AD-A137 557 SIMULATION OF BIOLUMINESCENCE IN DINOFLAGELLATES BY CONTROLLED PRESSURE CHANGES(U) NAVAL RESEARCH LAB WASHINGTON DC T Q DONALDSON ET AL. 16 DEC 83 NRL-8772 1/1

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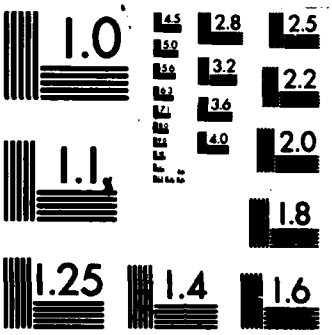

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NRL Report 8772

Stimulation of Bioluminescence in Dinoflagellates by Controlled Pressure Changes

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December 16, 1983

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NRL Report 8772	2. GOVT ACCESSION NO. A137557	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) STIMULATION OF BIOLUMINESCENCE IN DINOFLAGELLATES BY CONTROLLED PRESSURE CHANGES		5. TYPE OF REPORT & PERIOD COVERED Interim report on a continuing NRL problem. 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Thomas Q. Donaldson,*† Stevens P. Tucker,* and Richard V. Lynch III		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Research Laboratory Washington, DC 20375		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 43-1118-0-3
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Research Laboratory Washington, DC 20375		12. REPORT DATE December 16, 1983 13. NUMBER OF PAGES 7
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

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†Present address: COMNAVOCEANCEN, NAV STA Marianas

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Bioluminescence
Dinoflagellates
Gonyaulax
Pyrocystis
Stimulation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Bioluminescence in two species of dinoflagellates, *Pyrocystis lunula* and *Gonyaulax polyedra*, was stimulated when small pressure changes occurred. At an initial pressure of 1 atm, a pressure decrease rate of 0.25 ± 0.04 atm s^{-1} was the mean decrease threshold for *P. lunula*. Small variations in this threshold occurred at a higher initial pressure and at different times during the circadian cycle. Pressure decreases were much more effective than pressure increases. For *G. polyedra*, a pressure change rate of at least 0.95 atm s^{-1} was required to stimulate bioluminescence. Pressure increases and decreases were both effective, but decreases were more effective than increases. The variation in results did not lend itself to statistical analysis.

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STIMULATION OF BIOLUMINESCENCE IN DINOFLAGELLATES BY CONTROLLED PRESSURE CHANGES

INTRODUCTION

It has long been known that bioluminescence in dinoflagellates can be stimulated mechanically; however, the exact nature of the mechanism remains unknown. Studies have been hindered by the difficulty in quantitatively varying the mechanical stimulus. However, quantifiable stimulation has been achieved by the generation of a shock wave using a ceramic piezoelectric crystal [1,2] and by flow through a capillary tube [3]. In the first case, the stimulus may be deformation of the cell membrane; in the second, a shear gradient across the cell.

Another means of generating a quantifiable mechanical stimulus is through the use of controlled pressure changes. Relatively few investigations on the effects of pressure and pressure changes on dinoflagellate bioluminescence have been performed. Gooch and Vidaver [4] found that very high pressures (300 to 600 atm) increased the intensity of bioluminescence glow, altered the kinetics of this glow, and increased the frequency of spontaneous flashing in *Gonyaulax polyedra*. Swift et al. [5] found that pressures from 10 to 100 atm did not affect the total mechanically stimulable bioluminescence of *Pyrocystis lunula*, but that pressures greater than 25 atm measurably affected the spontaneous flashing rate. Krasnow et al. [6] found that sinusoidal pressure changes up to 1 atm did not affect the bioluminescence of *G. polyedra*.

MATERIALS AND METHODS

Cultures of *P. lunula* and *G. polyedra* were grown in *f*/2 medium [7] made with seawater from Monterey Bay. Prior to use, this medium was filtered through a 100 μm filter, autoclaved at 120°C for 20 min, and cooled overnight under sterile conditions. The cultures were grown at 21° ± 1°C, in 1-liter Ehrlenmeyer flasks filled to a depth of about 7 cm. A 12 h/12 h light/dark cycle was maintained by three 15-W cool white fluorescent lamps (General Electric F15T8CW) which were placed in parallel about 25 cm above the surface of the culture. The beginning of photophase was taken as circadian time (CT) 0000.

Only active, exponentially growing cultures were used in the experiments. 1 ± 0.006 ml of culture was pipetted during photophase into a test tube, which was then put back into the culture chamber. All test tubes were used within three days of preparation. For each experiment, a test tube was placed in a pressure test cell capable of holding 14 atm internal pressure. Transfer was performed during scotophase with minimum handling, and the light emission was monitored to detect any premature stimulation during transfer. Once in place, the samples were continuously monitored for spontaneous flashing prior to the experimental run. Cell concentrations were approximately 11×10^3 cells cm^{-3} for *P. lunula* and 2×10^3 cells cm^{-3} for *G. polyedra*. These concentrations were determined by counting the cells in an aliquot of culture medium (taken after gentle stirring) using a Fuchs-Rosenthal Ultra-Plane corpuscle counting chamber under 100X magnification.

Light emission was detected by using a 20-mm diameter, 10-stage, end window, EMI type 9524B photomultiplier tube (PMT) with a cathode sensitivity of 90 μA m^{-1} and a constantly applied high voltage of 1000 V for *P. lunula* and 1200 V for *G. polyedra*. Light from the pressure chamber reached the

Manuscript approved October 21, 1983.

PMT through a 2.5-cm-thick plexiglass window. The output of the PMT was amplified by a Keithley Model 417 high-speed picoammeter with a 3-V full-scale output and set for a full-scale input of 1×10^{-5} A for *P. lunula* and 3.3×10^{-6} A for *G. polyedra*. Signals were monitored and recorded using a Tektronix Type R564B oscilloscope which could store a signal for up to 1 h. The pressure signals were monitored on the screen simultaneously with the light signals.

Test cell pressure was measured with a 17-atm Statham Model PG146Tq strain gage pressure transducer. Differential pressure was measured with a 2-atm Servonic Model L-64 potentiometer pressure transducer. Both were excited by PMC Model BPA200 regulated voltage sources. Commercial-grade dry argon was used as the pressurizing gas.

Pressure change was induced in the following manner. Each sample was subjected to a slight pressure increase, which was then released. All pressure changes took place over 1 s; thus a cycle with a period of approximately 2 s was established (Fig. 1). After each oscilloscope sweep, which consisted of an average of three cycles, the pressure changes were increased in increments of approximately 0.02 atm for *P. lunula* and 0.03 atm for *G. polyedra* until stimulation occurred.

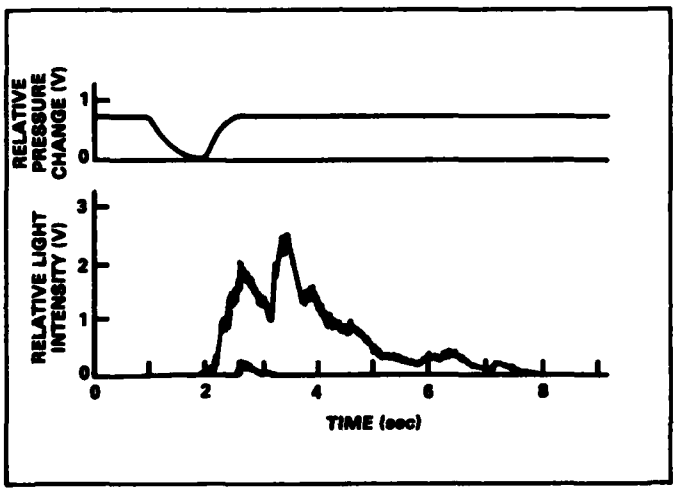

Fig. 1 — Responses of *P. lunula* to pressure changes. The lower trace shows the response to the pressure decrease of 0.61 atm shown on the upper trace. Superimposed on the lower trace is the much smaller response of the same organisms to a pressure increase of equal magnitude (not shown). Any response at all to a pressure increase was uncommon, occurring in only 2% of the experiments.

RESULTS

Figure 1 shows a typical bioluminescent response to a pressure decrease. Figure 2 summarizes the results of 739 experiments performed on *P. lunula* at an initial pressure of 1 atm. A pressure decrease was found to be far more effective in stimulating bioluminescence than a pressure increase. In fact, 98% of the responses were due to decreases. Consequently, responses due to pressure increases were omitted from subsequent analyses. Eighty-one percent of the initial responses occurred at a pressure decrease rate between 0.22 atm s^{-1} and 0.28 atm s^{-1}. The mean decrease threshold was 0.25 ± 0.04 atm s^{-1}.

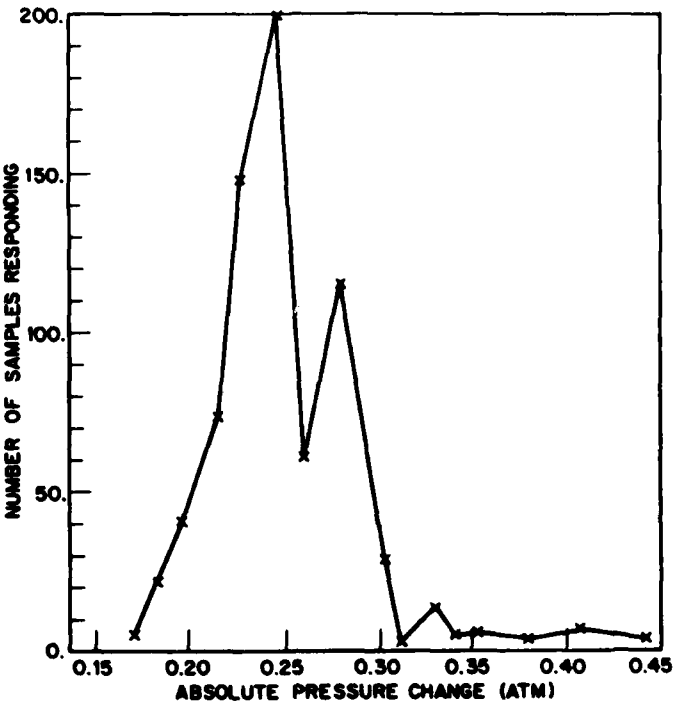

Fig. 2 — Distribution of sample responses of *P. lunula* to pressure decreases of the indicated magnitude at an initial pressure of 1 atm

Figure 3 summarizes the results of 156 experiments performed on *P. lunula* at an initial pressure of 2 atm. At this pressure, slightly greater pressure decrease rates were required to induce bioluminescence. Seventy-nine percent of the initial responses occurred at a pressure decrease rate between 0.25 and 0.30 atm s^{-1}. The mean decrease threshold was 0.28 ± 0.03 atm s^{-1}.

To test the effect of circadian rhythm on sensitivity to stimulation, 100 experiments using *P. lunula* at an initial pressure of 1 atm were performed at each of three times: CT 1430, CT 1830, and CT 2230. Although results were consistent with those presented in Fig. 1, the decrease threshold was 0.02 atm s^{-1} less at CT 1830 than at the other two times.

In contrast to *P. lunula*, *G. polyedra* responded to both positive and negative pressure changes. However, negative pressure changes were more effective. Moreover, much greater change rates (between 0.95 atm s^{-1} and 2.38 atm s^{-1}) were required to stimulate bioluminescence at an initial pressure of 1 atm. Because of the distribution of the data, no statistical threshold for stimulating bioluminescence could be established. Responses to both negative and positive pressure changes of 121 experiments are plotted in Fig. 4.

DISCUSSION

Because all experiments were performed with a constant duration of 1 s for the pressure changes, it is not possible from these data to conclude whether the magnitude or the rate of pressure change is stimulating bioluminescence. However, preliminary data at a shorter duration suggest that a smaller magnitude of change stimulates bioluminescence. Insufficient experiments were performed to confirm this point. This fact in turn suggests that the stimulation is rate-dependent; this is recorded in this report.

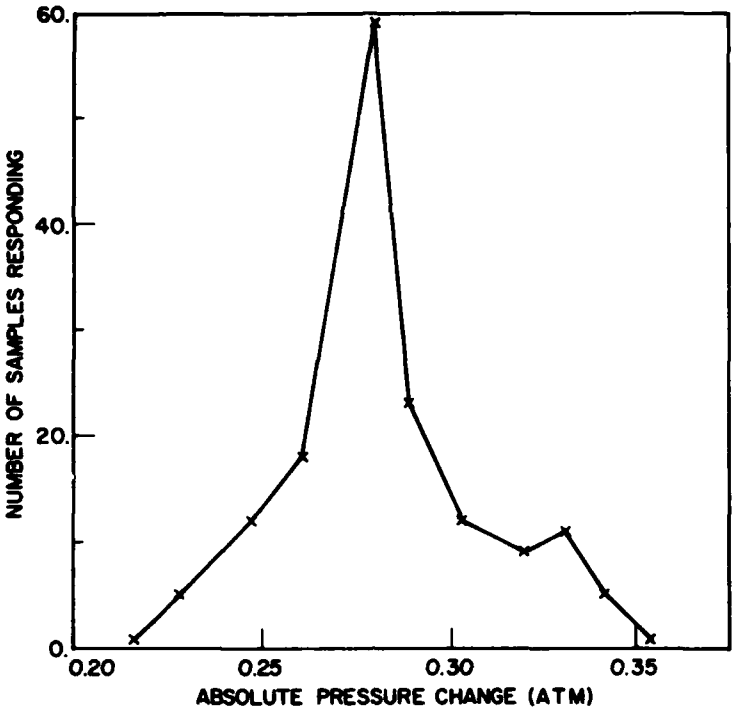

Fig. 3 — Distribution of sample responses of *P. lunula* to pressure decreases of the indicated magnitude at an initial pressure of 2 atm

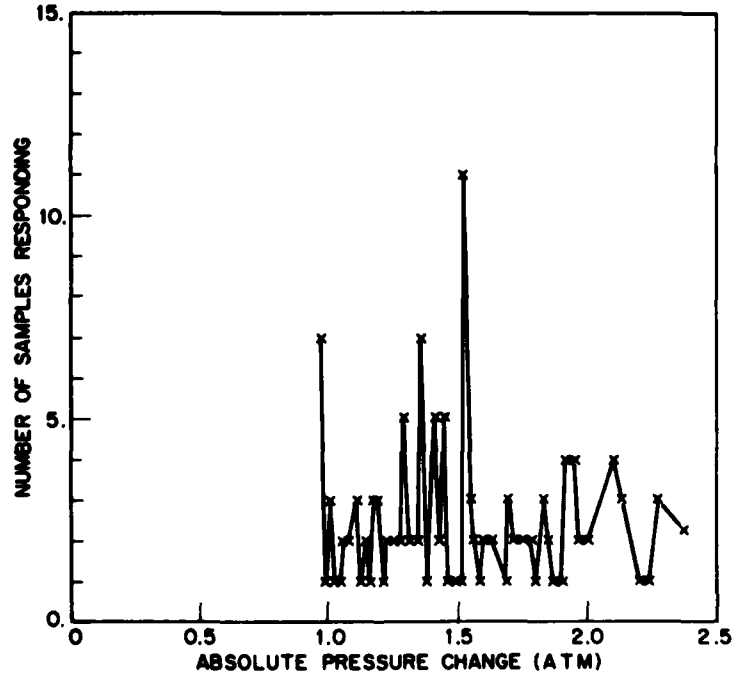

Fig. 4 — Distribution of sample responses of *G. polyedra* to pressure decreases of the indicated magnitude at an initial pressure of 1 atm

An interesting question is raised by the shape of the curves in Figs. 2 and 3. These curves (especially Fig. 2) appear to be bimodal. At this time it is not known if this effect is real and, if so, what its cause may be. Also, it is not known if the decrease in the amplitude of the second peak is related to the increase in the initial pressure.

Another interesting question is raised by the differences in the responses between *P. lunula* and *G. polyedra*. *P. lunula* is a large lunate, nonmotile (in its lunate cyst phase), unarmored dinoflagellate whereas *G. polyedra* is small, spherical, motile, and armored. Are these physical differences sufficient to account for the different responses to pressure changes? This question needs to be investigated by repeating these experiments using other species of dinoflagellates.

The suggestion has been made that bioluminescence stimulated by pressure decreases is due to the formation and movement of decompression gas bubbles. The pressure magnitudes and rates of change used in these experiments, especially with *P. lunula*, make such a mechanism unlikely.

Although individual experiments showed a greater variation in sensitivity to stimulation than is seen in aggregate at the different initial pressures and circadian times, those small differences nevertheless seem real.

ACKNOWLEDGMENTS

The authors thank Dr. B. M. Sweeney of the University of California at Santa Barbara for supplying cultures of *G. polyedra* and *P. lunula* and Dr. Elijah Swift of the University of Rhode Island for supplying cultures of *P. lunula*.

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