

# Bioluminescent response of the dinoflagellate *Lingulodinium polyedrum* to developing flow: Tuning of sensitivity and the role of desensitization in controlling a defensive behavior of a planktonic cell

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## Abstract

Dinoflagellate bioluminescence is believed to serve a defensive function, decreasing grazing at night. Previous characterization of bioluminescence stimulated by fully developed flows might have underestimated the true sensitivity of bioluminescence by not observing the initial response. Also, it has been suggested that bioluminescence may be more sensitive to time-varying flow than to constant flow conditions. We used developing laminar Couette flow to characterize the sensitivity of the initial bioluminescent response of the dinoflagellate *Lingulodinium polyedrum* in time-varying flow. Both the absolute sensitivity (threshold) and dynamic sensitivity were consistent with that determined previously in fully developed flows, although there were differences between different cultured isolates of the same species and between those isolates and cells harvested from a unialgal bloom of the same species. When the rate of increase of shear was varied while keeping the maximum shear level similar, the threshold was independent of the rate of increase of shear. Surprisingly, the integrated bioluminescence was strongly dependent on the rate of increase of shear. The mechanism behind the preferential response to rapidly increasing shear was determined to be desensitization. Desensitization may influence which naturally occurring flows strongly stimulate bioluminescence either by allowing cells to avoid producing a primary response in certain slowly changing flows or, more generally, to avoid the cost of repeated stimulation when entrained in environmental flows containing above-threshold shears.

Many planktonic organisms perform defensive behaviors. The well-known escape jump of copepods can be triggered by flow conditions with strain rates  $>0.2 \text{ s}^{-1}$ , where shear strain and extensional strain are equivalently stimulatory (Kjørboe et al. 1999). Flow-stimulated escape jumping is also seen in members of several groups of planktonic protists (Jakobsen 2001, 2002). In ciliates the threshold strain rate is  $2 \text{ s}^{-1}$ , while in the flagellates, including one dinoflagellate that can be stimulated to jump, the threshold is  $9\text{--}12 \text{ s}^{-1}$ . Jumping is clearly triggered by the flow conditions found within feeding currents and functions to allow individual cells to escape being eaten.

Bioluminescence is another defensive behavior present in many planktonic organisms. Bright luminescent displays may serve to distract an attacking animal or may serve as a

“burglar alarm,” exposing the animal to predation by visual predators and creating a selective pressure for the avoidance of luminescent food (Morin 1983; Young 1983). Dinoflagellates are among the most common sources of bioluminescence in coastal waters (Morin 1983). Dinoflagellate bioluminescence is often seen as light associated with swimming organisms or in breaking waves (Rohr et al. 1998). In individual cells it is expressed as a bright flash ( $10^7$  to  $10^{10}$  photons  $\text{s}^{-1}$  cell $^{-1}$ , depending on species) (Biggley et al. 1969; Latz and Lee 1995) that is triggered within 20 ms of mechanical stimulation and lasts on the order of 100 ms (Eckert 1965; Widder and Case 1981). Dinoflagellate bioluminescence reduces grazing (Esaias and Curl 1972; White 1979) by affecting the behavior of animals that feed on dinoflagellates (Buskey et al. 1983; Buskey and Swift 1983). Bioluminescence represents the most visible defensive behavior of a planktonic protist.

Dinoflagellate bioluminescence can be stimulated by direct contact with the cell surface (Eckert 1965; Widder and Case 1981) or by flow (Anderson et al. 1988). Previous studies found that the minimum shear strain rate (henceforth “shear”) required to stimulate bioluminescence in steady laminar flows varies between 20 and  $200 \text{ s}^{-1}$ , depending on the species tested (Latz et al. 1994, 2004). Sufficient shear levels are found around swimming animals (Latz et al. 1994; Rohr et al. 1998). In the flows generated by grazing cope-

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Pods, strain rates of this magnitude are not found in the far-field flow upstream of the copepod (van Duren et al. 2003), so it is unclear how flow-stimulated bioluminescence could function ecologically in dinoflagellates.

The studies of shear-stimulated bioluminescence in steady laminar flows could potentially underestimate the actual sensitivity of dinoflagellate bioluminescence. First, the time scale for mechanically stimulated dinoflagellate bioluminescence is much more rapid than that for flows to develop. Therefore, it is possible that the response observed in steady laminar flows is not the primary response and that the sensitivity of the primary response might be quite different. Second, the qualitative results of Anderson et al. (1988) suggest that dinoflagellates might be more sensitive to rapidly changing flows than to steady flows. This study addresses the following question: What is the sensitivity of dinoflagellate bioluminescence to developing flows?

## Methods

*Dinoflagellates*—*Lingulodinium polyedrum* isolate LP1-04 (CCMP1932) was originally isolated from Scripps Pier in La Jolla, California, in May 1998. The recent isolate 104 was selected because bioluminescent properties may change with the length of time an isolate has been in culture (Sweeney 1986). Isolate HJ has been in culture much longer and is of unknown origin but was used for comparison since this isolate has been used in previous studies of flow-stimulated bioluminescence (Rohr et al. 1998; Latz and Rohr 1999). The bioluminescent capacity of isolate 104 was typically  $5 \times 10^8$  photons cell<sup>-1</sup>, while that of isolate HJ was  $1 \times 10^8$  photons cell<sup>-1</sup> (von Dassow unpubl. data). Similar values were found in a previous comparison of a younger isolate versus an older isolate of this species (Sweeney 1986). Monocultures were grown in f/4 seawater medium minus silicate at  $20^\circ\text{C} \pm 0.5^\circ\text{C}$  in an environmental chamber on a 12:12 h light:dark cycle. Cell abundance and condition were monitored by counting samples under a dissecting microscope. Experiments were performed using cultures in the exponential phase of growth (1,000–6,000 cells ml<sup>-1</sup>).

Dense unialgal blooms of *L. polyedrum*, reaching concentrations  $>5,000$  ml<sup>-1</sup> in surface waters, occurred in the coastal waters of southern California in June–July 2001 and September 2003. Surface samples were collected during mid-afternoon by bucket from the Scripps Institution of Oceanography pier on 17 July 2001 and 4 September 2003. Samples were passed through 60- $\mu\text{m}$  nitex mesh to remove larger organisms, and then *L. polyedrum* cells were collected on 20- or 40- $\mu\text{m}$  nitex mesh and rinsed gently with glass fiber (GF/F) filtered seawater.

*Bioluminescence measurements and imaging*—Bioluminescence measurements were made within spherical integrating light collecting chambers as described previously (von Dassow and Latz 2002). Light emission was measured using a photon-counting photomultiplier tube (PMT; RCA 8575v2). A pulse from the servomotor controller (see section *Couette flow*) triggered the start of a 100-s record. A neutral density filter (1.0–2.0) and an aperture (3.1 to 1.25 cm) attenuated the amount of light illuminating the photocathode

to prevent saturation of the detector. Records with signals outside of the linear range of the detector were discarded. The detector was cross calibrated for quantum emission against a Quantalum 2000 calibrated photometer (Zefaco) using a C<sup>14</sup> phosphor. Luminescence was measured from the middle of the Couette chambers, away from influence of the ends, which were covered. Measured parameters from each record were obtained using custom data analysis software and included the integrated bioluminescence, maximum luminescent intensity, and time to reach half integrated bioluminescence (median response time) recorded in user-specified sections of the record.

Imaging was performed with a low-light GenIISYS intensified CCD video camera (Dage MTI) equipped with a 25-mm lens (Fujinon) set at f/1.4 and a +2 diopter lens (Fujinon). Images were collected at video rate (30 Hz) on digital magnetic tape.

*Couette flow*—In simple Couette flow, fluid contained in the gap between two concentric cylinders is sheared by rotating the outer cylinder while keeping the inner cylinder still. Nonswimming cells or cells that swim weakly can be gently kept in suspension by rotating the outer cylinder prior to testing at a rate slow enough that the shear in the gap is well below the threshold for stimulation of bioluminescence. Small volume Couette flow chambers were constructed with a polished cast acrylic outer cylinder (inner radius 12.700 mm) and a white delrin inner cylinder (length 100.8 mm, outer radius 12.070 mm), yielding a gap width of 0.635 mm. The ratio of the gap width to outer cylinder radius of 0.05 ensured a stable flow with a narrow range of shear across the gap when flow became fully developed (Taylor 1936; van Duuren 1968). Outer cylinder rotation was driven by a computer-controlled SilverMax servomotor (QuickSilver Controls).

The time-dependent shear was calculated as follows: during the phase of the flow when the outer cylinder is accelerating, the flow field was modeled by solving the partial differential equation:

$$\frac{\partial u}{\partial t} = \nu \frac{\partial^2 u}{\partial y^2} \quad (1)$$

with the boundary conditions  $u(0, t) = 0$  and  $u(L, t) = At$ , where  $u$  is the flow velocity at position  $y$  in the gap (from the stationary wall) at time  $t$ ,  $L$  is the gap width ( $6.35 \times 10^{-4}$  m),  $A$  is the tangential acceleration of the outer cylinder wall, and  $\nu$  is the kinematic viscosity ( $1.047 \times 10^{-6}$  m<sup>2</sup> s<sup>-1</sup> at 20°C). The inner and outer cylinder walls were modeled as infinite parallel plates. The outer wall accelerated at a constant rate, and the inner wall held still. The solution to this partial differential equation is

$$u(y, t) = \frac{Aty}{L} + \sum_{n=1}^{\infty} \frac{2AL^2}{n^3\pi^3\nu} (-1)^n \sin\left(\frac{n\pi y}{L}\right) \times \left[ 1 - \exp\left(-\frac{n^2\pi^2\nu}{L^2}t\right) \right] \quad (2)$$

(details of this solution are provided in Web Appendix 1 at

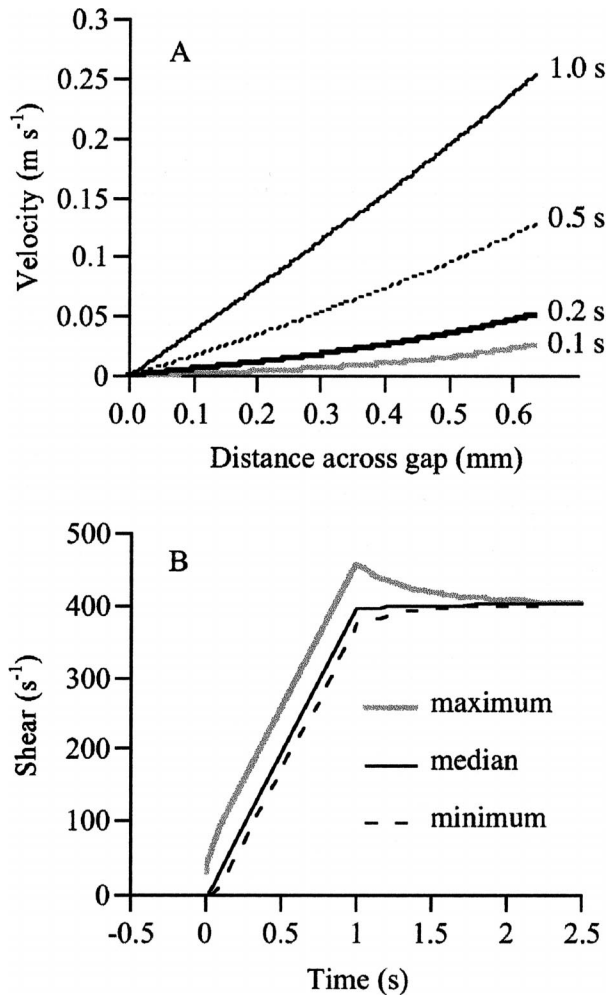


Fig. 1. Developing Couette flow. (A) Results from solutions for the velocity field across the gap at different times during acceleration of the outer cylinder at  $3.2 \text{ rps s}^{-1}$  over 1 s. Zero distance is at the wall of the inner cylinder (held stationary). (B) Minimum, median, and maximum shear in the gap over time during the acceleration of the outer cylinder at  $3.2 \text{ rps s}^{-1}$  over 1 s to a final rotation rate of  $3.2 \text{ rps}$  (A). After the acceleration phase, the minimum and maximum shear are assumed to approach the mean steady shear.

[http://www.aslo.org/lo/toc/vol\\_50/issue\\_2/0607a1.pdf](http://www.aslo.org/lo/toc/vol_50/issue_2/0607a1.pdf). Estimated shear in the gap during flow development was

$$\begin{aligned}
 S(y, t) &= \frac{\partial u(y, t)}{\partial y} \\
 &= \frac{At}{L} + \sum_{n=1}^{\infty} \frac{2AL}{n^2 \pi^2 \nu} (-1)^n \cos\left(\frac{n\pi y}{L}\right) \\
 &\quad \times \left[ 1 - \exp\left(-\frac{n^2 \pi^2 \nu}{L^2} t\right) \right] \quad (3)
 \end{aligned}$$

Once the outer cylinder reached the final rotation rate, maximum and minimum shear levels were assumed to exponentially approach the steady state mean shear level at that rotation rate (calculated as  $U_F/[6.35 \times 10^{-4}] \text{ m}$  at the end of

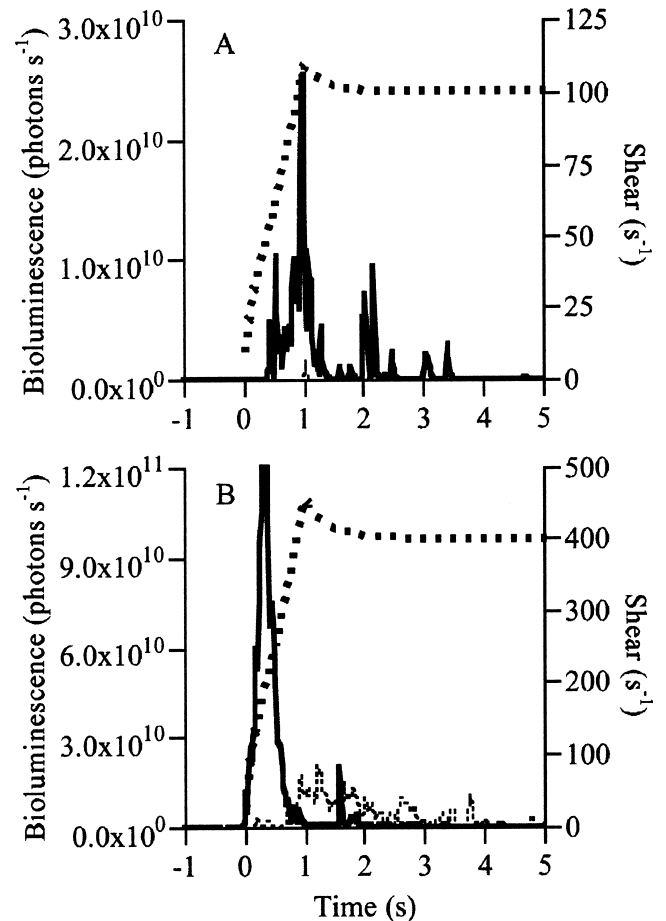


Fig. 2. The luminescent response of a recently isolated isolate (104) and an older isolate (HJ) of *L. polyedrum* to developing Couette flow. Thick black lines indicate the response of isolate 104, thin dashed lines indicate the response of isolate HJ, and thick dashed lines indicate the maximum shear in the gap with time (see *Methods*). (A) Acceleration of the outer cylinder over 1 s at  $0.8 \text{ rps s}^{-1}$  to  $0.8 \text{ rps}$ , a final shear of  $100 \text{ s}^{-1}$ . The response of isolate 104 was seen in all tests, but the weak single flash response of isolate HJ was not consistently observed. (B) Acceleration of the outer cylinder over 1 s at  $3.2 \text{ rps s}^{-1}$  to  $3.2 \text{ rps}$ , a final shear of  $402 \text{ s}^{-1}$ .

start-up, where  $U_F$  is the final tangential velocity of the outer cylinder) with time constant  $\tau = L^2 \nu^{-1}$ . Calculated velocity and shear profiles at different times are shown (Fig. 1).

The bulk Reynolds number in the flow, calculated as  $Re = LU_F \nu^{-1}$ , under the range of rotation rates tested (0.2 to 6.4 rotations per second [rps]) varied between 10 and 320. Under these conditions flow was expected to be nonturbulent (van Duuren 1968). Visualization with Kallirosopic fluid confirmed the flow was laminar.

The flow profiles involved a programmed 8-s delay between the start of the PMT record and the servomotor. The outer cylinder was first accelerated to a velocity of 0.05 rps (equivalent to a shear rate of  $6.1 \text{ s}^{-1}$ ) over 1 s and the velocity was held constant for 49 s (time = -50 to 0 s). This warm-up rotation period served to suspend any nonswimming cells and to lubricate the chamber bearings with seawater to promote smooth acceleration. The steady shear of

6.1 s<sup>-1</sup> during this period was well below the response threshold for this species (Latz et al. 1994; Latz and Rohr 1999) and little or no luminescence was stimulated then. At  $t = 0$  s, the outer cylinder was accelerated over 1–16 s to a rotation rate between 0.2–6.4 rps (shear rate = 25–804 s<sup>-1</sup>) that was maintained for the remainder of the 20-s period beginning with the start of acceleration. To test for desensitization, the outer cylinder was then slowed to 0.05 rps for 1 s and then accelerated over 1 s to a final rotation rate of 3.2 rps (shear rate = 402 s<sup>-1</sup>) maintained for 19 s more.

**Loading of Couette chambers**—On each day of testing, a small volume of culture (50–100 ml) was removed from the culture vessel during the final hour of the light phase, and cell abundance and condition were determined. Couette flow chambers were loaded first with 3.5 ml of seawater (buffered with 5 mmol L<sup>-1</sup> *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) pH 7.8 and 0.2- $\mu$ m filtered). Then, the volume of cell culture calculated to contain 500 cells was added. The inner and outer cylinders were slowly counterrotated by hand to distribute the cells before filling the remainder of the chamber completely with seawater and closing the chamber (ensuring no bubbles were trapped). For video recordings, the ends were left uncovered in order to verify that luminescence was not stimulated only at the Couette ends. Loading was always completed in the light between the last 15 min of the light phase and the first 45 min of the dark phase, when bioluminescence is minimally stimuable (Biggley et al. 1969) and loaded Couette chambers were stored horizontally in the dark before testing. At the time of testing (3 to 6 h into the dark phase), chambers were inserted into the integrating sphere and coupled to the servomotor and then allowed to rest 15 min prior to testing to allow for recovery from any mechanical stimulation during handling. Samples in which bioluminescence was stimulated during handling were discarded.

**Statistical analysis**—All statistical analysis was performed using Statview (SAS Institute). Statistical tests included least-squares linear regression and analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) post hoc test for pairwise comparisons. Statistical significance was determined based on a  $p$  value of 0.05.

## Results

**Response to developing Couette flow: Initial characterization**—Two isolates of *L. polyedrum* were compared. Isolate 104 always responded at lower shear levels than isolate HJ, and the response of isolate 104 was more rapid, much of it occurring during the developing phase of the flow (Fig. 2). In several tests at a range of outer cylinder velocities, there was no response to either final rotation rate when the inner cylinder was allowed to rotate with the outer cylinder, generating a flow field with acceleration but no shear (data not shown). An initial characterization of the sensitivity of these two isolates was done by maintaining a constant length of time of the acceleration phase at 1 s while varying the final shear between 25 and 862 s<sup>-1</sup> (Fig. 3). The threshold for

isolate 104 was a final rotation rate of 0.4 rps (shear of 50 s<sup>-1</sup>). At this rotation rate, a response of several flashes was consistently observed. The threshold for isolate HJ was 0.8–1.6 rps (shear of 100–201 s<sup>-1</sup>). A weak response, consisting of a single flash, was sometimes observed at 0.8 rps. In the case of isolate 104, most of the bioluminescence occurred during the accelerating phase of the flow at all flow rates above a final rotation rate of 0.8 rps. In contrast, even at flow rates well above threshold for isolate HJ, this isolate responded mostly after the accelerating phase of the flow (Fig. 3B). To compare the results in developing flow with previous studies in fully developed flows, integrated bioluminescence (population response) was expressed as a function of the instantaneous maximum shear at the median response time (the time at which half of the integrated response had occurred). Above 0.4 rps the integrated bioluminescence of isolate 104 varied with the 1.8 power of shear, while above 0.8 rps the integrated bioluminescence of isolate HJ varied with the 2.0 power of shear (Fig. 3C).

**The spatial distribution of stimulated cells flashing in the Couette chamber**—In the initial characterization, it was assumed that cells were homogeneously distributed within the Couette gap. To check the validity of this assumption, the bioluminescent response to a shear stimulus (accelerated at 3.2 rps s<sup>-1</sup> to 3.2 rps, a final shear of 402 s<sup>-1</sup>) was visualized. Flashes were distributed throughout the length of the Couette chamber. The velocity distribution of flashes should reflect the radial distribution of stimulated cells, with cells stimulated near the inner cylinder wall traveling very slowly and cells stimulated at the outer cylinder wall traveling fast. Flashes at different velocities were observed, with slowly moving flashes being passed by faster moving flashes in consecutive frames (Web Appendix 2 at <http://www.aslo.org/lo/toc/vol.50/issue.2/0607a2.pdf>). Our initial interest had been to obtain the radial distribution of stimulated cells in the Couette gap in order to view the onset of stimulation as the front of above-threshold shear propagates across the gap. The errors in measurement at video-rate time resolution (33 ms) prevented this from being done with confidence. However, the range of velocities was consistent with cells being stimulated at different radial positions within the Couette gap and not only at the cylinder walls.

**Comparison between response of cultured and environmental *L. polyedrum* cells**—The pronounced differences in the response kinetics and the absolute sensitivity of the two isolates of *L. polyedrum* might be due to length of time in culture. *L. polyedrum* cells from two naturally occurring blooms of this species were concentrated and tested for responses to a low (accelerated over 1 s to a final shear of 100 s<sup>-1</sup>) and a high (accelerated over 1 s to a final shear of 402 s<sup>-1</sup>) stimulus as done for isolates 104 and HJ (Fig. 4). Similar to isolate 104 but not to isolate HJ, bloom *L. polyedrum* cells responded consistently well to both stimuli.

Two parameters, the time at which the first flash was stimulated and the proportion of the response that occurred during the 1-s accelerating phase of the flow, were chosen for a quantitative comparison of the responses of the two different isolates of *L. polyedrum* and the bloom *L. polyedrum*

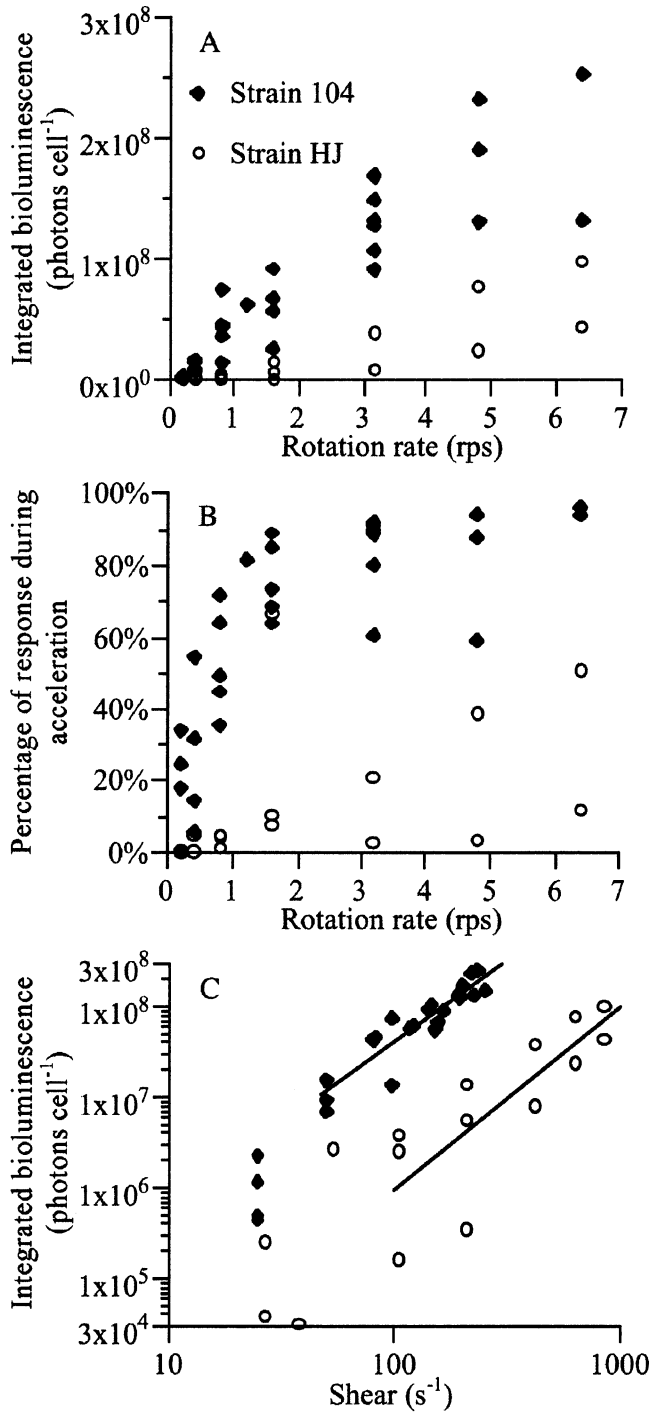


Fig. 3. Characterization of the sensitivities of *L. polyedrum* isolate 104 (closed diamonds) and isolate HJ (open circles) to Couette flow. The Couette outer cylinder was always accelerated linearly over 1 s to the final rotation rate, which was varied from 0.2 to 6.4 rps. Each symbol represents the value from a single test. (A) Integrated bioluminescent response normalized per cell versus final rotation rate. (B) The percentage of the total luminescent response to the flow protocol that occurs during the acceleration phase versus final rotation rate. (C) The integrated bioluminescent response per cell versus the estimated shear in the gap at the median response time. Note the log–log scale. The lines represent least-square power

(Table 1). The kinetic parameters of the responses to both low and high shear stimuli were similar between samples from both blooms, and so these results were pooled for statistical analysis. The time at which the first flash was stimulated was much greater for isolate HJ than for isolate 104 or for bloom *L. polyedrum* cells in response to both the low and high shear stimuli, while there was no significant difference between isolate 104 and bloom *L. polyedrum*. In response to both low and high shear stimuli, the proportion of the response that occurred during the 1-s accelerating phase of the flow was always greater in the bloom *L. polyedrum* than in isolate HJ but less than in isolate 104, but the difference between bloom cells and isolate HJ was significant only at the higher stimulus.

*The effect of rate of change of shear*—Since most of the response of isolate 104 occurred during the accelerating phase of the flow at all flows tested, the effect of rate of change of shear on the bioluminescent response was determined. Generally, rapidly increasing shear stimulated more bioluminescence (Fig. 5). The maximum shear at the time at which the first flash was stimulated was independent of the rate of change of shear and was consistent with a threshold of  $65.4 \pm 29.5 \text{ s}^{-1}$  ( $n = 29$ ) (Fig. 6A). This was similar to the threshold of approximately  $50 \text{ s}^{-1}$  when the final shear stress varied and the acceleration time was held constant (see *Response to developing Couette flow: Initial characterization*). This indicates that the threshold shear required to stimulate bioluminescence does not vary with the rate of change of shear.

At all rates of change of shear tested, the proportion of bioluminescence stimulated during the accelerating phase of the flow remained high, at  $80.1 \pm 15.0\%$  (Fig. 6B). Bioluminescence integrated over either the accelerating phase (Fig. 6C) or the full 20-s test phase (not shown) increased with the rate of change of shear. Bioluminescence stimulated over the accelerating phase was  $(8.1 \pm 3.0) \times 10^7$  photons cell<sup>-1</sup> when shear was increased at  $402 \text{ s}^{-2}$  but only  $(1.3 \pm 0.7) \times 10^7$  photons cell<sup>-1</sup> when shear was increased at  $25 \text{ s}^{-2}$  even though in the latter case cells were exposed to above-threshold shears for >14 times as long during the accelerating phase over which bioluminescence was integrated. The levels of bioluminescence stimulated by 25 and  $50 \text{ s}^{-2}$  were significantly different from the levels stimulated by 200 and  $402 \text{ s}^{-2}$  (Fisher's PLSD post hoc test:  $p < 0.01$  in each pairwise comparison).

*Desensitization in response to slowly increasing shear flow*—Two alternative hypotheses are proposed to explain the dependence of the integrated bioluminescence from the population of cells on the rate at which shear was increased. First, the rapidly changing flow could be more stimulatory. As the shear is increased more rapidly, the gradient in ve-

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law regressions fit to the data points from rotation rates at which a response was consistently observed (and so considered to be above the threshold for stimulation of bioluminescence). For isolate 104,  $y = 1.1 \times 10^4 S^{1.8}$ ;  $r^2 = 0.85$ . For isolate HJ,  $y = 81 S^{2.0}$ ;  $r^2 = 0.65$ .

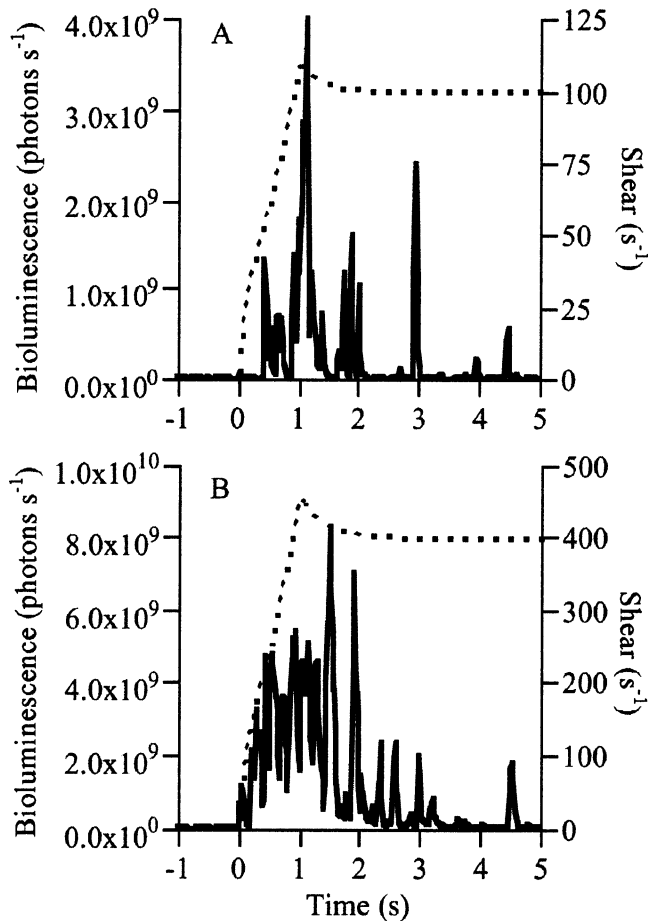


Fig. 4. Luminescent response of *L. polyedrum* cells harvested from a natural bloom on 17 July 2001 and tested as in Fig. 3. (A) Acceleration of the outer cylinder over 1 s at 0.8 rps  $s^{-1}$  to 3.2 rps, a final shear of 100  $s^{-1}$ . (B) Acceleration of the outer cylinder over 1 s at 3.2 rps  $s^{-1}$  to 3.2 rps, a final shear of 402  $s^{-1}$ . The solid line indicates the bioluminescent response while the dashed line indicates the maximum shear in the gap (plotted on the right axis).

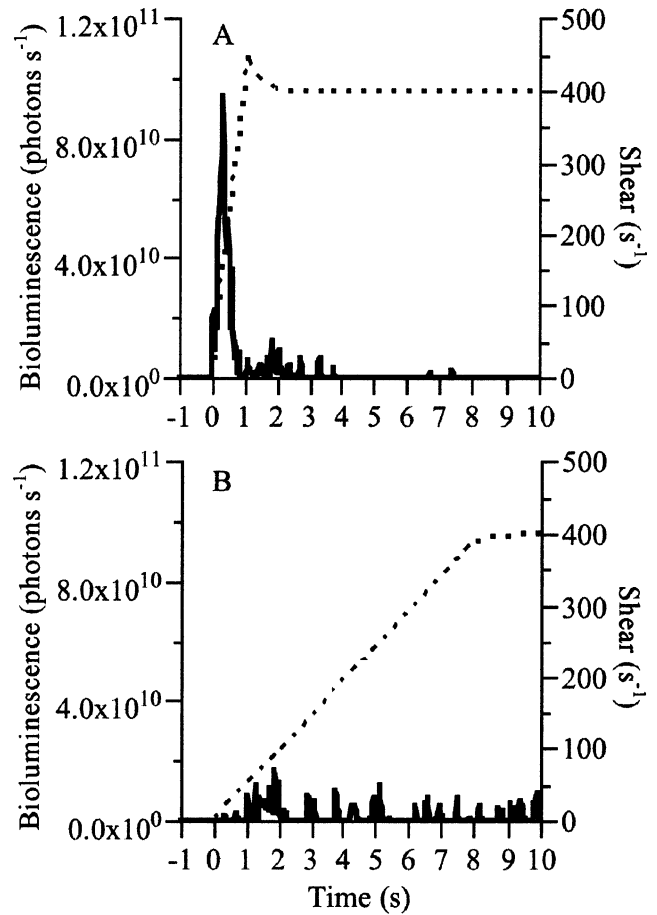


Fig. 5. The luminescent responses of *L. polyedrum* isolate 104 to varying rates of increase of shear keeping the final shear constant. (A) Acceleration of the outer cylinder over 1 s at 3.2 rps  $s^{-1}$  to 3.2 rps, a final shear of 402  $s^{-1}$ . (B) Acceleration of the outer cylinder over 8 s at 0.4 rps  $s^{-1}$  to 3.2 rps, a final shear of 402  $s^{-1}$ . The solid line indicates the bioluminescent response, while the dashed line indicates the maximum shear in the gap (plotted on the right axis).

Table 1. Kinetic parameters of the response of *Lingulodinium polyedrum* cells from cultured isolates and from *L. polyedrum* cells harvested from blooms of *L. polyedrum*. Time of first flash takes into account a 25-ms lag between stimulation and response. Statistical comparisons were performed for each parameter within each stimulus tested. For statistical tests, time of first flash was ln transformed while proportion of response during flow acceleration was arcsine-squareroot-transformed. *F*, *df*, and *p* signify, respectively, the *F* statistic, degrees of freedom, and statistical significance based on analysis of variance (ANOVA). Statistical significance of pairwise comparisons was determined by Fisher's protected least significant difference post hoc test with a significance threshold of 0.05.

<i>Lingulodinium polyedrum</i> cells	Low stimulus (0.8 rps $s^{-1}$ to 0.8 rps)			High stimulus (3.2 rps $s^{-1}$ to 3.2 rps)		
	Time of first flash (s)	Proportion of response during flow acceleration	<i>n</i>	Time of first flash (s)	Proportion of response during flow acceleration	<i>n</i>
Blooms	0.412 ± 0.060*	0.108 ± 0.054†	4	0.110 ± 0.058*	0.472 ± 0.162†*	5
Isolate 104	0.419 ± 0.153*	0.500 ± 0.150*	4	0.092 ± 0.074*	0.838 ± 0.121*	6
Isolate HJ	3.458 ± 3.028†	0.024 ± 0.022†	3	0.317 ± 0.126†	0.112 ± 0.097†	3
ANOVA						
<i>F</i>	13.2	30.3		4.85	30.9	
<i>df</i>	2, 8	2, 8		2, 11	2, 11	
<i>p</i>	0.0029	0.0002		0.0309	<0.0001	

\* Significantly different from isolate HJ.

† Significantly different from isolate 104.

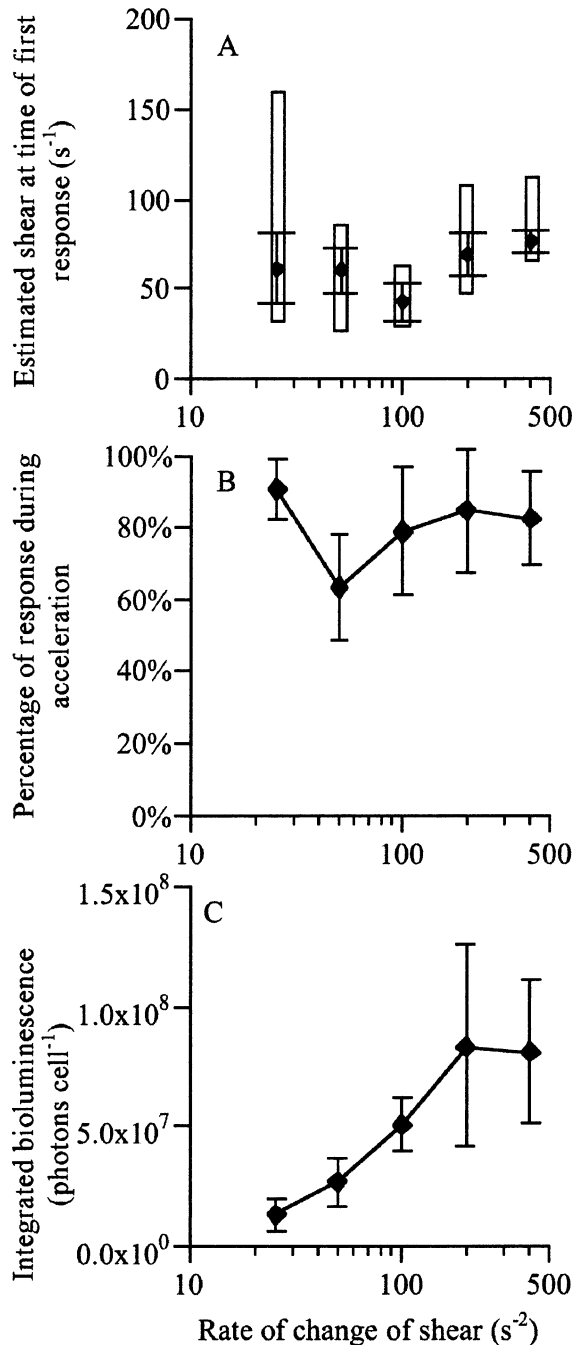


Fig. 6. Dependence of the luminescent response of *L. polyedrum* isolate 104 on the rate of change of shear. (A) The estimated maximum shear ( $s^{-1}$ ) in the gap at the time of the first recorded response (adjusted by a 25-ms lag between stimulation and bioluminescence) versus the rate of change of shear ( $s^{-2}$ ). Symbols represent the mean  $\pm$  standard error of  $n = 3$ –11 replicates, while the boxes outline the range (minimum and maximum). Regression analysis showed that the estimated maximum shear in the gap at the time of the first recorded response was independent of the rate of change of shear ( $y = 54.7 + 41.7x$ ;  $r^2 = 0.08$ ;  $p = 0.14$ ). (B) The proportion of the luminescent response that occurred during the accelerating phase of the flow protocol. Symbols represent mean  $\pm$  standard deviation. There was no significant effect of rate of change on the proportion of luminescent response occurred during the accelerating phase (one-way ANOVA:  $F_{4,24} = 2.5$ ,  $p = 0.07$ ). (C) The

locity and the gradient in acceleration across the gap become larger. The stronger response to more rapidly increasing shear than to slowly increasing shear could result if cells were sensitive not only to shear but to gradients in shear or acceleration across the cell. Alternatively, many metazoan sensory cells (including mechanoreceptors and photoreceptors) are tuned to respond more strongly to rapidly changing stimuli in part by adaptation or desensitization, where stimulatory signal transduction pathways are downregulated by slower inhibitory pathways activated by the same stimulus (Torre et al. 1995). The difference in integrated bioluminescence as a function of rate of change of shear may be due to desensitization. The following two-step flow protocol (Fig. 7A) was used to test between these two alternative explanations: The first flow test consisted of an acceleration phase where the shear was increased at either 25, 100, or 402  $s^{-2}$  to a constant final shear of 402  $s^{-1}$ . This was held for the remainder of the 20-s total of the test period. The flow was then stopped over 1 s, held at rest for 1 s, and in all cases the shear was increased at 402  $s^{-2}$  to 402  $s^{-1}$  for a total of 20 s. In the absence of desensitization, populations that respond poorly to shear increasing at 25 or 100  $s^{-2}$  should still respond to the second test with shear increasing at 402  $s^{-2}$  nearly as strongly as other populations responded to shear increasing at 402  $s^{-2}$  in the first step. The capacity for bioluminescence in dinoflagellates is exhaustible. Therefore, the expected responses in the second test from populations exposed in the first test to shear increasing at 25 or 100  $s^{-2}$  were estimated as differences between the average responses in the first test of populations tested with shear increasing at 402  $s^{-2}$  and those of populations exposed to shear increasing at 25 or 100  $s^{-2}$ .

There was a significant difference in integrated bioluminescence as a function of the different rates of change of shear during the first test (one-way ANOVA:  $F_{2,23} = 8.4$ ,  $p = 0.0019$ ) (Fig. 7B). Populations subject to the 25 and 100  $s^{-2}$  increasing shear responded significantly less during the developing phase of the flow than populations subjected to the 402  $s^{-2}$  increasing shear (Fisher's PLSD:  $p = 0.0012$  and 0.0049, respectively), even though the integrated time of exposure to above-threshold shear was much less in the latter case ( $\approx 1$  s vs. 3.5 and 14 s). The difference between 25 and 100  $s^{-2}$  was not significant (Fisher's PLSD:  $p = 0.88$ ). The proportion of bioluminescence stimulated during the accelerating phase,  $90\% \pm 9\%$  for 25  $s^{-2}$  ( $n = 11$ ),  $76\% \pm 12\%$  for 100  $s^{-2}$  ( $n = 5$ ), and  $70\% \pm 16\%$  for 402  $s^{-2}$  ( $n = 10$ ), was significantly different (one-way ANOVA:  $F_{2,23} = 8.0$ ,  $p = 0.0023$ ). The difference in percentage between 25  $s^{-2}$  and 100 and 402  $s^{-2}$  was significant (Fisher's PLSD:  $p = 0.026$  and  $p = 0.0008$ , respectively).

The responses to the second test were very low compared with the responses to the first step regardless of the rate of

←

integrated luminescent response during the accelerating phase of the flow (normalized to cell number) versus the rate of change of shear ( $s^{-2}$ ). ANOVA analysis indicated that the effect of rate of change was significant ( $F_{4,24} = 9.1$ ,  $p < 0.0001$  for accelerating phase;  $F_{4,24} = 12.1$ ,  $p < 0.0001$  for the full 20 s).

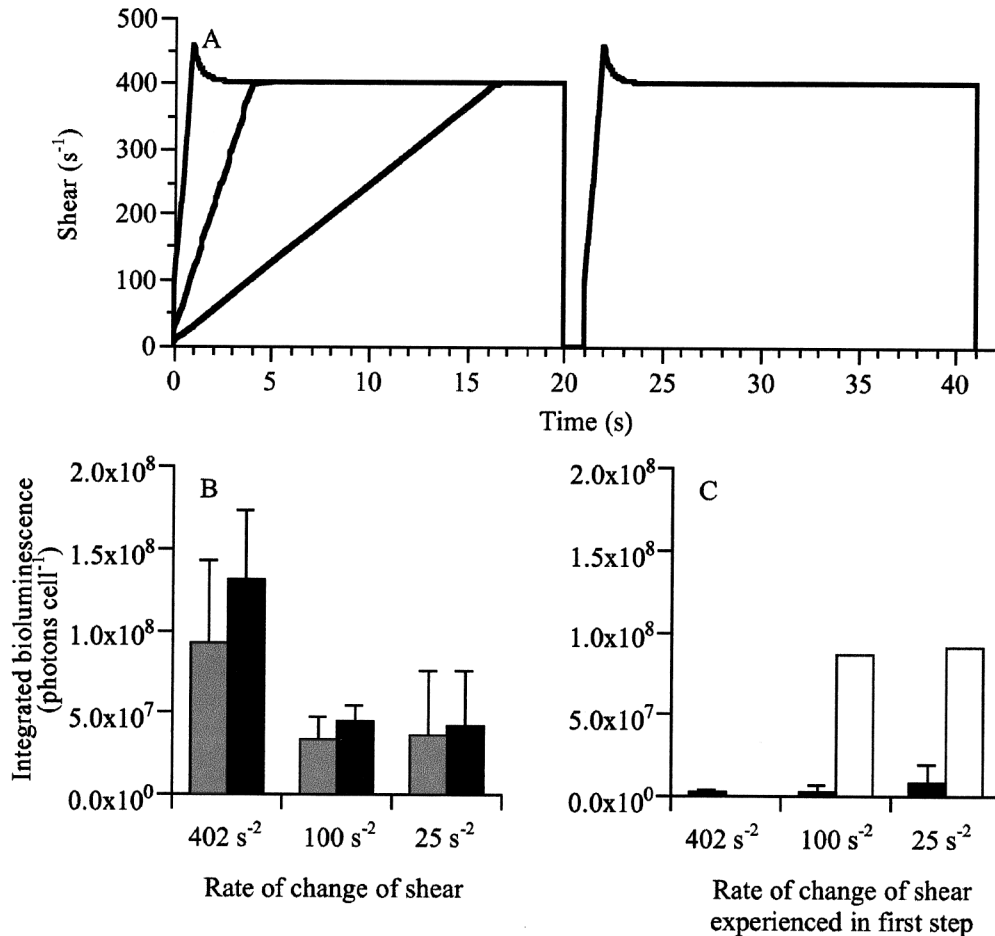


Fig. 7. Test for desensitization as the mechanism of tuning to respond to rapidly increasing shear. (A) Two-step flow protocol used. In the first test period, the rate at which shear was increased was either 402, 100, or 25 s<sup>-2</sup> to a final level of 402 s<sup>-1</sup> and held there for the remainder of the 20-s period. Then flow was decreased to warm-up levels (6 s<sup>-1</sup>) for 1 s. In the second test period, shear was always increased at 402 s<sup>-1</sup> to a final level of 402 s<sup>-1</sup>. (B) The response during the first test period. The gray bars show the integrated bioluminescent response over the accelerating phase (either 1, 4, or 16 s total for 402, 100, and 25 s<sup>-2</sup>, respectively) while the black bars show the integrated bioluminescent response over the full 20-s period. (C) The bioluminescent response during the second test period. The black bars indicate the integrated bioluminescence during the full 20-s period. A conservative estimate for the expected bioluminescence in the second test from samples that had been exposed to the 100 and 25 s<sup>-2</sup> increasing shear in the first test period is the difference between the integrated bioluminescence over the full 20-s first test period of those samples and of samples exposed to 402 s<sup>-2</sup> increasing shear in the first test period. The white bars indicate the missing bioluminescence in the second test. Values in (B) and (C) represent means  $\pm$  standard deviation.

change of shear experienced during the first step. Populations subjected to 25 and 100 s<sup>-2</sup> increasing shear during the first test responded similarly to the second test as those subjected to 402 s<sup>-2</sup> increasing shear during the first test (Fig. 7C) ( $F_{2,23} = 1.5$ ,  $p = 0.25$ ). In the absence of desensitization, populations exposed first to a slowly increasing shear, with resulting low exhaustion of bioluminescence, should have the capacity to produce a much stronger response during the second test (Fig. 7C). These results are consistent with the hypothesis that desensitization results in the dependence of the population-level bioluminescent response on the rate of change of shear.

In no case was bioluminescence stimulated when the flow was rapidly stopped. When the moving outer cylinder is rapidly decelerated, the gradients in shear and acceleration across the gap are in all cases high while the fluid comes to

rest. If cells were particularly sensitive to the gradient in shear or the gradient in acceleration across the gap, strong stimulation of bioluminescence would be expected to occur when the outer cylinder was stopped. This observation also supports the hypothesis that the dependence of the bioluminescent response on the rate of change of shear is due to desensitization that occurs when the shear increases slowly.

Notably, bioluminescence could be stimulated from desensitized samples by strongly hitting the Couette chamber. This demonstrates that desensitized cells can still be stimulated by exposure to forces much greater than those found in the Couette flow.

*Strain and rate of change of strain associated with natural flows*—We have reviewed information available on representative animal-generated flows to predict which will stim-

Table 2. Strain and rate-of-increase of strain characterizing some animal-generated flows.  $a$  is the animal radius (modeled flows) or half the animal length,  $U$  is the velocity in the feeding appendages or of the swimming animal. For the copepod feeding current, the strain is that found 0.5–0.6 mm upstream of the animal (van Duren et al. 2003). For the euphausiid current, values are presented 0.9–1 mm from the pleopods, the closest distance for which information is available (Goldthwait et al. 2004), and  $U$  refers to the maximum velocity in the aft-directed jet. For simplicity, modeled results are only presented for the center-to-center distance from the animal  $r = 1$  mm (a distance-to-surface of 0.5 mm) and  $\theta = 0$  (directly head on).

Flow	Scales		Strain	Rate of increase of strain
	$a$ (mm)	$U$ (mm s <sup>-1</sup> )		
Measured				
Copepod feeding current (far-field) <sup>1</sup>	0.36–0.5	4.4–7.7	6.1–7.6 s <sup>-1</sup>	Not available
Copepod burst swim <sup>2</sup>	0.36–0.5	25–108	Not available	Not available
Euphausiid currents <sup>3</sup>	85	80	60–100 s <sup>-1</sup>	>100 s <sup>-2</sup>
Modeled				
Feeding current <sup>4</sup>	0.5	5	9.4 s <sup>-1</sup>	91 s <sup>-2</sup>
Swimming (slow) <sup>5</sup>	0.5	1	1.1 s <sup>-1</sup>	0.47 s <sup>-2</sup>
Swimming (rapid) <sup>5</sup>	0.5	50	56 s <sup>-1</sup>	1.2 × 10 <sup>3</sup> s <sup>-2</sup>

<sup>1</sup> Values given for *Temora longicornis* (van Duren et al. 2003).

<sup>2</sup> Values given for *Temora longicornis* (van Duren and Videler 2003) and *Acartia hudsonica* (Buskey and Swift 1983).

<sup>3</sup> Goldthwait et al. 2004.

<sup>4</sup> Scales for *Temora longicornis* (van Duren et al. 2003).

<sup>5</sup> Scales chosen to be representative of slow or fast (escape) swimming of copepods.

ulate bioluminescence (Table 2). The rate of change of fluid strain has not been determined in empirical studies of animal-generated flows. The strain near the surface of a swimming animal can be estimated by modeling the axially symmetric velocity field around a translating sphere with the equation

$$u = U \begin{pmatrix} -\cos \theta \\ \sin \theta \end{pmatrix} + \frac{3}{4} \frac{aU}{r} \begin{pmatrix} 2 \cos \theta \\ -\sin \theta \end{pmatrix} - \frac{1}{4} \frac{a^3 U}{r^3} \begin{pmatrix} 2 \cos \theta \\ \sin \theta \end{pmatrix} \quad (4)$$

(Visser 2001), where  $u$  is velocity,  $r$  is the radial distance from the animal center,  $\theta$  is the zenith angle,  $a$  is the radius, and  $U$  is swimming speed. This low Re equation is expected to approximate the upstream flow close to the sphere. The rate-of-strain tensor is, in polar coordinates (Batchelor 1967):

$$\begin{bmatrix} e_{rr} = \frac{\partial u}{\partial r} & e_{r\theta} = \frac{r}{2} \frac{\partial}{\partial r} \left( \frac{u_\theta}{r} \right) + \frac{1}{2r} \frac{\partial u_r}{\partial \theta} & e_{r\phi} = 0 \\ e_{\theta r} = e_{r\theta} & e_{\theta\theta} = \frac{1}{r} \frac{\partial u_\theta}{\partial \theta} + \frac{u_r}{r} & e_{\theta\phi} = 0 \\ e_{\phi r} = 0 & e_{\phi\theta} = 0 & e_{\phi\phi} = \frac{u_r}{r} + \frac{u_\theta \cot \theta}{r} \end{bmatrix} \quad (5)$$

The magnitude of the strain,  $G$ , is the sum of the magnitude of the eigenvalues of the rate-of-strain tensor. The instantaneous rate of change of strain ( $dG/dt$ ) experienced by a Lagrangian particle can be calculated as  $(dG/dt) = u_r(\partial G/\partial r) + u_\theta(\partial G/\partial \theta)$ . A cell directly in front of a 1-mm diameter spherical animal swimming at 50 mm s<sup>-1</sup> would cross the threshold strain ( $\geq 50$  s<sup>-1</sup>) 0.58 mm from the surface of the animal, and the instantaneous rate of increase of shear would be 1,200 s<sup>-2</sup> (the values of  $a$  and  $U$  in Table 2 are typical

of copepods performing rapid and slow swimming behavior, see, e.g., van Duren and Videler 2003). Similar results are obtained using the self-propelled sphere equations of Visser (2001). At the same distance from an animal moving at 1 mm s<sup>-1</sup>, the strain is only 1.1 s<sup>-1</sup>, increasing at 0.5 s<sup>-2</sup>.

The upstream far-field feeding current of a copepod can be modeled with the equation:

$$u = -\frac{3}{4} \frac{aU}{r} \begin{pmatrix} 2 \cos \theta \\ -\sin \theta \end{pmatrix} - \frac{1}{4} \frac{a^3 U}{r^3} \begin{pmatrix} 2 \cos \theta \\ \sin \theta \end{pmatrix} \quad (6)$$

(Visser 2001), where  $U$  is the velocity of the feeding current. At a distance of 0.5 mm from the animal surface and directly upstream of an animal with a feeding current velocity of 5 mm s<sup>-1</sup>, the rate of strain would be 9.4 s<sup>-1</sup> and the rate of increase of strain would be 91 s<sup>-2</sup>, both increasing monotonically as the animal is approached. This is similar to the value of shear determined in the feeding current of the copepod *Temora longicornis* at this position (van Duren et al. 2003). This equation predicts strain will increase to above-threshold levels for stimulation of bioluminescence very near the animal (results not shown), but this has not yet been measured, and it is not clear how well this equation approximates velocity when  $r \approx a$ . In the copepod *Eucalanus pileatus*, the velocity 150 μm from a stationary second maxillae was 10 mm s<sup>-1</sup> (Koehl and Strickler 1981), which suggests strain rates of >67 s<sup>-1</sup>. This suggests that strain above threshold for stimulation of bioluminescence exists within the region where fluid is handled by copepod appendages.

Finally, from the velocity traces of the euphausiid current shown in Goldthwait et al. (2004), a cell passing through this current might be expected to experience a strain rate increasing to 60–100 s<sup>-1</sup> over a time of <0.5 s, resulting in a rate of increase of shear of >100 s<sup>-2</sup>.

## Discussion

This is the first study to characterize the bioluminescent response throughout the development of a quantified flow where the parameters of flow development can be varied. Our main results are that (1) the shear threshold and the relationship between stimulated bioluminescence and shear were similar in developing flow to that found in fully developed flows; (2) there were differences in the kinetics and absolute sensitivities of different strains and naturally occurring *L. polyedrum*; (3) above threshold, there was a preferential response to rapidly increasing shear over slowly increasing shear; (4) the decreased response to slowly increasing shear was due to desensitization, which could prevent repeated stimulation as well; (5) in animal-generated flows, strains that are above threshold for stimulation of bioluminescence are associated with a high rate of increase of strain.

*Comparison of shear sensitivity between developing and fully developed flow*—The absolute shear sensitivities of *L. polyedrum* isolates 104 and HJ determined here were of similar magnitude to the shear sensitivity of *L. polyedrum* isolate 70A determined previously using fully developed laminar Couette flow (Latz et al. 1994) and of isolate HJ and naturally occurring *L. polyedrum*-dominated dinoflagellate populations using fully developed laminar or turbulent pipe flow (Rohr et al. 1998, 2002). The shear threshold of isolate HJ was 100–200 s<sup>-1</sup> in developing Couette flow (this study), which is close to that found for the same isolate in fully developed pipe flow (Latz and Rohr 1999). When the rate of change of shear was varied the bioluminescent response of isolate 104 was always initiated approximately when a threshold shear  $\approx 65$  s<sup>-1</sup> was crossed, which was not significantly different from the threshold for this isolate determined by varying the final shear (50 s<sup>-1</sup>). It is the instantaneous shear, not the rate of change of shear, which controls whether a flow is stimulatory or not.

The relationship between the total bioluminescent response and the shear stimulus was similar between the two isolates and similar to that previously determined in fully developed flow. Integrated bioluminescence varied with the 1.8 power of shear in isolate 104 and with the 2.0 power of shear in isolate HJ. In fully developed laminar Couette flow, bioluminescence varied with the 1.9 power of shear in isolate 70A (Latz et al. 1994).

*Differences between isolates and comparison with naturally occurring *L. polyedrum**—The two isolates of *L. polyedrum* tested in this study had quite different response kinetics and bioluminescent capacities and moderately different absolute sensitivities. The kinetics of the luminescent response of isolate 104, the recent isolate, were much faster than that of isolate HJ, which is older. Isolate 104 had a response threshold at shear levels 2–4 times less than that of isolate HJ. These intraspecies differences may be due in part to differences in length of time since isolation (Sweeney 1986). The responses of *L. polyedrum* cells harvested from blooms of this species were qualitatively more similar to that of isolate 104 than to isolate HJ, with a lower response

threshold and faster kinetics, even though prior exposure to mechanical stresses (filtration during harvesting) is predicted to increase the threshold shear required for stimulation (Latz and Rohr 1999). The bioluminescence intensity of natural *L. polyedrum* cells was less than that of isolate 104 in response to the same stimuli. This may be an effect of the stress of harvesting the cells. These considerations may account in part for the differences between isolate 104 and *L. polyedrum* cells harvested from natural blooms.

Rohr et al. (2002) noted that the threshold for stimulation of *L. polyedrum*-dominated natural assemblages was typically two to fourfold less than the threshold for stimulation of cultured *L. polyedrum* isolate HJ. This might reflect a loss of absolute sensitivity in isolate HJ compared with natural *L. polyedrum* cells. It is also possible that natural populations of *L. polyedrum* contain subpopulations with different response kinetics and sensitivities. The intraspecies variability seen between these two isolates complicates the interpretation of interspecies comparisons (e.g., Latz et al. 2004). Further studies comparing the shear sensitivity and response kinetics of multiple isolates (recently isolated) are needed.

*Sensitivity to the rate of increase of shear due to desensitization*—Above the shear threshold, the bioluminescence stimulated from a population of cells varied strongly with the rate of increase of shear. The population response is a function of the number and intensity of the all-or-nothing flash responses of individual cells. The intensity of the stimulated flash, which is controlled by an action potential (Eckert 1965), does not vary strongly with shear at the individual cell level in *L. polyedrum*, and most of the variation in population response is due to differences in the number of cells flashing (Latz and Rohr 1999). Therefore, the probability of an individual cell responding is a function of both the instantaneous shear level and the rate of change of shear.

Sensitivity to the rate of increase of shear resulted from desensitization as populations exposed first to a slowly increasing shear could not respond well to a subsequent rapidly changing shear. The following explanation is proposed: shear stimulates a rapid excitatory signal transduction pathway (e.g., an increase in intracellular Ca<sup>2+</sup>, von Dassow and Latz 2002) controlling the flash-triggering action potential that results in bioluminescence. In addition, an inhibitory pathway is also stimulated (e.g., activation of Ca<sup>2+</sup> pumps to decrease intracellular Ca<sup>2+</sup>). When shear increases rapidly, the excitatory pathway outpaces the inhibitory pathway. When shear increases slowly, the inhibitory pathway is able to catch up to the excitatory pathway, decreasing the probability of response at the level of the single cell and decreasing the total bioluminescence produced by a population of cells. Similar mechanisms of desensitization are present in the tuning of a variety of metazoan sensory cells (Torre et al. 1995).

*Bioluminescence in animal-generated flows*—Our results allow prediction of which animal-generated and environmental flows could trigger light production (Table 2). The strains found in the far-field (>0.5 mm) upstream flow of copepod feeding currents (<10 s<sup>-1</sup>) are too low to trigger bioluminescence (Van Duren et al. 2003), but biolumines-

cence should be stimulated by higher strains within the region of the moving feeding appendages. High-speed video observations of cell capture by copepod feeding appendages suggest that fluid volumes containing cells might be handled for >100 ms before the cell contacts the animal (at the mouth) and, furthermore, that a cell can be rejected up to 200 ms after capture (Price et al. 1983). This suggests that the range of sensitivity of bioluminescence may still permit a flashing cell to escape ingestion, as copepods stop feeding and jump in response to the flash (Buskey et al. 1983; Buskey and Swift 1983).

Other animal-generated flows are predicted to stimulate bioluminescence. Rapidly swimming animals, such as copepods performing escape jumps, will stimulate bioluminescence (Table 2) (Buskey et al. 1983). The aft-directed jet of the euphausiid *Euphausia pacifica* (Goldthwait et al. 2004) also stimulates bioluminescence. Bioluminescence triggered by swimming animals might repel grazing animals from thin layers of bioluminescent cells at night (Young 1983). This behavior would presumably only have selective advantage if there is genetic relatedness between neighboring bioluminescent cells, since cells that flash might not be the same cells as those directly threatened by the animal. Whether or not stimulation of bioluminescence by swimming animals can provide a selective advantage to bioluminescent cells, this phenomenon may expose animals to greater risk of visual predation (Mensing and Case 1992).

The rate of increase of strain experienced by a cell entrained in an above-threshold animal-generated flow will be very high and the time of exposure will be very brief. In these circumstances, desensitization will not decrease the probability of an initial flash and the exposure time may be so brief that repeated stimulation would not occur anyway.

*Stimulation of bioluminescence in environmental flows*—Cells entrained in environmental flows containing above-threshold shears may be repeatedly exposed to high shears long enough to exhaust the cell. Cells that remain near the surface at night or are mixed into near-surface waters may be exposed repeatedly to breaking waves, which contain above-threshold shear levels (Stokes et al. 2004). The rate of change of shear in breaking waves is not known. In the highest shear region of an overturning wave, the rate of change of shear is likely to be very high (D. Stokes pers. comm.). Therefore, desensitization is unlikely to inhibit the production of the first flash in a breaking wave but might inhibit the production of repeated flashes.

Persistent shear levels in shallow benthic boundary layers are often >50 s<sup>-1</sup> (Shimeta et al. 2003). High bottom shears will change at time scales associated with the forcing mechanism generating them (e.g., tidal currents, internal waves, or ocean swells), and these time scales range from as long as hours to as short as several seconds. The rate of increase of shear experienced by a Lagrangian particle in such a flow cannot be predicted exactly unless seabed topography and currents are precisely specified. However, the increase in the mean shear of a particle entrained in flow over a smooth bottom is expected to be very low even when the shear is above threshold. In these flows, desensitization is expected

to inhibit the production of a primary flash in addition to inhibiting repeated stimulation.

While the laminar boundary layer over the continental shelf represents a very small volume of the ocean, dinoflagellates may accumulate in or near this region under certain conditions. A major portion of the population vertically migrates downward 10–20 m at night (Eppley et al. 1968) and could intersect shallow benthic layers. Levels of wind-induced turbulence with  $\varepsilon = 10^{-5}$ – $10^{-4}$  m<sup>2</sup> s<sup>-3</sup> may result in loss of motility in dinoflagellates (Thomas and Gibson 1990; Zirbel et al. 2000), causing them to accumulate at the benthos in shallow seas. Finally, dinoflagellates often form benthic cyst beds and would encounter the boundary layer during cyst formation and hatching from cysts (Lewis and Hallett 1997). Rapid desensitization might prevent repeated stimulation of the flash-triggering action potential in cells near benthic boundary layers.

*Tuning the sensitivity of bioluminescence to flow*—The bioluminescent response of *L. polyedrum*, with a threshold shear strain of 50 s<sup>-1</sup>, is 5–250 times less sensitive than the escape jump of other plankton, which is triggered by strains of 0.2–10 s<sup>-1</sup> (Kjørboe et al. 1999; Jakobsen 2001, 2002). A puzzle remains as to why bioluminescence is less sensitive to fluid forces than escape jumping in other plankton. The copepod *Pleuromamma xiphias* is capable of both escape jumping and bioluminescence in response to a threat, and the threshold stimulus for eliciting an escape jump is less than that for eliciting bioluminescence. It was proposed that bioluminescence is much more costly than jumping because it exposes the animal (as well as its predator) to potential visual predation (Hartline et al. 1999). Dinoflagellates are not subject to visual predation, and bioluminescence is thought to be the primary defensive behavior in *L. polyedrum*, so it might be expected that the sensitivity of bioluminescence would be similar to that of escape jumping in other flagellates to minimize risk of ingestion. One possible explanation is that the energetic cost of bioluminescence may still be high. The cost of a flash has not yet been estimated. *L. polyedrum* cells can produce more than one flash per cell (Latz and Lee 1995; von Dassow unpubl. data). Repeated stimulation of the flash-triggering action potential may be stressful due to cellular acidification (Nawata and Sibaoka 1979). The action potential might be stimulated even after luciferin has been exhausted, so the cost of stimulation could continue beyond production of flashes. In *Pyrrocystis fusiformis*, the action potential occurs even during the day phase when the scintillons are uncoupled from the stimulation pathway (Widder and Case 1981). This shows that acidification of the cytoplasm might occur any time the cell encounters high shear conditions, even if bioluminescence is not produced. Desensitization may allow energetic savings by decreasing the occurrence of energetically costly cellular events downstream of the signaling pathway, including the action potential and luminescent chemistry. The need for sensitivity of bioluminescence to respond to animal-generated flows might be balanced against a risk of being entrained in an above-threshold environmental flow that would stimulate the cell. The near-surface wind-driven ocean turbulence can have energy dissipation rate  $\varepsilon$  up to  $1 \times 10^{-4}$

$\text{m}^2 \text{s}^{-3}$  (Zirbel et al. 2000). Thus, the mean background shear estimated by  $\sqrt{\varepsilon/7.5\nu}$  (Lazier and Mann 1989) is up to  $4 \text{ s}^{-1}$  near the surface. The threshold for stimulation of bioluminescence is set at over an order of magnitude above the background mean shear level due to upper ocean turbulence.

The observation of rapid desensitization of bioluminescence is consistent with the hypothesis that this behavior has a high cost since this property may decrease the number of times a cell is expected to flash in above-threshold environmental flows. The sensitivity of population bioluminescence to the rate of increase of shear might be important in some, but not all, environmental flows, reducing the probability that even a first flash is produced. More generally, desensitization may inhibit repeated stimulation in above-threshold environmental flows, preventing exhaustion.

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