of each type measured.

Flash parameter	Flash type		
	Single-fast	Single-slow	Double
Max I (photons · s ⁻¹) ^a	2.30 ± 0.37 × 10 ¹¹ (1.69 × 10 ⁹ –1.76 × 10 ¹²)	1.21 ± 0.58 × 10 ¹¹ (9.48 × 10 ⁹ –1.33 × 10 ¹²)	3.32 ± 0.79 × 10 ¹¹ (7.09 × 10 ⁹ –1.47 × 10 ¹²)
Quantum emission ^b (photons · flash ⁻¹)	1.77 ± 0.29 × 10 ¹⁰ (1.62 × 10 ⁸ –1.56 × 10 ¹¹)	2.37 ± 0.64 × 10 ¹⁰ (3.76 × 10 ⁹ –1.46 × 10 ¹¹)	7.65 ± 1.99 × 10 ¹⁰ (1.13 × 10 ⁹ –4.60 × 10 ¹¹)
% TSL ^b	7.7 ± 1.8 (0.2–53.0)	4.7 ± 1.4 (0.7–16.4)	14.1 ± 2.9 (0.1–49.7)
Rise (ms) ^c	63.9 ± 4.9 (20–240)	283.5 ± 56.0 (50–1110)	80.3 ± 13.2 (20–390)
E-fold (ms) ^d	78.1 ± 7.6 (10–390)	281.3 ± 45.6 (40–800)	208.5 ± 30.0* (30–760)
97% decay (ms) ^e	303.1 ± 30.4 (50–1440)	1080.9 ± 186.6 (150–3970)	1148.8 ± 226.9* (90–6500)
Duration (ms) ^e	378.8 ± 34.6 (80–1560)	1521.3 ± 262.0 (430–5140)	1598.5 ± 310.2 (160–8150)
N	83	23	34

* Decay of second flash component.

^a Double flashes different from slow flashes (Fisher's PLSD, $P < 0.05$).

^b Double flashes different from fast and slow flashes ($P < 0.05$).

^c Slow flashes different from fast and double flashes ($P < 0.05$).

^d Each flash type different ($P < 0.05$).

^e Fast flashes different from slow and double flashes ($P < 0.05$).

(Table I). Single slow flashes were observed 15% of the time and were characterized by a slower rise (> 280 ms) and decay (≈ 1 s) than fast flashes. Total duration of the slow flashes was 1.5 s, with each flash averaging 5% of TMSL. $\approx 1/4$ of all flashes were the double component flashes previously described (Latz et al., 1987a). The first component of these flashes exhibited the rapid kinetics of the fast flashes, while the second component resembled the slow flashes. Double flashes averaged 1.6 s in duration and constituted 14% of TMSL (Table I). Their similarity to double flashes elicited by single electrical pulses (Latz et al., 1987a) suggests that the double flash results from a single mechanical stimulus rather than multiple stimuli causing two sequential flashes.

TOTAL STIMULABLE LUMINESCENCE OF *P. XIPHIAS*

Recovery of bioluminescence following collection

Initial results indicated that recently collected organisms produced very few flashes compared with specimens tested 24 h following collection (Fig. 3), resulting in a greatly

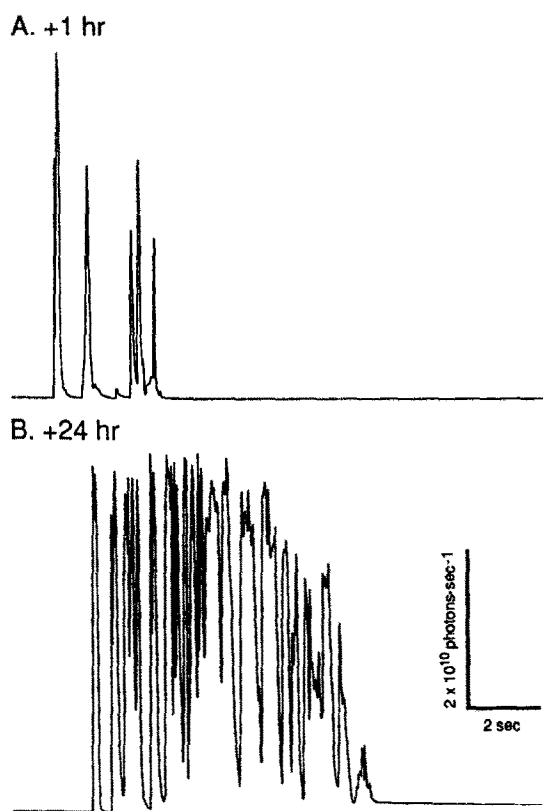


Fig. 3. Depletion of bioluminescence of *P. xiphias* during collection and handling. Light emission was stimulated by maintained stirring at 2000 rpm. (A) Emission 1 h after collection. Only five flashes are produced; light emission is produced over a period of 3 s. TMSL = 1.5×10^{10} photons $\cdot$ ind $^{-1}$. (B) Emission of a different specimen measured 24 h after collection. ≈ 25 flashes are produced in 9 s. TMSL = 2.0×10^{11} photons $\cdot$ ind $^{-1}$.

reduced TMSL. This suggests that the decrease in luminescence is due to either substrate depletion during collection and handling or, possibly, a change in sensitivity to excitation. TMSL was measured only once from each specimen and measurements were taken at regular intervals over 24 h in order to monitor the temporal recovery of luminescence (Fig. 4A). ≈ 16 h was required for maximum TMSL values to be reached. During the first 18 h, the increase in bioluminescence capacity (TMSL) as a function of time (T) was best fit by the exponential equation:

$$\text{TMSL} = 2.32 \times 10^{10} * e^{0.04T}$$

($r^2 = 0.87$). Maximal TMSL values measured 16–24 h after collection represented the summation of ≈ 20 flashes, and averaged $1.1 \pm 0.5 \times 10^{11}$ photons $\cdot$ ind $^{-1}$. During recovery, both the number of flashes contributing to TMSL and the quantum emission of single flashes increased (Table II), since recently collected specimens produced fewer

TABLE II

Recovery of *P. xiphius* bioluminescence for selected times after collection. Total mechanically stimulated luminescence (TMSL) of specimens maintained in the dark for specified time was elicited by constant stirring at 2000 rpm. $\bar{x}$ values with SE of $\bar{x}$ are shown; N refers to number of organisms tested at each time period. Total response duration is length of time to total exhaustion of light emission (i.e., from beginning of first flash to end of last flash in record). Average equivalent flash quantum emission is estimated by dividing TMSL by average number of flashes produced.

Time (h)	Number of flashes $\cdot$ ind $^{-1}$	Total response duration (s)	Equivalent quantum emission (photons $\cdot$ flash $^{-1}$)	N
1.0	13.0 ± 1.3	8.3 ± 1.7	2.0×10^9	20
6.3	13.8 ± 1.9	5.0 ± 0.8	3.5×10^9	10
10.8	14.7 ± 1.3	10.3 ± 1.6	3.1×10^9	11
12.7	18.1 ± 1.8	6.3 ± 0.5	4.1×10^9	9
15.3	20.3 ± 1.8	8.7 ± 2.0	5.8×10^9	12
19.5	18.9 ± 2.8	7.7 ± 1.3	5.3×10^9	7
21.0	19.2 ± 2.8	8.2 ± 1.4	6.6×10^9	6
24.0	19.7 ± 1.8	12.0 ± 1.4	6.1×10^9	20

flashes and only 24% of the maximum TMSL. The equivalent flash quantum emission, estimated by dividing the TMSL by the number of flashes, increased during the period 1–15 h after collection. Since some temporal summation of light emission occurred, not all the bioluminescence was expressed in discrete flashes (Fig. 3B); therefore, the equivalent flash quantum emission underestimates the actual single flash emission (Table I). The increase in flash number and quantum emission during recovery resulted in an increase in the average duration of the bioluminescence response (slope of linear regression of response duration on time vs. zero slope; $t = 2.09$, $P < 0.05$). At maximal TMSL values, light emission was maintained for ≈ 12 s (Table II).

Long-term recovery

Long-term recovery of *P. xiphias* was monitored in specimens maintained up to 3 days in the shipboard laboratory during a cruise in the mid-Atlantic ($\approx 30^\circ\text{N } 32^\circ\text{W}$) during March 1989. Although organisms were not fed during this time, seawater was replaced by siphoning every 24 h to minimize the effects of O_2 depletion and metabolic waste buildup in the vials. Specimens exhibiting any luminescence during transfer were discarded. Mortality for the three groups tested was 0/9 after 1 day, 5/16 for the 2nd day, and 5/17 the 3rd day.

TMSL of *P. xiphias* measured after 1 day (17 h) of recovery was $3.1 \pm 0.2 \times 10^{11}$ photons $\cdot \text{ind}^{-1}$, higher than the value of $0.9 \pm 0.1 \times 10^{11}$ photons $\cdot \text{ind}^{-1}$ obtained in the 17-h test during August 1986 in the Sargasso Sea (Student's t test, $t = 7.93$, $P < 0.05$). The disparity in TMSL values may be explained by differences in nutritional or other physiological states, or improved handling protocols between 1986 and 1989. After 2 days (45 h) of recovery the TMSL was $7.0 \pm 1.2 \times 10^{11}$ photons $\cdot \text{ind}^{-1}$, and after 3 days (68.3 h) TMSL equalled $5.2 \pm 0.7 \times 10^{11}$ photons $\cdot \text{ind}^{-1}$. The 1-day TMSL value was significantly different from the 2- and 3-day readings, which were not significantly different from each other (Fisher's PLSD, $P > 0.05$). There was no significant trend for increasing TMSL after 1 day of recovery, since the linear regression of TMSL (after 17 h) vs. time of recovery did not exhibit a slope significantly different from zero ($t = 1.817$, $P > 0.05$). Therefore, most of the recovery of bioluminescence of *P. xiphias* in the shipboard laboratory environment occurred in < 1 day of collection.

It was not possible to assess the impact of mortality on TMSL of the longest recovery times. However, there was no evidence of bias due to mortality since log-normal histograms of TMSL at each recovery period were not skewed.

Recovery following total depletion

The bioluminescence of *P. xiphias* was totally depleted through maintained stirring until exhaustion of light emission. Specimens were then allowed to recover for specified time periods before retesting of TMSL (Fig. 4B). Specimens tested 1 h after depletion produced 6% of maximal TMSL (based on the 24-h recovery experiment). Recovery of luminescence (% TMSL) over time (T) for 24 h was best described by the logarithmic regression:

$$\% \text{TSL} = 5.86 * T^{0.89}$$

($r^2 = 0.96$) rather than an exponential regression. After 22 h, bioluminescence had recovered to $\approx 85\%$ of maximum TMSL, although the 50% upper confidence interval was 1.0×10^{11} photons $\cdot \text{ind}^{-1}$, equivalent to the maximum TMSL level. If the recovery after total depletion were described by an exponential regression, the rate of recovery for the period of 2.5–10 h increased at only a slightly greater exponential rate of $0.06 \cdot \text{h}^{-1}$ than for the previous collection recovery experiment, although the TMSL was not as well correlated ($r^2 = 0.77$) as for the logarithmic regression given above.

The number of flashes produced during recovery from total depletion was similar to the flash number during the 24-h recovery after collection, increasing from 11.3 ± 1.2 flashes measured 4.3 h after depletion to 25.3 ± 3.9 flashes after 22 h of recovery. The exception was that 1 h following total depletion, the number of flashes was significantly

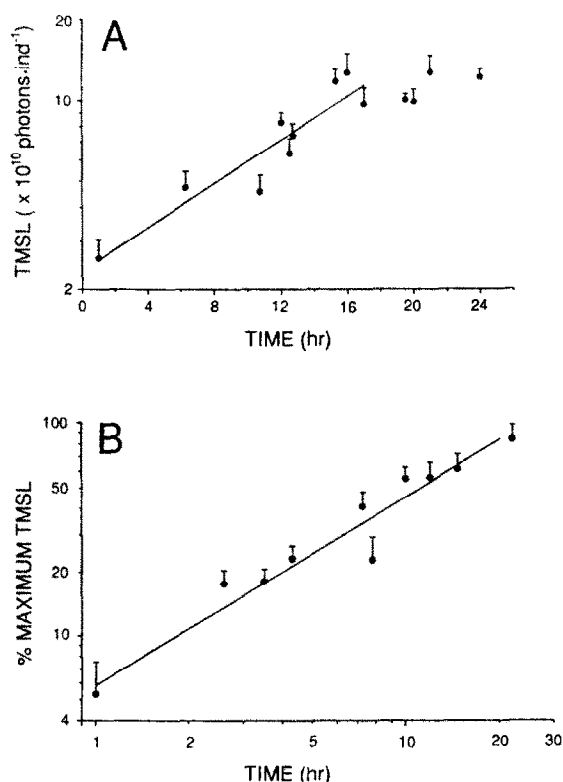


Fig. 4. Recovery of *P. xiphias* bioluminescence. For each group tested, $\bar{x}$ values are shown; error bars represent SE of $\bar{x}$. (A) Recovery after collection. Total mechanically stimutable luminescence (TMSL) increased with time and reached a maximum average value of $1.1 \pm 0.5 \times 10^{11}$ photons $\cdot$ ind⁻¹ after 15 h of recovery. Line represents calculated least-squares exponential regression of TMSL on time, according to equation: $y = 2.32 \times 10^{10} \cdot e^{0.04x}$, $r^2 = 0.87$. (B) Recovery following total laboratory depletion. Percent total stimutable luminescence (% TMSL) of maximal TMSL measured from previous experiment (1.1×10^{11} photons $\cdot$ ind⁻¹) increased with time after depletion. Line represents calculated least-squares logarithmic regression of TMSL on time, according to equation: $y = 5.86 \cdot x^{0.89}$, $r^2 = 0.96$.

different; only 4.8 ± 0.9 flashes were produced compared to 13.0 ± 1.3 flashes during the collection recovery experiment (Student's *t* test, $t = 2.972$, $P < 0.05$).

Effect of stimulation rate

Since previous work has demonstrated that the rate of electrical stimulation affects flash duration and total quantum emission in *P. xiphias* (Latz et al., 1987a), the effect

TABLE III

Effect of rate of stimulation on bioluminescence of *P. xiphias*. Total mechanically stimulated luminescence (TMSL) of specimens maintained in dark for > 16 h was elicited by constant stirring at various motor speeds. $\bar{x}$ values with SE $\bar{x}$ are shown; N refers to number of organisms tested at each time period. Total response duration is length of time to total exhaustion of light emission. TMSL values for 1000, 2000, and 3000 rpm stimulation rates were equivalent (one-factor ANOVA, $P > 0.05$).

Motor speed (rpm)	Number of flashes $\cdot$ ind $^{-1}$	Total response duration (s)	TMSL (photons $\cdot$ ind $^{-1}$)	N	TMSL + retest (photons $\cdot$ ind $^{-1}$)	N_r
500	8.0 ± 1.1	$> 21.9 \pm 3.2^a$	$7.7 \pm 2.0 \times 10^{10}$	11	13.4×10^{10}	6
1000	17.7 ± 1.5	13.4 ± 1.8	$11.1 \pm 0.8 \times 10^{10}$	19	13.3×10^{10}	6
2000	18.8 ± 1.5	9.9 ± 1.8	$10.8 \pm 0.8 \times 10^{10}$	19	—	—
3000	15.9 ± 1.2	7.1 ± 1.3	$8.9 \pm 1.2 \times 10^{10}$	12	9.2×10^{10}	6

^a For some flash records, luminescence persisted beyond 40-s data collection period.

^b 4 min following initial testing, specimens were retested using a motor speed of 2000 rpm to determine fraction of TMSL not released during the first stimulation. Values represent mean sums of initial TMSL and retest measurements; N_r refers to number of specimens retested.

of rate of mechanical stimulation on TMSL was measured. While levels of excitation were not directly quantified, the rate of excitation was considered to be related to the rotational speed of the stirrer.

The excitation threshold was close to the stirring rate of 500 rpm, since at that speed only 50% of the specimens tested emitted bioluminescence. At higher stirring rates bioluminescence was successfully elicited from all specimens.

The total bioluminescence produced with suprathreshold stimulation did not vary with stimulation rate (Table III). TMSL values for motor speeds of 1000, 2000, and 3000 rpm averaged $1.0 \pm 0.1 \times 10^{11}$ photons $\cdot$ ind $^{-1}$ and were not significantly different from one another (one-factor ANOVA, $P > 0.05$). The slowest stirring rate of 500 rpm yielded a lower TMSL of $7.7 \pm 2.0 \times 10^{10}$ photons $\cdot$ ind $^{-1}$.

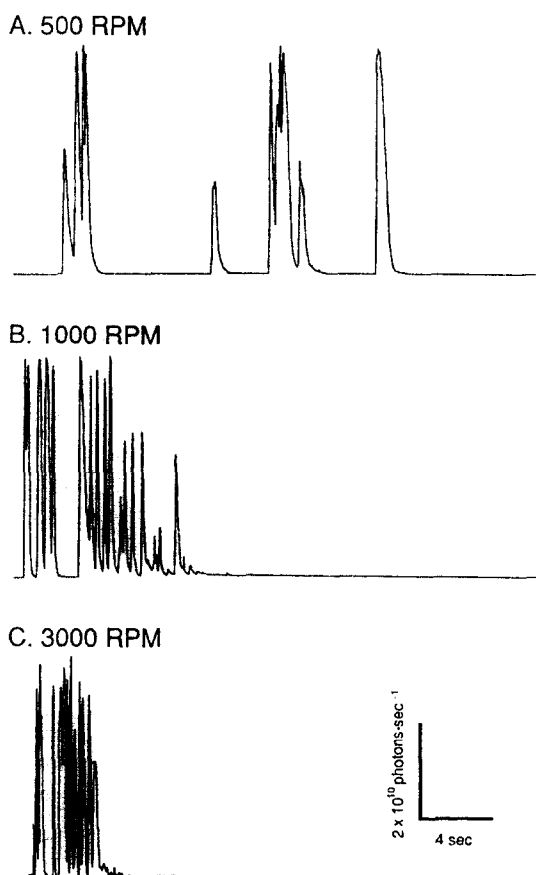


Fig. 5. Effect of rate of stimulation on bioluminescence for three specimens of *P. xiphius*. (A) Stimulation at a motor speed of 500 rpm. This stimulation rate was just supra-threshold; nine flashes were produced over a period of 19 s. (B) Stimulation at 1000 rpm. Flashes were produced at a faster rate; 19 flashes occurred in 10 s. (C) Stimulation at 3000 rpm; 22 flashes were produced in 5 s.

The number of flashes produced followed a similar pattern (Table III). Higher stimulation rates of 1000–3000 rpm generated an average of 17.7 ± 0.8 flashes $\cdot$ ind $^{-1}$, while the slowest motor speed of 500 rpm produced only 8.0 ± 1.1 flashes $\cdot$ ind $^{-1}$.

The lower TMSL and flash numbers for 500 rpm stimulation represented incomplete expression of bioluminescence due to the low rate of stimulation. Total exhaustion of light emission did not occur during the 40-s measurement period, as immediate restimulation at 2000 rpm generated additional light emission. The sum of the original plus the retest values approximately equaled the maximal TMSL levels found for the higher stimulation rates (Table III).

The most prominent effect of differential stimulation rates was in the response duration (i.e., the period of time during which bioluminescence was expressed). The response duration was inversely proportional to the rate of stimulation (Fig. 5). The slowest stirring speed yielded the longest response duration; flashes were produced over a period of > 21 s (Table III). Increasing the motor speed, and hence the rate of excitation, decreased the response duration due to an increase in the flashing rate. At the standard motor speed of 2000 rpm, the duration of light emission was ≈ 10 s. At the highest motor speed tested (3000 rpm), all flashes were produced in only 7 s.

BIOLUMINESCENCE OF OTHER COPEPOD SPECIES

Recovery of bioluminescence

The pattern of recovery of luminescence of *L. flavicornis* during the first 24 h after collection was similar to that for *P. xiphias*. Maximal TMSL values measured > 10 h after collection averaged $1.2 \pm 0.2 \times 10^{10}$ photons $\cdot$ ind $^{-1}$. Organisms tested immediately after collection produced only 38% of this value; TMSL subsequently increased exponentially with time (T) according to the equation:

$$\text{TMSL} = 4.50 \times 10^9 * e^{0.04T}$$

($r^2 = 0.91$). Total recovery occurred in ≈ 10 h (Fig. 6A). The rate of recovery was similar to that for *P. xiphias* (t test of slopes, $P > 0.05$).

Long-term recovery of *L. flavicornis* was measured during the March 1989 cruise in the mid-Atlantic. TMSL was measured after 1 day (19 h), 2 days (41.3 h), and 3 days (65.5 h) after collection. Mortality during the course of the experiment was not monitored. There were no significant differences between these measurements (one-factor ANOVA, $P > 0.05$); the overall mean TMSL was $1.2 \pm 0.1 \times 10^{10}$ photons $\cdot$ ind $^{-1}$. Therefore, recovery of bioluminescence in *L. flavicornis* occurred in < 1 day.

The large bathypelagic *G. princeps* exhibited a similar pattern of recovery, with total recovery of bioluminescence occurring in ≈ 1 day (Fig. 6B). Assuming an exponential increase in bioluminescence over time, the increase in TSL as a function of time (T) is described by the equation:

$$\text{TSL} = 1.36 \times 10^{11} * e^{0.03T}$$

($r^2 = 0.73$). TSL increased at a similar rate as in the other two species. A TSL of 7×10^{11} photons $\cdot$ ind $^{-1}$ was reached in 28 h, with little further increase evident up to 800 h after collection. Individuals examined 1 h after capture produced only 14% of the TSL which was produced after 28 h.

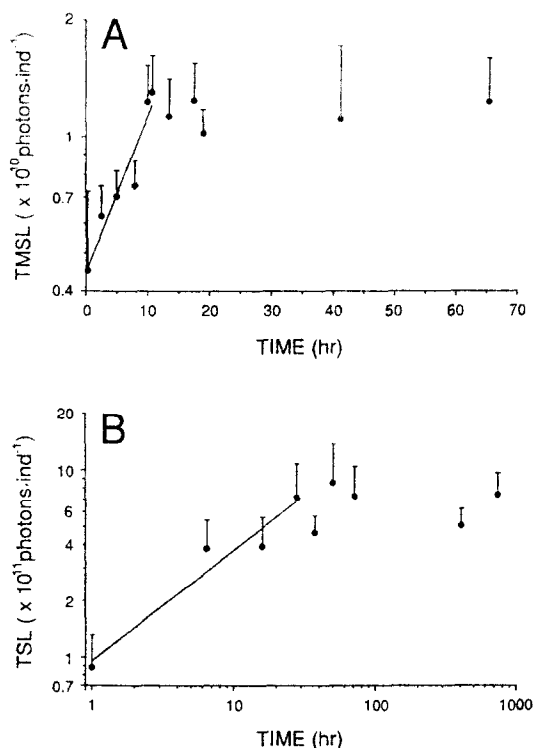


Fig. 6. Recovery of bioluminescence of other copepod species following collection. As in Fig. 4. (A) *L. flavicornis*. Line represents least-squares exponential regression of TMSL for times from 0.25–11 h, according to equation: $y = 4.50 \times 10^9 * e^{0.04x}$, $r^2 = 0.91$. Maximum TMSL value was $\approx 1.2 \pm 0.1 \times 10^{10}$ photons $\cdot$ ind $^{-1}$. (B) *G. princeps*. Bioluminescence recovered in < 30 h. Maximum TSL value measured 24 h after collection was $6.5 \pm 1.3 \times 10^{11}$ photons $\cdot$ ind $^{-1}$. Line represents calculated least-squares logarithmic regression of TSL for times from 1 to 28 h, according to equation: $y = 9.55 \times 10^{10} * x^{0.59}$, $r^2 = 0.94$.

Total duration of the luminescent response of *G. princeps* increased significantly during the initial 38 h of recovery (t test vs. zero slope, $t = 4.22$, $P < 0.05$), after which no further increase in response duration occurred (Table IV). The number of flashes remained unchanged throughout the recovery period. Average response intensity, estimated by dividing TSL by the duration of light emission, increased during the initial 28 h after collection (Table IV). Therefore, the increase in TSL during recovery was due to an increase in both response duration and average intensity.

TABLE IV

Recovery of *G. princeps* bioluminescence after collection. Total stimulated luminescence (TSL) of specimens maintained in dark for specified time was elicited with electrical stimuli at 2 Hz. $\bar{x}$ values with SE of $\bar{x}$ are shown; *N* is number of organisms tested at each time. Average intensity is obtained from dividing TSL (refer to Fig. 6B) by duration of flash.

Time (h)	Total response duration (s)	Average intensity (photons $\cdot$ s $^{-1}$)	<i>N</i>
1.0	14.2 $\pm$ 3.6	0.7 $\pm$ 0.3 $\times 10^{10}$	8
6.5	14.3 $\pm$ 4.4	2.4 $\pm$ 1.0 $\times 10^{10}$	8
16.0	23.5 $\pm$ 1.9	1.7 $\pm$ 0.8 $\times 10^{10}$	8
28.0	19.3 $\pm$ 3.2	3.0 $\pm$ 1.5 $\times 10^{10}$	8
37.5	36.7 $\pm$ 6.7	1.9 $\pm$ 0.8 $\times 10^{10}$	8
50.5	36.3 $\pm$ 5.6	2.4 $\pm$ 1.0 $\times 10^{10}$	8
72.0	16.0 $\pm$ 3.3	3.9 $\pm$ 0.9 $\times 10^{10}$	7
408.0	33.0 $\pm$ 4.6	1.5 $\pm$ 0.3 $\times 10^{10}$	7
744.0	29.5 $\pm$ 6.4	2.2 $\pm$ 0.7 $\times 10^{10}$	6

Effect of rate of stimulation

The effect of rate of mechanical stimulation for *G. princeps* was generally similar to that for *P. xiphius*. Motor speeds of 500–3000 rpm stimulated an average TMSL of $4.3 \pm 0.7 \times 10^{12}$ photons $\cdot$ ind $^{-1}$, and were not significantly different from one another (Fisher's PLSD, $P > 0.05$). The slowest stimulation rate of 100 rpm produced a TMSL

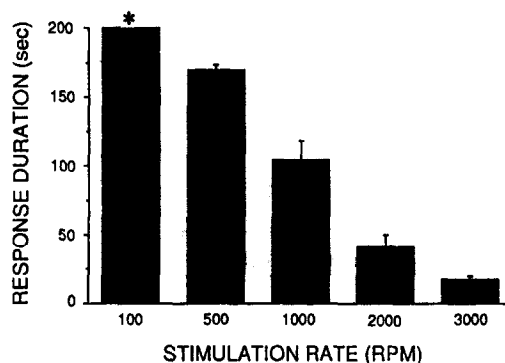


Fig. 7. Total response duration of *G. princeps* bioluminescence when mechanically stimulated at different stirring rates. Response duration decreases with increasing stimulation rate. All values are significantly different from one another (Fisher's PLSD, $P < 0.05$). Bar height represents $\bar{x}$; error bars represent SE of $\bar{x}$. Asterisk (*) indicates that response duration for 100 rpm stimulation exceeded 200-s period of collection, such that bioluminescence was not exhausted.

of $3.5 \pm 1.2 \times 10^{12}$ photons $\cdot$ ind $^{-1}$ and a response duration which exceeded the collection period of 200 s; therefore, at this low stimulation rate the bioluminescence capacity was not exhausted. The response duration was significantly decreased by each successive increase in the stimulation rate (Fisher's PLSD, $P < 0.05$; Fig. 7). Response durations ranged from > 200 s at 100 rpm to 17 s at 3000 rpm.

DISCUSSION

INDIVIDUAL FLASHES OF *P. XIPHIAS*

Three types of flashes were produced in response to mechanical stimulation: (1) single fast flashes, which accounted for $\approx 50\%$ of all flashes measured, (2) single slow flashes, and (3) double flashes. Double flashes consisted of a first component which resembled a fast flash, and a second component similar to a slow flash. The double flashes were similar to those stimulated by electrical pulses (Latz et al., 1987a), although the mechanically excited flashes were of a shorter duration (averaging 1.6 s) than the electrically stimulated flashes (averaging 2.6 s). Previous measurements of electrically excited flashes also underestimated levels of quantum emission, since flashes were stimulated before full recovery of bioluminescence had occurred (Latz et al., 1987a). Therefore, the values reported in this study more accurately reflect the emissions of specimens of *P. xiphias* that have fully recovered after collection and handling, and may better represent in situ flashes.

Maintained mechanical stimulation (i.e., constant stirring) did not result in as much temporal summation or bioluminescence as did repetitive electrical stimuli (Latz et al., 1987a). The bioluminescent response to mechanical stirring consisted of trains of flashes, with a direct relationship between the rate of flashing and the stirring speed. The temporal summation of flashes triggered with repetitive electrical stimuli could have been caused by a direct electrical effect on the light organs or on the neural circuitry controlling the light organs (Baguet & Case, 1971; Baguet, 1975; Baguet et al., 1980).

RECOVERY OF BIOLUMINESCENCE

Several investigators have observed that freshly collected copepods have reduced luminescence. Clarke et al. (1962) described how luminescence from several copepods of the family Metrididae increased over the course of 24 h. David & Conover (1961) showed flash records demonstrating that the flash quantum emission of *M. lucens* tested 6 h after collection was less than after long-term laboratory maintenance. Finally, Barnes & Case (1972) observed that the bioluminescence of *G. princeps* was largely restored in <24 h after testing.

The present study provides the first quantitative analysis of the recovery process for any luminescent zooplankton. Light emission after collection increased exponentially, with maximum TSL values reached in $< \approx 1$ day. All three species recovered at the same rate. Net collection and subsequent handling depleted up to 85% of the available bioluminescence. This most likely occurred through mechanical agitation in the collecting net, sorting basins, transfer pipettes, etc. The amount of excitation may very well be related to the type of plankton net used or sampling strategy (i.e., net closing), although this was not investigated.

Recovery of copepod bioluminescence after total laboratory depletion was best described by a logarithmic regression; dinoflagellates also recover from laboratory

depletion at a logarithmic rate. Calculated recovery rates of cultures of the dinoflagellates *Gonyaulax polyedra*, *Pyrodinium bahamense*, and *Pyrocystis lunula* (Fig. 12 in Biggley et al., 1969) and single cells of *Pyrocystis fusiformis* (Figs. 7–8 in Widder & Case, 1981) indicate that while recovery of these dinoflagellate species occurred at similar logarithmic rates (Fig. 8), their recovery rates are lower than for *P. xiphias* (present

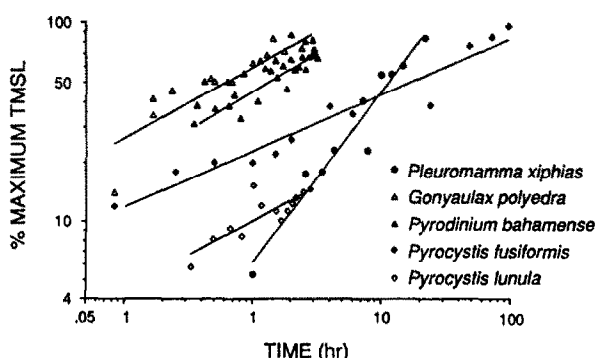


Fig. 8. Comparison of recovery of bioluminescence of *Pleuromamma xiphias* to recovery of dinoflagellate luminescence after total depletion. Data for *P. xiphias* taken from Fig. 4B. Data for *G. polyedra*, *P. bahamense*, and *P. lunula* are taken from Fig. 12 of Biggley et al. (1969), and are based on stirring 3-ml vols. of dinoflagellate cultures at a speed of 1800 rpm. Data for *P. fusiformis*, taken from Figs. 7 and 8 of Widder & Case (1981), are obtained from single cells loaded into plastic tubing and mechanically stimulated at a rate of 0.33 Hz by a solenoid until exhaustion of luminescence. Lines represent calculated least-squares logarithmic regressions of TMSL vs. time, according to equations: *G. polyedra*, $y = 14.0 * x^{0.35}$ ($r^2 = 0.80$); *P. bahamense*, $y = 9.4 * x^{0.38}$ ($r^2 = 0.84$); *P. lunula*, $y = 2.4 * x^{0.35}$ ($r^2 = 0.70$); *P. fusiformis*, $y = 22.8 * x^{0.28}$ ($r^2 = 0.94$).

study). A wide range of predicted times for full recovery is obtained from solving the regression equations. Full recovery times were on the order of days for *P. fusiformis* and *P. lunula*, 24 h for *P. xiphias*, and as brief as 5–8 h for *G. polyedra* and *P. bahamense*. The time to full recovery is apparently not directly or inversely related to the amount of luminescence produced (TSL). *G. polyedra* and *P. bahamense* have the lowest TSL values yet the fastest calculated recovery times (Biggley et al., 1969), while *P. xiphias* has a larger TSL and faster recovery (present study) than *P. fusiformis* and *P. lunula* (Biggley et al., 1969; Swift et al., 1973, 1985a; Latz et al., 1988).

The physiological mechanisms involved in the recovery of bioluminescence are not understood. Exhaustion of luminescence may result from causes other than the total depletion of luminescent substrate, as copepods stimulated to exhaustion still contain luminescent material (Barnes & Case, 1972). Mechanically depleted dinoflagellates will also emit luminescence upon chemical stimulation (Latz & Case, unpubl. data), and are able to produce 12–14% of TMSL after just 5 min of recovery (Biggley et al., 1969; Widder & Case, 1981). This suggests that behavioral or physiological habituation or fatigue is partially responsible for the cessation of light emission. Measured recovery

rates may therefore reflect more than rates of de novo synthesis or mobilization from storage of luciferin/luciferase, for while luminescent competency will not be achieved until the materials are properly packaged within dinoflagellate membranes (Sweeney, 1976) or in copepod luminescent glands (Clarke et al., 1962), there may be synaptic or other systemic or central nervous system processes involved in recovery.

Organism size was not a factor in the rate of recovery of luminescence, as all three copepod species examined had similar recovery rates. Since the magnitude of TSL did vary with species size, the time for complete recovery increased with increasing size. TSL recovery for *G. princeps* was not related to the number of flashes produced, as was the case for *P. xiphias*, although this was probably due to temporal summation of the electrically stimulated flashes (Latz et al., 1987a).

Estimates of light budgets for various taxa have been calculated using average light output and abundance data for each group (Lapota et al., 1988b; Batchelder & Swift, 1989). If individuals are allowed recovery times which are insufficient for the total recovery of all groups, the bioluminescence of organisms which recover faster will be relatively overestimated in the total light budget, and the light budget as a whole will be underestimated. Thus, for light budgets to be accurate, recovery times must be of sufficient duration to allow complete recovery of all taxa involved.

Previous studies of copepod bioluminescence have allowed 4–6 h of recovery prior to testing (Swift et al., 1985b; Latz et al., 1987a; Batchelder & Swift, 1989). The present study indicates that these measurements underestimate maximum TSL by $\approx 50\%$ due to incomplete recovery of bioluminescence. One method to circumvent this problem has been to estimate maximum TMSL by using a most probable TSL value (MPV) from partially recovered organisms by discarding the dimmer half of the values (Latz et al., 1987b; Batchelder & Swift, 1988, 1989). This method (1) provides only an estimate of postrecovery TSL values, and (2) arbitrarily assumes that 50% of the measured variability is due to damage or incomplete recovery, thus probably underestimating the natural variability found in the population. In fact, the recovery data presented in this study are represented by a near-Gaussian distribution expected of a random sampling approach.

TABLE V

Comparison of most probable value (MPV) estimates of maximum total stimuable luminescence (TSL) to measured values of TSL measured after full recovery of bioluminescence. MPV is calculated by discarding dimmer half of measurements made at specified time during recovery period (refer to text). $\bar{x}$ values with SE of $\bar{x}$ are shown.

	Time (h)	Light emission (photons $\cdot$ ind $^{-1}$)	MPV estimate of max TSL (photons $\cdot$ ind $^{-1}$)	Measured max TSL (photons $\cdot$ ind $^{-1}$)
<i>P. xiphias</i>	6.3	$4.8 \pm 0.7 \times 10^{10}$	$6.4 \pm 1.0 \times 10^{10}$	$11.4 \pm 0.5 \times 10^{10}$
<i>G. princeps</i>	6.5	$2.5 \pm 0.9 \times 10^{11}$	$4.3 \pm 0.8 \times 10^{11}$	$6.5 \pm 1.3 \times 10^{11}$
<i>L. flavicornis</i>	5.0	$7.0 \pm 1.1 \times 10^9$	$10.6 \pm 1.2 \times 10^9$	$12.5 \pm 1.9 \times 10^9$

To illustrate the limits of this method, present recovery data for *P. xiphias* provide an MPV estimate of maximum TMSL measured 6.3 h after collection. The MPV of $6.4 \pm 1.0 \times 10^{10}$ photons $\cdot$ ind $^{-1}$ underestimates the maximum TMSL of $11.4 \pm 0.5 \times 10^{10}$ photons $\cdot$ ind $^{-1}$ by $\approx 40\%$ (Table V). The calculated MPV for *G. princeps* also underestimates the TSL at full recovery by 34%. For *L. flavicornis*, the calculated MPV effectively estimates the TMSL of 1.2×10^{10} photons $\cdot$ ind $^{-1}$. A 1-day recovery approach provides a more accurate measure of TSL for two of the three species, and conserves all measurements.

In contrast, it is possible that light budgets developed from laboratory measurements may be overestimates if the recovered state exceeds the in situ luminescent capacity of organisms, which must be dependent on their unknown natural rate of luminescent activity (Buskey & Swift, 1987). Since direct in situ measurements of TSL from identified organisms are not yet possible, it is difficult to assess the accuracy of laboratory TSL measurements and how well they represent actual levels of bioluminescence potential. Trauma associated with collection and handling may cause long-term physiological effects such that complete recovery of bioluminescence is not possible. In addition, maximal bioluminescence measured in the shipboard laboratory may represent an equilibrium between recovery and restimulation. Sources of restimulation may include ship vibration and motion, encounters with surfaces during organism swimming, as well as "spontaneous" emissions. In a reduced stress environment, levels of bioluminescence may be greater. Preliminary experiments with *G. princeps* indicate that specimens maintained in the shore laboratory on luciferin-free food have a higher TSL than on board ship (Bowlby & Case, unpubl. data).

There are no quantitative studies on the mechanical excitation of copepod bioluminescence. The present study suggests that the rate of maintained mechanical stimulation determines the rate of flashing and the response duration. As the rate of stimulation increased, the rate of flashing increased and the response duration decreased. This was probably caused by increased shear levels, turbulence, and contacts with the stirring rod and vial surfaces at the higher stimulation rates. In any metazoan bioluminescent system, rate of excitation may be expected to complexly affect emission by way of time dependent central and peripheral nervous system processes such as facilitation, adaptation, habituation, or fatigue.

This study suggests that rather than total bioluminescence (TSL), it is the rate of light emission and response duration that more accurately reflect the excitation field experienced by organisms in situ. Knowledge of the physical and biological factors stimulating light emission is needed before mechanisms and functions of bioluminescence can be understood, or before accurate estimates of bioluminescence potential of oceanic luminescent communities may be derived by the photon budget technique. While it remains clearly necessary to conduct laboratory studies on identified sources, it is equally clear that in situ measurements of spontaneous luminescence and luminescence caused by defined in situ physical stresses are fundamental to meaningful modeling efforts. Neither of these categories of in situ measurement have yet to be obtained for any significant fraction of the oceans.

ACKNOWLEDGEMENTS

We are grateful to the captains and crews of the *USNS Bartlett*, *RV Endeavor*, *RV New Horizon*, and *USNS Kane*, and to E. Widder, S. Bernstein, and D. Ondercin for technical assistance. Supported by grants from the Office of Naval Research (Contract numbers NOOO14-84-K-0314 and NOOO14-87-K-0044 to J. F. Case and NOOO14-89-J-1477 to M. I. Latz).

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