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Luminescent response of the red tide dinoflagellate *Lingulodinium polyedrum* to laminar and turbulent flow

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Abstract

While it is universally accepted that plankton continually experience a dynamic fluid environment, their sensitivity to the features of the surrounding flow field at the relevant length and time scales of the organism is poorly characterized. The present study uses bioluminescence as a tool to understand how the red tide dinoflagellate *Lingulodinium polyedrum* (= *Gonyaulax polyedra*) responds to well-characterized hydrodynamic forces present in fully developed laminar and turbulent pipe flow. The response of *L. polyedrum* to hydrodynamic stimulation was best characterized by wall shear stress; at similar values of wall shear stress, the response was similar for laminar and turbulent flows.

The response threshold occurred in laminar flow at a wall shear stress of approximately 0.3 N m^{-2}. At these low flow rates, video analysis of the velocity of flash trajectories revealed that responding cells were positioned only near the pipe wall, where local shear stress levels were equal to or greater than threshold. For cell concentrations ranging over four orders of magnitude, threshold values of wall shear stress were restricted to a narrow range, consistent with an antipredation function for dinoflagellate bioluminescence. For laminar flows with above-threshold wall shear stress values $\leq$ 1 N m^{-2}, mean bioluminescence increased with wall shear stress according to a power (log-log) relationship, with the slope of the power function dependent on cell concentration. The increase in bioluminescence within this range was due primarily to an increasing population response rate and, to a lesser extent, an increase in maximum flash intensity per cell and the increased flux of organisms with higher flow rates. For wall shear stress levels $>$ 1 N m^{-2}, the maximum intensity per cell remained approximately constant with increasing wall shear stress, even as the flow transitioned from laminar to turbulent, and the smallest turbulent length scales became less than the average cell size.

All plankton experience a dynamic fluid environment due to the effects of wind, waves, tides, and currents. For phytoplankton, turbulence regulates the vertical transport of cells (Lewis et al. 1984; Cowles and Desiderio 1993), which affects primary production through changes in the average level of incident illumination (reviewed by Kiørboe 1993), the sedimentation rate of cells from the mixed layer (Ruiz et al. 1996), the coagulation of cells caused by shear-induced collisions between suspended phytoplankton (Jackson 1990), and the encounter rate of phytoplankton to grazers and microbes (Bowen et al. 1993). It also relaxes diffusion limitation of nutrient uptake, which can lead to enhanced growth (Pasciak and Gavis 1975; reviewed by Kiørboe 1993 and Karp-Boss et al. 1996). Turbulence can negatively affect phytoplankton growth and cell division (Thomas and Gibson 1990*a,* 1995; Berdalet 1992; reviewed by Estrada and Berdalet 1997) and alter cell motility (Thomas and Gibson 1990*a*).

The present study applies bioluminescence as a tool to characterize how dinoflagellates, one of the most shear-sensitive groups of marine phytoplankton (Thomas and Gibson 1990*b;* Estrada and Berdalet 1997), sense and respond to fluid shear at the small temporal and spatial scales relevant to individual organisms. Common sources of stimulated bioluminescence in near-surface waters (Staples 1966; Tett 1971), dinoflagellates respond to mechanical stimulation within 20 ms with a light flash ≥100 ms in duration, depending on species (Eckert 1965; Widder and Case 1981; Latz and Lee 1995). Based on typical values of oceanic turbulence (Mackenzie and Leggett 1993), the Kolmogorov length and time scales, which characterize the smallest turbulent eddies, are on the order of centimeters and seconds, respectively (Tennekes and Lumley 1972). Because most dinoflagellates are smaller than the energy-containing eddy scales in the ocean, they experience oceanic turbulence as laminar shear (Lazier and Mann 1989).

It has been generally accepted that turbulent flow is most effective at stimulating dinoflagellate bioluminescence. Bathyphotometers, which measure in situ levels of bioluminescence, routinely use highly turbulent flow to stimulate light emission (Losee et al. 1985; Ondercin 1989; Widder et al. 1993). However, it should not be inferred that laminar flow does not stimulate bioluminescence (discussed by Rohr et al. 1997). Using Couette flow, characterized by a constant shear field in the gap between concentric cylinders, Latz et al. (1994) found that the response threshold for bioluminescence stimulation of cultured dinoflagellates and plankton samples occurs in laminar flow at a shear stress level of approximately 0.1 N m^{-2}. A similar threshold has been ob-

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tained for mixed plankton samples tested in fully developed pipe flow (Rohr et al. 1997, 1998).

In the present study, fully developed pipe flow was chosen to stimulate bioluminescence because (1) the hydrodynamic properties of the flow field are well characterized through simple measurements of the volumetric flow and pressure drop along the pipe, (2) it offers a wide (and sometimes overlapping) range of laminar and turbulent shear stress stimuli, (3) it provides continuous replacement of organisms, (4) organisms experience the flow field for only a brief time period, and (5) previous bioluminescence measurements have not appeared to be significantly degraded by the inlet and upstream length of pipe (Losee and Lapota 1981). This approach extends the previous study of Latz et al. (1994) on the red tide dinoflagellate *L. polyedrum* (= *G. polyedra*) through examining a different flow field over a wider range of laminar and turbulent flow stimuli. Furthermore, in addition to mean bioluminescence intensity, the fraction of organisms responding and the characteristics of individual flashes were studied as a function of shear. The present results for *L. polyedrum* further demonstrate that this organism's response is best characterized by the shear stress and not the laminar or turbulent nature of the flow. Preliminary accounts of this study have been reported by Latz and Rohr (1993) and Latz et al. (1995).

Materials and methods

Experimental organism—Cultures of *L. polyedrum* (= *G. polyedra*) Stein were grown in f/4 medium (Guillard and Ryther 1962) minus silicate and maintained at 20 ± 0.5°C in a Percival environmental chamber on a 12 : 12 light : dark cycle. Daytime illumination levels were 240 μE m^{-2} s^{-1} as measured with a Biospherical Instruments QSL-100 scalar irradiance meter. Cell abundance was determined by counting samples under a dissecting microscope. Two hours prior to the end of the day phase, when cells are mechanically unexcitable (Biggley et al. 1969), aliquots from cultures exhibiting exponential growth were added to 0.45-μm filtered seawater in the head tank so that the calculated cell concentration was 15 cells ml^{-1} or as otherwise stated. For most experiments, the contents of the head tank were slowly stirred at 0.5 rpm with a 20-cm-wide paddle to create a homogenous distribution of cells; stirring was terminated prior to the beginning of testing. At the beginning of the dark phase, the apparatus was covered with opaque sheeting. Testing commenced 2.5 h into the dark phase, when the bioluminescence of *L. polyedrum* is maximal (Biggley et al. 1969).

Flow-field measurements—Organisms were tested in a pipe flow apparatus described by Rohr et al. (1990). The apparatus consisted of a 75-liter transparent acrylic head tank coupled to a clear acrylic pipe. The pipe was 91 cm in length with an internal diameter of 0.635 cm. Cells in the head tank were drawn into the pipe through a gentle contraction where, as revealed by dye studies, the flow always remained laminar. Flow through the pipe was manually controlled by an adjustable valve fitted onto flexible tubing connected to the end of the pipe. Volumetric flow rate was measured by weighing the discharge fluid collected for a known time period. Average flow velocity was determined by dividing the volumetric flow rate by the pipe cross-sectional area. Average flow velocity was used to calculate the Reynolds number (Re), defined as

$$\mathrm{Re} = \frac{\rho U_{\mathrm{avg}} D}{\mu} \tag{1}$$

where ρ is the fluid density, U_{avg} is the average fluid velocity, D is the pipe diameter, and μ is the dynamic viscosity. The pressure drop along the pipe was determined by two pressure ports, located 65 and 128 cm downstream of the pipe inlet, connected by tubing to a variable reluctance differential transducer (Validyne). The pressure drop was used to calculate the Darcy friction factor (λ), defined as

$$\lambda = \frac{\Delta p \frac{D}{L}}{\frac{1}{2}\rho U_{\mathrm{avg}}^2} \tag{2}$$

where Δp is the pressure drop along a length L of pipe (Schlichting 1979).

The flow is considered fully developed when the mean velocity profile across the pipe becomes essentially constant downstream; under these conditions, the shear stress at the pipe wall is balanced by the pressure gradient along it. For fully developed pipe flow, the shear stress at the pipe wall (τ_{wall}) is calculated as

$$\tau_{\mathrm{wall}} = \frac{\Delta p D}{4L} \tag{3}$$

and the shear stress $\tau(r)$ profile across the pipe is

$$\tau(r) = \tau_{\mathrm{wall}} \frac{2r}{D} \tag{4}$$

where r is the radial position from the center of pipe (Schlichting 1979). Equation 4 indicates that shear stress is greatest at the pipe wall and zero at the pipe centerline. The average shear stress across the pipe diameter in fully developed flow is ⅔ τ_{wall}.

The relationship between Darcy friction factor and Reynolds number indicates whether flow is laminar, turbulent, fully developed, or transitional (Fig. 1A). Laminar flow measurements showed excellent agreement with the theoretical relationship $\lambda = 64/\mathrm{Re}$ (Schlichting 1979) for Re < 2,000. For higher flows with Re > 2,000, the measured relationship began to deviate from the theoretical curve, indicating that flow was not fully developed, although dye observations confirmed that the flow remained laminar until approximately Re = 7,000. At these higher laminar flows, the length of pipe upstream of the pressure ports was insufficient to allow fully developed flow (White 1979). Therefore, for laminar flows as Re increased beyond 2,000, the measured pressure drop increasingly overestimated theoretical values of wall shear stress, with the worse case being an overestimate of 60% at the highest laminar flow of 2.5 N m^{-2}. Turbulent flow measurements followed the accepted empirical relationship $\lambda = 0.316/\mathrm{Re}^{0.25}$ (Blasius 1913; Schlichting 1979) for

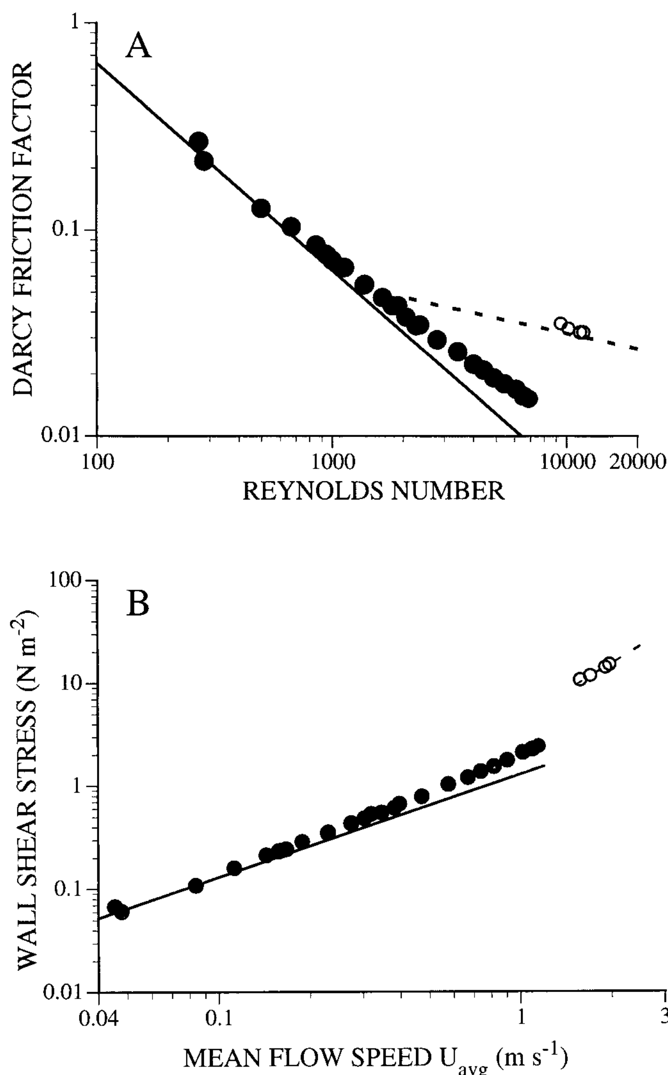

Fig. 1. Hydrodynamic characterization of pipe flow. The solid line shows the theoretical relationship for laminar flow, while the dashed line represents the accepted empirical relationship for turbulent flow (*see* text for details). Each point represents a single flow rate. Solid symbols represent measurements where flow was laminar, and open symbols are for turbulent flows. (A) Relationship between Darcy friction factor (λ) and Reynolds number for pipe flows from a typical experiment. At laminar flows with Re $>$ 2,000, the flow was not entirely fully developed, accounting for the deviation of the points from the solid line. (B) Relationship between wall shear stress and mean flow speed (U_{avg}) for the same experiment. When laminar flow was not fully developed for $U_{avg} > 0.33$ m s^{-1}, the measured shear stress slightly overestimated theoretical values.

fully developed pipe flow. Transitional flows, where the flow was intermittently laminar and turbulent, were not considered in this study.

Bioluminescence measurements—Light emission was measured using two photon-counting photomultiplier detectors (Rohr et al. 1990), the lower one always located 67 cm (ca. 106 pipe diameters) from the pipe inlet. On occasion, a second photomultiplier was positioned either 6 cm (ca. nine pipe diameters) from the inlet or opposite the downstream photomultiplier. Each detector, coupled to the pipe with a light-shielded adapter, viewed a 5-cm length of pipe. Unless otherwise stated, photomultiplier data presented in this paper were from the lower detector, where turbulent flow was always fully developed and laminar flow was fully developed for Re $\leq$ 2,000. The frequency of square wave (TTL) pulses produced by the photomultiplier discriminator was proportional to the number of photons detected. Mean bioluminescence was measured by pulse counting performed by an EG&G Ortec 874 counter controlled by an IBM-PC–compatible computer through software that also monitored the voltage of the pressure transducer. Using a pair of EG&G Ortec ACE-MCS multichannel scalars mounted in another IBM-PC–compatible computer, pulses were also counted in 5-ms time bins in order to resolve individual flash events and determine maximum flash intensity for each flow rate.

The photomultiplier detectors were calibrated for absolute quantum emission by filling the pipe with a chemiluminescent fluid from Cyalume® lightsticks (American Cyanamid). Simultaneous readings of the quantum emission of a 1-ml fluid sample were measured in a Quantalum 2000 (Zefaco) calibrated photometer.

Bioluminescence was measured at each flow rate for 10–100 s, with the period adjusted to keep the volume of water sampled approximately equal. For each experiment, flow rates were presented first in a decreasing series, from maximum turbulent to below threshold in the laminar range, and then in a reverse order from laminar to turbulent. There was generally no difference in luminescent response at equivalent flow rates as a function of the temporal sequence of flows (Rohr et al. 1994, 1997, 1998; present study). For each flow rate, Reynolds number, friction factor, and the following bioluminescence parameters were determined: mean and maximum bioluminescence intensity, proportion of cells responding, and, for some experiments, the decay rate of individual flashes. A response threshold was calculated for each experiment using two approaches. A regression method was based on the power regression of mean bioluminescence vs. wall shear stress. For each experiment, a least-squares regression of log mean bioluminescence vs. log wall shear stress was calculated for the range of flows with $\tau_{wall} \leq 1$ N m^{-2} where a response was noted; within this range, the power regression was generally linear. Using the regression equation, the wall shear stress value at which the regression line intercepted the background level of bioluminescence was calculated. For comparison, threshold was also determined using a flash criterion method, by identifying the flow with the lowest shear stress value that still elicited a response.

Mean bioluminescence intensity, the time-averaged emission from a population of cells, was calculated by integrating light emission over the entire record and then dividing by the elapsed time. Mean intensity is therefore a function of the total number of flashes produced and their intensity. Maximum intensity is defined as the brightest emission measured during a sampling period. A mechanically stimulated flash of a *L. polyedrum* cell has a duration of approximately 150 ms and a maximum intensity of 2×10^8 quanta s^{-1} (Latz and Lee 1995). Because cells are advected past the detector,

an entire flash may not be sampled; e.g., the brightest part of the flash may have already occurred upstream, and only part of the flash decay is measured. Therefore, the maximum intensity for the entire sampling record is considered to approximate the maximum intensity of a single flash. Although each mechanically stimulated *L. polyedrum* cell is capable of only a few flashes (Latz and Lee 1995), for the present study, it is assumed that a cell will produce no more than one flash within the limited field of view of the detector.

Flash intensity in dinoflagellates follows a simple exponential decay (Seliger et al. 1961; Eckert 1967). The instantaneous decay rate of a flash was measured from the bioluminescence time series (5-ms integration time). Custom software was used to calculate the least-squares linear regression of the logarithm of the intensity as a function of time. The slope of this regression modeled the exponential decay of the flash. Approximately 40 flashes were measured at each flow rate that was analyzed.

The proportion of cells responding (i.e., population response proportion) was defined as the number of flashes measured by the photomultiplier divided by the estimated number of cells advected past the detector during the measurement period. This parameter was calculated by multiplying the flash rate measured from the multichannel scalar records (units of flashes per second) by the volumetric flow rate (milliliters per second) calculated from the mass flow measurement to obtain the volumetric flash rate (flashes per milliliter). The volumetric flash rate was then divided by the cell concentration (cells per milliliter), measured from subsamples of the discharge fluid, to obtain the proportion of cells responding in view of the detector (flashes per cell). The response proportion reflected the instantaneous rate of flashes occurring in view of the detector. For experiments with the standard cell concentration of 15 cells ml^{-1}, flows with $\tau_{wall} > 1$ N m^{-2} resulted in coincident flashes within the 5-ms resolution multichannel scalar record, which underestimated the actual flash rate. Experiments with cell concentrations <1 cell ml^{-1} minimized the problem of coincident responses and provided a more accurate record of flash rate at higher flows.

For several laminar flow experiments, the level of local shear stress for each responding cell was determined directly from video imaging of its radial position within the pipe. The pipe was viewed by an intensified SIT video camera (Cohu, model 55) fitted with a Bell & Howell 15-mm lens and extension tubes. Each flash appeared as a linear streak of light in the video record. During video playback, the distance traveled in one frame by the leading edge of the flash was measured in order to calculate the cell velocity, which is assumed identical to the velocity of fluid in that streamline. The radial position (r) of each responding cell at a particular laminar flow rate was calculated as

$$U(r) = U_{max}\left[1 - \left(\frac{r}{R}\right)^2\right] \tag{5}$$

where $U(r)$ is the flash velocity measured from the video, the maximum fluid velocity $U_{max} = 2 \cdot U_{avg}$, and R is the pipe radius. The local value of the shear stress at a particular radial position was calculated according to Eq. 4. Because the swimming of *L. polyedrum* is weak (Kamykowski et al. 1992) compared to bulk water motion considered in this study, the cells were considered to be passive tracers within the flow.

To further assess the effect of cell radial position on flow-stimulated bioluminescence, cells in one experiment were injected into the flow at the pipe inlet. The head tank was filled with Whatman GF/F-filtered seawater. Cell injection from a 60-ml plastic syringe coupled to 1.5-mm ID Teflon tubing was controlled by a Harvard Apparatus model 22 infusion pump. At the injection rate of 10 ml min^{-1}, estimated to deliver 48 cells s^{-1}, no bioluminescence was observed to be stimulated in the injection tube. Stimulated bioluminescence was measured at the downstream photomultiplier position, as described above, for both laminar and turbulent flows. Comparisons between injection positions at centerline and the wall were made for replicate tests using the same sample of cells within a single syringe to minimize differences in cell abundance between syringes.

Unless otherwise stated, values represent the arithmetic mean with standard error of the mean. Statistical tests were performed using Statview software (Abacus Concepts), with statistical significance based on the $P = 0.05$ probability level.

Results

Response threshold—The response threshold for *L. polyedrum* always occurred in laminar flow. The maximum intensity of individual flash events at near-threshold flows was at least 10 times background levels. For all experiments ($n = 22$), there was no significant difference between the response threshold determined from the regression method, which occurred at a flow with a wall shear stress of 0.32 ± 0.03 N m^{-2}, and the threshold of 0.30 ± 0.03 N m^{-2} obtained by the flash criterion method (paired t-test, $P = 0.15$). Response thresholds based on the regression method were used for further analysis of the effects of prestirring, sampling time, cell concentration, and culture age.

There was no significant effect of gentle prestirring on the response threshold. Threshold occurred at a wall shear stress of 0.35 ± 0.04 N m^{-2} ($n = 8$) for the prestirred experiments and at 0.27 ± 0.03 N m^{-2} ($n = 14$) for the unstirred experiments (Mann–Whitney U-test, $P = 0.17$). Thus, gentle prestirring at a rotation rate that did not stimulate bioluminescence was used to help maintain homogeneous distributions of organisms in the head tank. Levels of bioluminescence for flows with subthreshold levels of wall shear stress were similar to background levels measured with no flow.

Because at the stimulation threshold the probability of response is low, the measured response threshold may be significantly affected by sampling time and cell concentration. Typical measurements for low flow rates used a 100-s sampling period. In one experiment, flow with a slightly subthreshold τ_{wall} value, which did not stimulate flashes for a 100-s period, also did not stimulate flashes for a 500-s sampling period. Therefore, the 100-s sampling period was considered adequate for characterizing the response threshold.

The response threshold was restricted to a τ_{wall} range of

0.14–0.57 N m^{-2} (n = 22 experiments) over a cell concentration of four orders of magnitude. Within this narrow range, the response threshold was significantly correlated with the logarithm of cell concentration (linear regression, $r^2 = 0.31$; the slope of the regression was significantly different from zero, $t = -2.8$, $P = 0.01$). There was a significant difference in thresholds when grouped into low (<1 cell ml^{-1}), medium (6–15 cells ml^{-1}), and high (65–1,100 cells ml^{-1}) ranges of cell concentration (one-way ANOVA, $P = 0.04$). The low cell concentration group had the highest thresholds ($\tau_{wall} = 0.43 \pm 0.05$ N m^{-2}, n = five experiments), with the high cell concentration group having the lowest ($\tau_{wall} = 0.21 \pm 0.003$ N m^{-2}, $n = 3$). Most experiments involved the medium cell concentration range of 6–15 cells ml^{-1}, which yielded a mean threshold of $\tau_{wall} = 0.30 \pm 0.04$ N m^{-2} ($n = 12$).

During March and April 1995, a red tide of *L. polyedrum* spread along the entire coast of southern California. Surface abundance of *L. polyedrum* at the Scripps Institution of Oceanography pier reached 4,000 cells ml^{-1} (Latz unpubl. data). Daytime surface-water samples containing approximately 1,200 cells ml^{-1} of *L. polyedrum* were tested at night in pipe flow and compared to two experiments with cultured cells at equivalent concentrations. The response threshold of $\tau_{wall} = 0.21$ N m^{-2} for the red tide sample was in excellent agreement with the $\tau_{wall} = 0.21$ N m^{-2} threshold values for laboratory cultures of *L. polyedrum* tested at high concentrations.

There was no significant effect of culture age, that is, the time after inoculation into fresh medium, on the response threshold (linear regression, $r^2 = 0.001$; the slope of the regression was not significantly different from zero, t-test, $P = 0.89$). The mean age of *L. polyedrum* cultures used for testing was 12 d (range = 3–32 d).

Response as a function of wall shear stress—Population and single-cell response varied with flow conditions (Fig. 2). Flows with a wall shear stress near the response threshold stimulated only a few flashes of dim intensity (Fig. 2A). As the flow rate and thus the wall shear stress increased, so did the rate of flashing and the bioluminescence intensity of individual flashes (Fig. 2B).

For laminar flows above threshold, mean bioluminescence increased up to approximately 1–2 N m^{-2}, after which it leveled off (Fig. 3A). The rate of increase of mean bioluminescence in laminar flow, within the τ_{wall} range between threshold and approximately 1–2 N m^{-2}, varied with cell concentration (power [log-log] regression, $r^2 = 0.42$; the slope of the regression was significantly different from zero, $t = 3.5$, $P < 0.01$). The low cell concentration (<1 cell ml^{-1}) group had the lowest mean bioluminescence vs. τ_{wall} slopes (2.3 ± 0.5, $n = 4$), while the high cell concentration (>15 cells ml^{-1}) group exhibited the highest (5.0 ± 1.0, $n = 3$); these values were significantly different according to a post hoc test (Scheffe test, $P = 0.04$). For standard cell concentrations of 6–15 cells ml^{-1}, mean bioluminescence over the same range of τ_{wall} increased as the 3.7 ± 0.4 (n = 12 experiments) power of τ_{wall}. For $\tau_{wall} > 2$ N m^{-2}, no further increase in mean bioluminescence was usually found, even when the flow became turbulent (Fig. 3A).

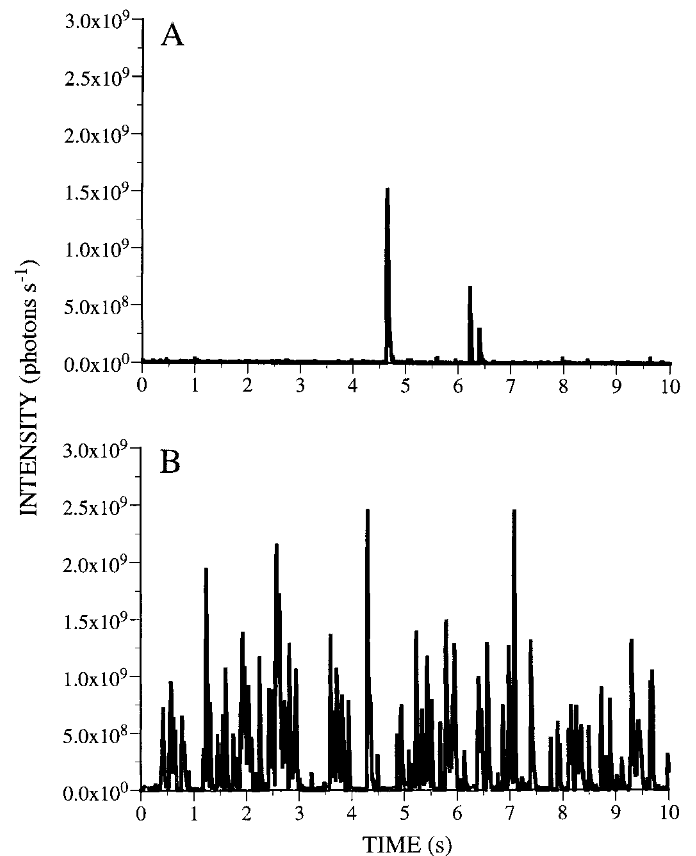

Fig. 2. Representative bioluminescence time series obtained for two different flow rates during a single experiment. Data represent the intensity of light emission as a function of time for a population of *L. polyedrum* at a concentration of six cells ml^{-1}. Only 10 s of the 20-s time series is shown. Each spike represents one luminescent flash at low flow rates or possibly up to several overlapping flashes at high flow rates. (A) Stimulated bioluminescence in laminar flow with a wall shear stress of 0.54 N m^{-2}. Only three flashes were produced. (B) Stimulated bioluminescence in high laminar flow with a wall shear stress of 1.04 N m^{-2}. For this record, 11% of the population produced flashes in view of the detector.

Mean bioluminescence intensity is a function of the number of organisms responding, their flash intensity, and possibly advection effects (Widder et al. 1993). An estimate of which component was most important can be made for the range of laminar flows with τ_{wall} values between threshold and 1 N m^{-2} where coincidence of flashes did not occur and single flash events in the time series could be resolved. Within this range for a representative experiment using the standard cell concentration of 15 cells ml^{-1}, maximum bioluminescence intensity per cell increased according to the 0.5 power of τ_{wall} (Fig. 3B), while the fraction of the population responding increased according to the 5.2 power of τ_{wall} (Fig. 3C). The influence of advection throughout this range of laminar flows would result at most in a linear increase in mean bioluminescence intensity with τ_{wall} (*see Discussion*). Therefore, the increase in mean bioluminescence within this range was primarily due to a 300-fold increase in population

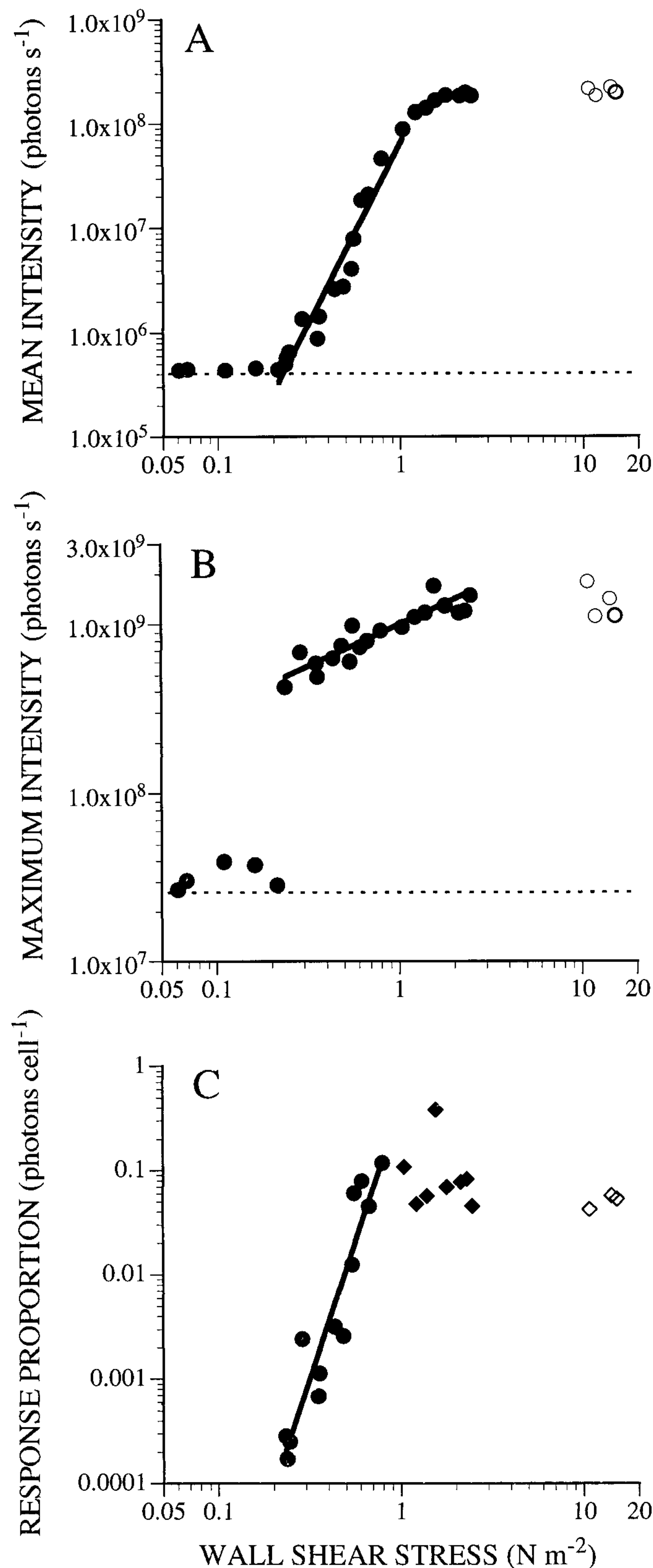

Fig. 3. Luminescent response of *L. polyedrum* to laminar and turbulent pipe flow. Data expressed as a function of wall shear stress for a representative experiment at a cell concentration of 15 cells ml^{-1} run on 26 April 1995. The gap between wall shear stress values of 2–10 N m^{-2} represents a region of transitional flow where no measurements were made. Solid symbols are for laminar flows, while open symbols are for turbulent flows. The dashed line represents the background level for no flow. (A) Average bioluminescence as a function of wall shear stress. The line represents the least-square power law regression between wall shear stress values of 0.2–1 N m^{-2} (for this experiment, $y = 2.8x^{3.4}$; $r^2 = 0.94$). (B) Maximum intensity of bioluminescence as a function of wall shear stress. The maximum intensity represents the brightest flash emission at each flow rate. The line represents the least-square power law regression between wall shear stress values of 0.2–2 N m^{-2} (for this experiment, $y = 162.8x^{0.6}$; $r^2 = 0.84$). At wall shear stress values $\leq$ 0.2 N m^{-2}, maximum intensity was similar to background because no flashes occurred. (C) Proportion of cells responding as a function of wall shear stress. *See* text for details. The line represents the least-square power law regression between wall shear stress values of 0.2–1 N m^{-2} (for this experiment, $y = (2.4 \times 10^{-6})x^{5.2}$; $r^2 = 0.89$). Diamond symbols represent flows in which the response rate was underestimated due to coincidence of individual flashes, making it difficult to resolve single flash events.

response proportion, compared to only a fivefold increase in maximum flash intensity.

In the case of experiments with the standard cell concentration of 15 cells ml^{-1} and flows with $\tau_{wall} > 1$ N m^{-2}, values of mean bioluminescence intensity, maximum bioluminescence intensity per cell, and the population response rate did not change between high laminar and turbulent flow. However, under these conditions, multiple flashes (i.e., coincidence) occurred in the detector field of view, making it difficult to resolve individual cell responses. To further investigate the effect of turbulence on the flash response of individual cells, separate experiments ($n = 4$) using low cell concentrations (<1 cell ml^{-1}) were conducted to minimize the effect of coincident flashes. In a representative experiment, there was no significant difference between maximum bioluminescence intensity per cell for high laminar flows (τ_{wall} = 0.8–1.4 N m^{-2}) compared to that for turbulent flows (τ_{wall} = 6–12 N m^{-2}) ($n = 10$, *t*-test, $P = 0.5$). These low concentration experiments demonstrated that in the absence of flash coincidence, levels of maximum intensity remained essentially unchanged through transition.

There was also no evidence that the flash shape, as modeled by an exponential decay, changed as a function of flow stimulus level (Fig. 4). The mean exponential decay rate of the flash, measured for a representative experiment from bioluminescence time series for both laminar and turbulent flows, was -0.11 s^{-1} ($n = 371$).

Effect of preagitation—Although gentle prestirring had no significant effect on the response threshold, more active mixing, sufficient to produce noticeable bioluminescence, may selectively stimulate the more sensitive cells. This hypothesis was tested by the following protocol: the bioluminescence vs. shear stress relationship for an unstirred population was first measured, then the remaining population in the head tank was retested after being stimulated by the reciprocating manual movement of a 0.5-cm mesh for 20 min.

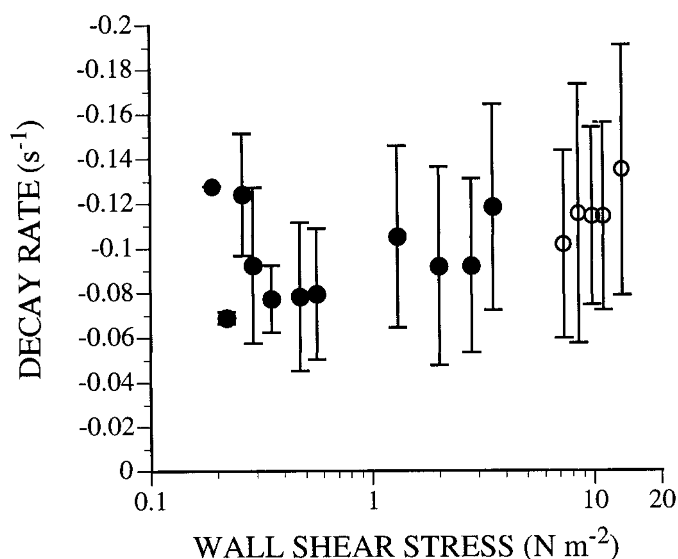

Fig. 4. Decay rate of *L. polyedrum* flashes as a function of wall shear stress for a representative experiment of 21 September 1993. Decay rates (per second) of 371 individual flashes were measured from high-resolution time series encompassing the full range of laminar and turbulent flows. There was no statistically significant relationship between decay rate and shear stress ($r^2 < 0.06$).

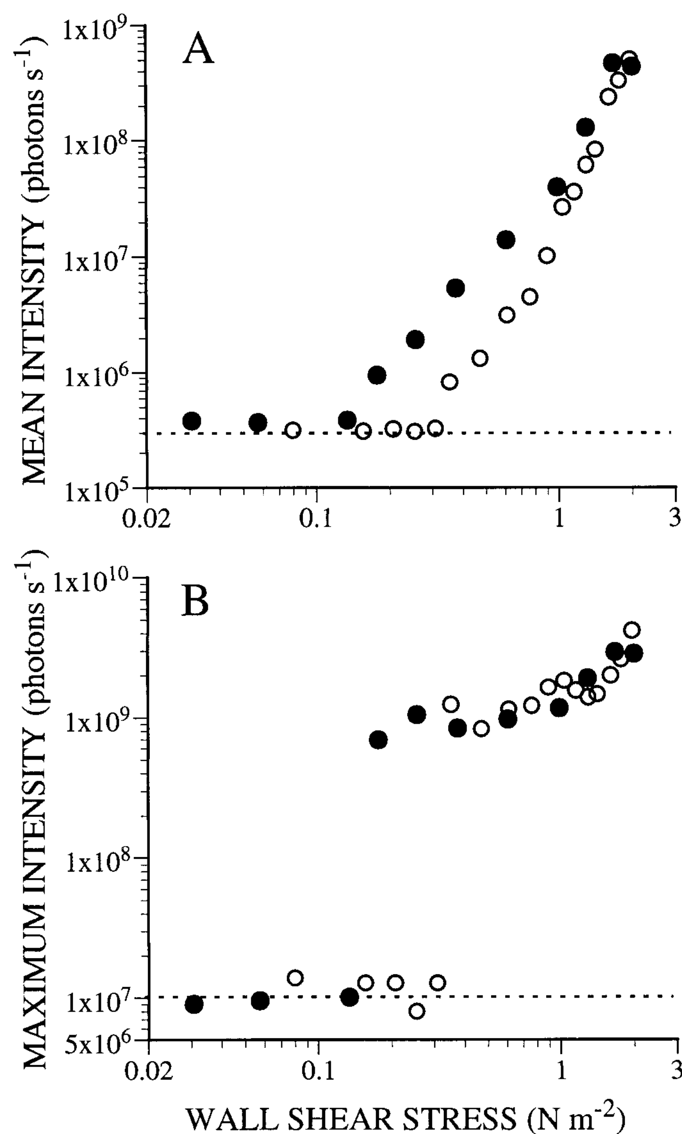

Fig. 5. Effect of hydrodynamic prestimulation on the population response of *L. polyedrum* to laminar pipe flow. The relationship between bioluminescence intensity and wall shear stress was determined for the unstirred condition (closed circles) and following a 20-min agitation with a 0.5-cm mesh (open circles); only data for laminar flows are shown because there was no difference for turbulent flows. The dashed line represents the background level with no flow. (A) Mean bioluminescence intensity as a function of wall shear stress. The shift in the population response following mesh stimulation included a higher response threshold and a diminished response at wall shear stress values < 1.5 N m^{-2}. (B) Maximum bioluminescence intensity as a function of wall shear stress. There was no significant change in maximum intensity per cell for the two conditions.

The movement of the mesh was observed to elicit flashes in the vicinity of the mesh surface.

As a result of the mesh agitation, there was a decrease in mean bioluminescence consistent with a depletion of the more sensitive cells. In one representative experiment, the response threshold shifted from approximately 0.18 N m^{-2} before agitation to approximately 0.35 N m^{-2} after mesh agitation (Fig. 5A). For all above-threshold flows with a τ_{wall} < 1.5 N m^{-2}, mean bioluminescence intensity was diminished after mesh agitation. However, the mean response was unchanged for both laminar and turbulent flows with τ_{wall} > 2 N m^{-2}. Maximum intensity per cell was unaffected by mesh stimulation (Fig. 5B). These results suggest that the decrease in mean intensity because of mesh agitation was due to selective prestimulation of a sensitive subpopulation of cells, not a change in the maximum flash intensity of individual cells.

Effect of cell position—Estimated mean responsiveness near threshold was equivalent to approximately 2 out of 10^4 cells advected past the detector. However, because of the gradient of shear stress within the pipe, cells in near-threshold flows would be expected to respond only to the highest shears, which occur near the pipe wall. To test this prediction, the radial position of cells responding to near-threshold flows was determined from video analysis of their instantaneous velocity and Eq. 5 for five flow rates with τ_{wall} between 0.22 and 0.38 N m^{-2}. Responding cells were always located near the pipe wall, with radial positions $\geq R/2$ (Fig. 6A), where $\tau > 0.17$ N m^{-2} (Fig. 6B). The mean value of local τ at the flash position was 0.27 ± 0.005 N m^{-2} (n = 81).

The cell injection experiment was designed to provide further evidence that responding cells were located near the pipe wall, as found by the video flash analysis. For laminar flows, there was no bioluminescence stimulated when cells were injected at the centerline (n = three replicates) (Fig. 7A). However, when injected at the pipe wall at the same flow rate, there were many flash responses (Fig. 7B). For turbulent flows, there was no significant difference in mean

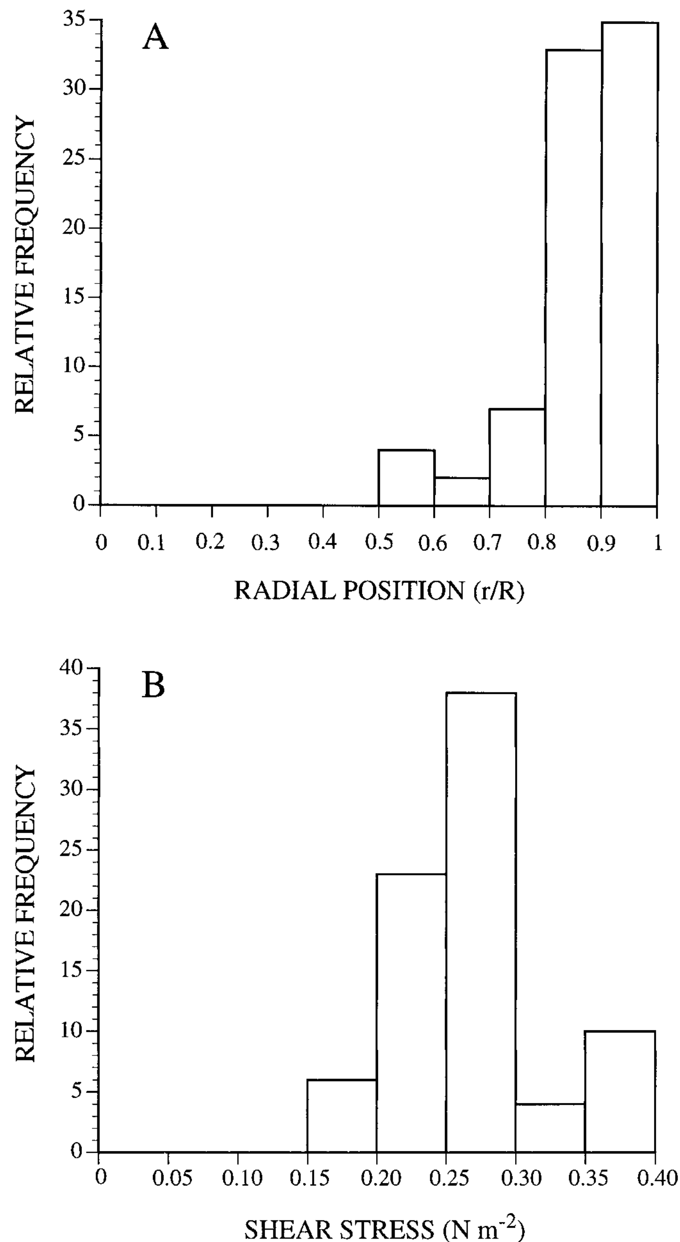

Fig. 6. Radial position of *L. polyedrum* cells responding to near-threshold pipe flows. Video analysis of flash response velocity provided the radial position; *see* text for details. Data represent five flow rates with wall shear stress values between 0.22 and 0.38 N m^{-2}. (A) Frequency distribution of relative radial position of each responding cell, where $r/R = 1$ is at the pipe wall and $r/R = 0$ is at centerline. (B) Frequency distribution of local values of shear stress calculated at each cell position. All flashes occurred near the pipe wall, where local shear stress levels ≥ 0.17 N m^{-2}.

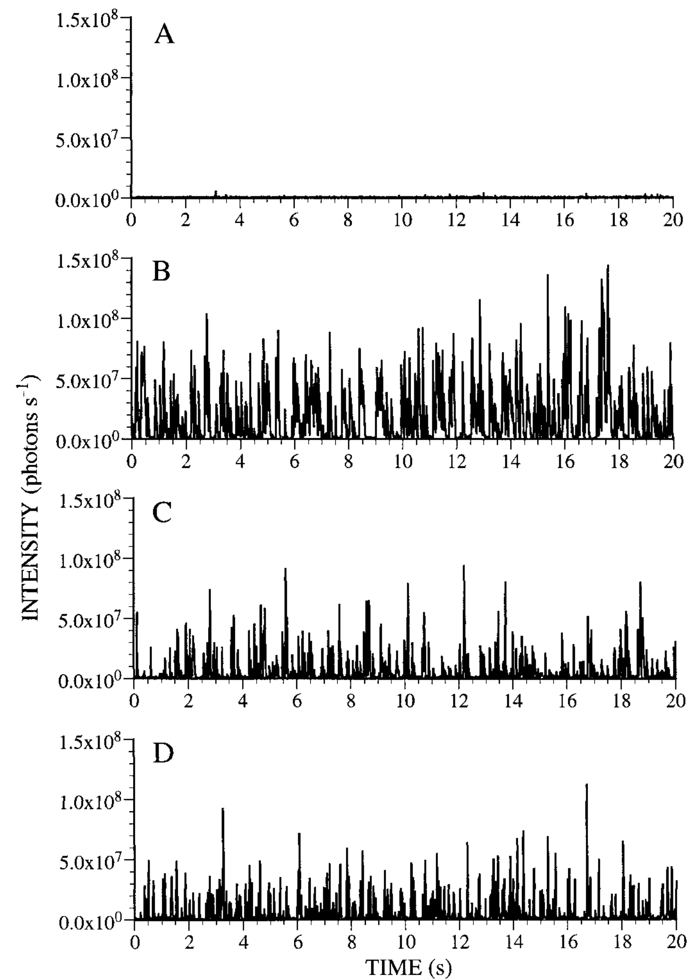

Fig. 7. Effect of cell injection position on the flow-induced response of *L. polyedrum.* Bioluminescence time series of flow-stimulated response as a function of time are shown for a single population of cells continuously injected either at centerline or the pipe wall for both laminar and turbulent flow. (A) Laminar flow, $\tau_{wall} = 0.9$ N m^{-2}, injected at centerline. No flashes were stimulated. Mean bioluminescence intensity similar to background levels. (B) Laminar flow, $\tau_{wall} = 0.9$ N m^{-2}, injected at the pipe wall. Many flashes were stimulated. Mean bioluminescence intensity = 1.85×10^7 photons s^{-1}. (C) Turbulent flow, $\tau_{wall} = 7.2$ N m^{-2}, injected at centerline. Mean bioluminescence intensity = 6.55×10^6 photons s^{-1}. (D) Turbulent flow, $\tau_{wall} = 7.3$ N m^{-2}, injected at the pipe wall. Mean bioluminescence intensity = 6.64×10^6 photons s^{-1}.

bioluminescence and maximum intensity (ANOVA, $P > 0.67$) for centerline (Fig. 7C) and pipe wall (Fig. 7D) injection positions. The cell injection results support the video analysis data that show cells located at the pipe centerline do not respond in laminar flow. For turbulent flow, because of mixing, there is no positional effect.

Laminar vs. turbulent flow—Two experiments were performed with an overlapping range of shear stress to determine whether the laminar or turbulent nature of the flow was important. After measuring the response of cells throughout the full range of laminar flow, the flow was forced to become turbulent at lower Reynolds numbers by placing a washer at the pipe entrance, and the identical Reynolds number range was reexamined (Fig. 8A). When expressed as a function of Reynolds number, mean bioluminescence intensity was notably higher in turbulent flow (Fig. 8B). However, for equivalent values of wall shear stress, mean bioluminescence was similar for laminar and turbulent flows (Fig. 8C). Maximum bioluminescence intensity exhibited a similar pattern, with

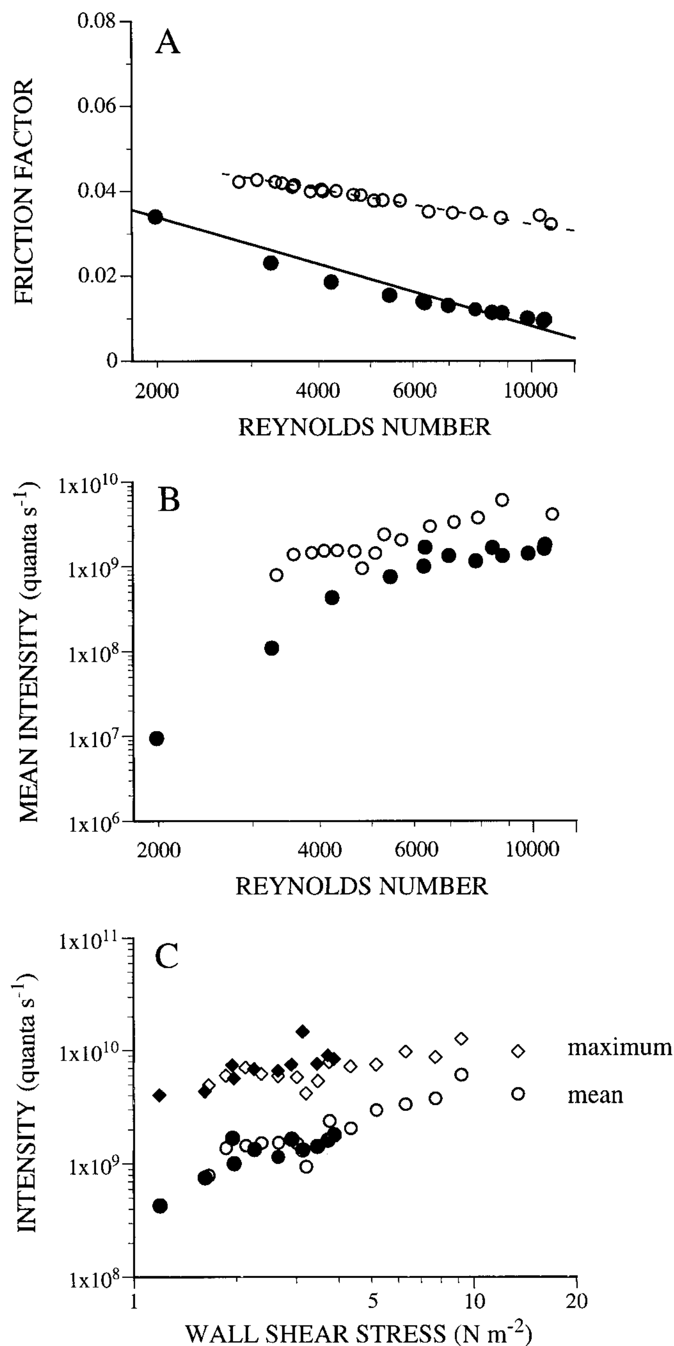

Fig. 8. Experiment using forced turbulent flow to achieve an overlapping range of shear stress with previously tested laminar flow. The bioluminescence of *L. polyedrum* was first measured for laminar flow, then measured again for the same range of flow rates after the flow was tripped. Closed symbols are for laminar flow, while open symbols are for tripped turbulent flow. (A) Relationship between friction factor and Reynolds number, showing the overlapping laminar and turbulent flow conditions. Symbols and lines as in Fig. 1. (B) Mean bioluminescence expressed as a function of Reynolds number, which is proportional to flow rate. At a given Reynolds number (i.e., flow rate), turbulent flow stimulated more bioluminescence than did laminar flow because it contained higher levels of shear stress. (C) Mean and maximum bioluminescence intensity expressed as a function of wall shear stress. At a given level of wall shear stress, there was no difference between responses in laminar and turbulent flows.

no detectable sensitivity to the nature of the flow field when expressed as a function of wall shear stress. These results suggest that organisms respond as a function of mean fluid shear and not the laminar or turbulent nature of the flow field.

Discussion

The present study examined organism response to well-characterized hydrodynamic conditions in pipe flow. Unlike Rohr et al. (1990), who used the same apparatus but without pressure drop measurements, the present study included pressure drop measurements along the pipe in order to determine if the flow was laminar or turbulent, fully developed or developing. This capability makes it possible to resolve whether the bioluminescent response of the organism was sensitive to the laminar or turbulent nature of the shear stress, as discussed by Rohr et al. (1990). In the present study, stimulated bioluminescence for each fully developed flow has been expressed as a function of shear stress at the pipe wall, where maximum levels occur. Tests of laminar and forced turbulent flows with an overlapping range of shear stress demonstrate that the flow-induced response of individual cells of *L. polyedrum* was best characterized by the shear stress alone and was insensitive to the laminar or turbulent nature of the flow. Although the mechanotransduction process whereby a hydromechanical stimulus results in the emission of light remains unknown, presumably the fluid shear force strains the cell membrane, thereby activating mechanoreceptors that initiate a signal transduction pathway, ultimately leading to a luminescent flash. The final steps of this sequence involve a tonoplast action potential (Eckert 1966; Widder and Case 1981), which leads to acidification of the cytoplasm due to proton flux from the vacuole to the cytoplasm, directly activating the luminescent chemistry (summarized by Fritz et al. 1990).

Response threshold—Response thresholds are notoriously difficult to measure because at threshold, the probability of response is very low. Nevertheless, the response threshold for *L. polyedrum* bioluminescence was quite robust, with a standard deviation of 0.13 N m^{-2} over a range of cell concentrations covering approximately four orders of magnitude (from 10^{-1} to 10^{3} cells ml^{-1}). The mean threshold τ_{wall} of 0.21 N m^{-2} for high concentration experiments was similar to the response threshold of 0.1 N m^{-2} obtained for similar concentrations of *L. polyedrum* experiencing Couette flow (Latz et al. 1994). The similarity in response thresholds of *L. polyedrum* measured for two independent flow conditions strongly suggests that the threshold is flow field independent.

For all experiments, the response threshold for *L. polyedrum* occurred in laminar flow at an average τ_{wall} of 0.32 N m^{-2}. This value is similar to those obtained for freshly collected plankton in the San Diego area, containing a mixture of *L. polyedrum* and other dinoflagellates, using the identical pipe flow apparatus (Rohr et al. 1997, 1998). The ecological significance of the threshold level of shear stress, which is several orders of magnitude greater than shear present in typical oceanic flows, has been discussed by Latz et al. (1994). A response threshold based on sensitivity to this lev-

el of shear stress is consistent with an antipredation function of dinoflagellate bioluminescence and accounts for the occurrence of bioluminescence under a variety of field and experimental conditions.

Cells experienced above-threshold shears for only a short time while entrained in the pipe flow. Based on mean flow rate, cells were exposed for approximately 20 s at near-threshold flows and only 0.4 s at the highest flow rates. Therefore, the experimental flow field represented a short-term exposure of cells to flow stimuli, with continuous replenishment of new cells into the field of view of the detector. There was no evidence of cell damage from these brief exposures. Microscopic examination of cells collected at the pipe exit confirmed that cells were intact and swimming after their transit down the pipe and through the exit valve. While not meant to mimic natural oceanic conditions, the pipe flow field is not unlike short-term conditions found in transient features such as breaking waves, where cells might experience high shear for brief periods on the order of seconds. Longer exposure is known to affect motility and cell division in dinoflagellates at much lower shear levels. A 15-min period of laminar Couette flow inhibits the population growth of red tide dinoflagellates at shear levels two orders of magnitude lower than the bioluminescence response threshold (Thomas and Gibson 1990*a;* Thomas et al. 1995). Therefore, dinoflagellates are sensitive to much lower levels of shear at longer periods than the brief exposure times used in the present study.

Gentle prestirring with a paddle rotating at 0.5 rpm, which did not stimulate flashes, had no significant effect on population response threshold. However, after exposure to more active mixing with a reciprocating mesh screen, which was observed to stimulate flashes, the resulting population tested in pipe flow exhibited a shift in the response pattern consistent with selective prestimulation of the more sensitive cells. These results suggest that energetic oceanic conditions that prestimulate a portion of the in situ population may have little effect on levels of stimulated bioluminescence at high shears. Although the escape response of copepods habituates to turbulent conditions (Hwang et al. 1994), it is unknown whether the mechanical sensitivity of dinoflagellates is modified by their turbulence prehistory.

Response in laminar flows—Both unialgal populations of *L. polyedrum* and mixed plankton samples showed an increase in mean bioluminescence intensity for laminar flows with τ_{wall} ranging from threshold to approximately 1 N m^{-2} (Rohr et al. 1997, 1998; present study). The results of the present study demonstrated that this increase was primarily due to an increase in the proportion of responding cells and, to a lesser extent, an increased flash brightness. The population response rate at the position of the detector increased several orders of magnitude, to a maximum of approximately 10% in high laminar flow at τ_{wall} = 1–2 N m^{-2}. Although levels of maximum intensity also increased within this range, the degree of increase was less than one order of magnitude.

In the dinoflagellate *Pyrocystis fusiformis,* cells stimulated at near-threshold shear levels in Couette flow produce dimmer flashes than those at higher stimulus levels (Latz et al. 1994), most likely because gentle mechanical stimulation results in localized luminescent activity (Widder and Case 1982) rather than a flash response involving the whole cell. Even though *L. polyedrum* is not known to produce a local response, the increase in maximum intensity in laminar flow suggests that the flash intensity of individual cells does vary somewhat with stimulus strength. There was no evidence that flash shape changed as a function of stimulus strength, because the rate of exponential flash decay did not vary with wall shear stress.

Advective vs. hydrodynamic effects—Increases in flow rate will not only increase shear stress levels but will also increase the number of cells advected past the fixed detector due to increasing mean flow velocity, U_{avg}. The effect of advection on mean bioluminescence is a function of the duration of the flash response relative to the residence time of the flash while in the field of view of the detector. As discussed by Widder et al. (1993) for bathyphotometers and Rohr et al. (1994) in the context of pipe flow, if the residence time of a flash in view of the detector is much less than the flash duration, then there is no explicit dependence between mean bioluminescence intensity and U_{avg}. This occurs because the increase in the flux of flashes due to a higher flow rate will be offset by a reduction in the duration of the flash within the detector field of view. By contrast, if the residence time of a flash is much greater than the flash duration, then there is a linear dependence between mean bioluminescence intensity and U_{avg}. Therefore, the increase in bioluminescence with increasing flow will have one component due to the effects of increased hydrodynamic stimulation and one due to advective effects.

For the present pipe flow conditions, Rohr et al. (1994) have shown that advective affects would cause an essentially linear increase in bioluminescence with increasing flow rate. Because wall shear stress increases linearly with flow rate in laminar, fully developed pipe flow, bioluminescence will increase linearly with the mean velocity due to advective effects alone. However, the increase in mean bioluminescence with laminar flow rate, due primarily to an increasing proportion of responding cells, was much greater than linear. Therefore, hydrodynamic stimulation due to the effect of shear was much greater than advective effects.

Relationship between responses of unialgal cultures and mixed plankton samples—The present results for cultured *L. polyedrum* and those for mixed plankton samples (Rohr et al. 1997, 1998) previously measured in the identical pipe flow apparatus allow a direct comparison of unialgal cultures to mixed field populations. The response threshold occurs at similar levels of shear stress, there is a similar increase in mean bioluminescence intensity with increasing shear stress, and there is a similar maximum in response that occurs in high laminar flow. Because *L. polyedrum* is one of the most abundant luminescent organisms in the coastal areas of southern California (Lapota et al. 1994), the response of mixed plankton samples may reflect that of *L. polyedrum.* The similar response in pipe flow between red tide seawater samples dominated by *L. polyedrum* and cultured cells at equivalent concentrations further suggests that the shear stress response of natural populations is similar to that of

cultured dinoflagellates and that the results of laboratory experiments may be extrapolated to field conditions.

Laminar vs. turbulent flow—Even though turbulent flow is routinely used in bathyphotometers to stimulate in situ bioluminescence (e.g., Swift et al. 1983; Losee et al. 1985; Widder et al. 1993), dinoflagellates also respond to laminar flows. The luminescent response threshold of cultured dinoflagellates and mixed plankton samples occurs in laminar Couette and pipe flow at shear stress levels of approximately 0.1–0.3 N m^{-2} (Latz et al. 1994; Rohr et al. 1997, 1998; present study). In the present study of *L. polyedrum,* experiments with low cell concentrations in which individual flashes could be discerned at all flow rates revealed that the maximum response of the individual cell was reached in high laminar flow and did not change with increasing shear stress upon transition to turbulence. The experiment using an overlapping range of laminar and forced turbulent flows demonstrated that bioluminescence was better characterized by wall shear stress than flow rate. At equivalent shear stress values, turbulent flow was no more stimulatory than laminar flow.

The size of the smallest turbulent eddy is on the order of the Kolmogorov length scale, L_k, defined as (Tennekes and Lumley 1972)

$$L_k = \left(\frac{\nu^3}{\epsilon}\right)^{1/4} \quad (6)$$

indicating that the kinematic viscosity (ν) and the rate of energy dissipation per unit mass (ϵ) govern small-scale motion. In fully developed pipe flow, L_k can be estimated based on the dissipation rate near the pipe wall (ϵ_{wall}) or averaged across the pipe (ϵ_{avg}) (Rohr et al. 1994). Because the position of cell injection in turbulent flow had no effect on downstream bioluminescence, it is likely that most cells experienced a range of L_k as they were mixed across the pipe.

Because dinoflagellates are much smaller than the Kolmogorov eddy scale for most oceanic flows, they experience turbulence as laminar flow that varies temporally in the magnitude and direction of shear (Lazier and Mann 1989). In fully developed pipe flow, the turbulent eddy scale L_k was less than the size of *L. polyedrum* cells throughout the range of turbulent flows (Fig. 9). However, eddies at the Kolmogorov scale do not contain appreciable shear energy; significant shear energy is considered to exist at scales 5–10 times greater than L_k (Lazier and Mann 1989). Thus, for *L. polyedrum* cell size to approach the smallest eddy size containing appreciable energy, τ_{wall} (and thus Re) would have to be much higher than possible in the present flow apparatus.

The length scales of the turbulent eddies have previously been believed to be a significant factor for effective bioluminescence stimulation (Rohr et al. 1990; Widder et al. 1993). When turbulent eddies are smaller than the organisms, they should be more effective in distorting rather than advecting them. For example, animal cells in suspension culture exhibit mechanical damage when the Kolmogorov scale becomes smaller than the cell size (reviewed by Hua et al. 1993).

The present experiments with *L. polyedrum,* along with

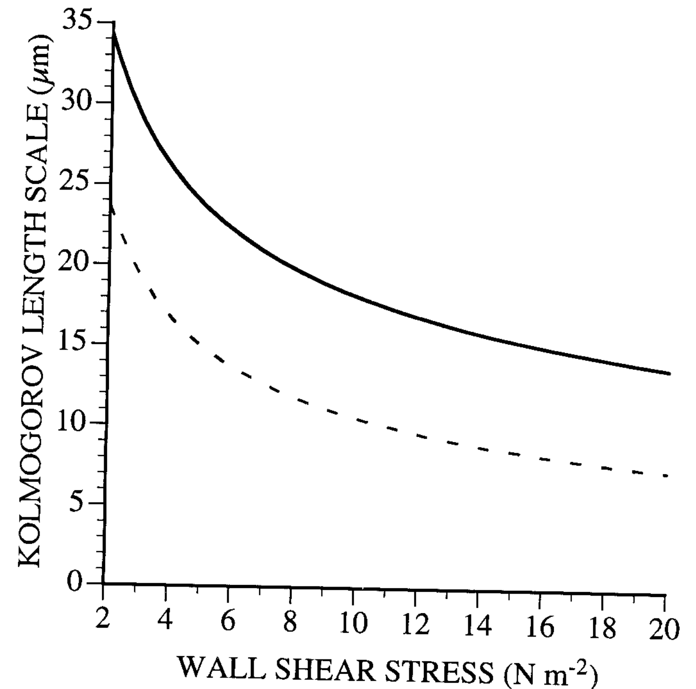

Fig. 9. Calculated Kolmogorov length scale (L_k) as a function of wall shear stress for fully developed pipe flow. Eddy scale was calculated according to Eq. 6 based on energy dissipation rate averaged across the pipe (ϵ_{avg}, solid line) and near the pipe wall (ϵ_{wall}, dashed line) (Rohr et al. 1994). L_k was smaller than the size of *L. polyedrum* cells for all turbulent flows.

those performed with mixed plankton samples (Rohr et al. 1997), suggest that the stimulation of bioluminescence is unaffected by turbulent length scale. Light emission was stimulated throughout a range of laminar flows, where no eddies and thus no length scales were present. The luminescent response of cells reached a maximum in the high laminar regime, for flows with $\tau_{wall} > 1$ N m^{-2}, with no further increase in maximum intensity and flashing rate at higher flow rates, including turbulent flow conditions. Future studies at higher turbulent flows are needed to determine whether length scales much smaller than the cell size affect bioluminescence.

Using the same governing parameters of small-scale motion, the Kolmogorov time scale, L_T, is defined as (Tennekes and Lumley 1972)

$$L_T = \left(\frac{\nu}{\epsilon}\right)^{1/2}. \quad (7)$$

The Kolmogorov time scale is representative of the turnover time of eddies at the Kolmogorov length scale. Estimates of L_T based on the energy dissipation rate near the pipe wall ranged from 1.2 ms (for Re = 2,400, τ_{wall} = 0.91 N m^{-2}) to 0.03 ms (for Re = 20,000, τ_{wall} = 37.2 N m^{-2}), whereas corresponding values of L_T based on the average energy dissipation rate across the pipe wall ranged from 2.1 to 0.11 ms. In either case, calculated L_T values were much less than the response time scales of the organism (20-ms latency and 150-ms duration). Although the response of organisms was

unaffected by the turbulent length and time scales of the flow, the biological implications of this interaction remain poorly understood.

It has previously been suggested that bioluminescence can be mechanically stimulated by changes in shear, acceleration, and pressure and not by constant values of these parameters (Anderson et al. 1988). While such flow fluctuations may be stimulatory, previous (Latz et al. 1994) and present results have demonstrated that such flow fluctuations are not required for stimulation. Furthermore, there was no change in cell response upon transition from laminar flow, where cells experienced constant shear conditions, to turbulent flow, where cells were subjected to fluctuating shear stresses.

The results of the present study for an internal flow are also applicable to external flows. Suprathreshold levels of shear are likely to exist within the boundary layers of moving objects such as fish or dolphins (Latz et al. 1995; Rohr et al. 1998). Increases in boundary layer thickness as a result of laminar to turbulent transition or flow separation will result in a brighter luminescent signature because of a greater stimulatory volume and hence, the larger number of organisms entrained in these high shear regions. Shear-stimulated bioluminescence is not only an excellent way to investigate the short-term response of dinoflagellates to fluid motion but, under suitable viewing conditions, is also an excellent candidate for flow visualization of laboratory and oceanic flows.

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