JOURNAL OF PLANKTON RESEARCH | VOLUME 26 | NUMBER 12 | PAGES 1529–1546 | 2004

Bioluminescence response of four species of dinoflagellates to fully developed pipe flow

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Received March 27, 2004; accepted in principle July 14, 2004; accepted for publication August 23, 2004; published online September 30, 2004

Dinoflagellate bioluminescence provides a nearly instantaneous index of flow sensitivity. This study compared flow sensitivity in four species of morphologically diverse luminescent dinoflagellates (Ceratium fusus, Ceratocorys horrida, Lingulodinium polyedrum and Pyrocystis fusiformis) using fully developed laminar and turbulent pipe flow. Bioluminescence response thresholds always occurred in laminar flows with wall shear stress levels that, depending on species, ranged from 0.02 to 0.3 N m^{-2}. With few exceptions, such as breaking waves and wave-forced bottom shears in shallow nearshore areas, these threshold shear stress levels are several orders of magnitude larger than typical oceanic ambient flows. For laminar flows above threshold, species also differed in the proportion of organisms responding and the minimum shear stress level where individual flashes reached their highest intensity. Following transition to turbulent flow, there was never a dramatic increase in bioluminescence, even when energetic turbulent length scales were similar to the cell size. On the basis of their bioluminescence response in laminar flow, these species were ranked in order of decreasing sensitivity as C. horrida > P. fusiformis > C. fusus > L. polyedrum. This ranking, though not conclusive, is consistent with increased flow sensitivity due to increasing size and the presence of spines. With the exception of a small fraction of the C. horrida population that is sensitive enough to flash within the feeding current of a predator, the present study suggests that flashes only occur with predator contact. Nevertheless, flow sensitivity may serve as an index of the response to mechanical agitation during predator contact/handling. Flow sensitivity may be constrained to maximize the response to predator contact/handling while minimizing stimulation by background oceanic flows to avoid depleting luminescent reserves.

INTRODUCTION

Morphological features affect how plankton interact with their immediate hydrodynamic environment. Cell shape affects boundary layer dynamics and hence nutrient flux (Pasciak and Gavis, 1975; Karp-Boss *et al.*, 1996; Pahlow *et al.*, 1997), drag (Hoerner, 1965) and sinking rates (Walsby and Xypolyta, 1977; Fogg, 1991; Sommer, 1996; Estrada and Berdalet, 1997; Margalef, 1997; Zirbel *et al.*, 2000). For non-spherical organisms, oscillatory and erratic motion between the organism and the fluid may also occur (Mason, 1954; King, 2002). Rotation and flow alignment depend on organism shape and stiffness (Mead and Denny, 1995; Karp-Boss and Jumars, 1998; Karp-Boss *et al.*, 2000). Flow sensitivity in plant cells grown *in vitro* is attributed to their relatively large cell size (20–150 μm diameter), rigid cell walls and large vacuoles (Joshi *et al.*, 1996). Therefore, the hydrodynamic stimulus experienced in the same flow field by cells with different morphologies may vary. Shear sensitivity of cells in bioreactors and agitated microcarrier cultures is also dependent on the length scales of the turbulence relative to cell size (Cherry and Papoutsakis, 1986, 1988, 1989; Croughan and Wang, 1989; Lakhotia and Papoutsakis, 1992; Hua *et al.*, 1993; Joshi *et al.*, 1996). Consequently, for the same cell morphology, the stimulatory nature of

doi:10.1093/plankt/fbh141, available online at www.plankt.oupjournals.org

laminar and turbulent flow may be significantly different. Cells can also change their morphology (Schöne, 1970; Zirbel *et al.*, 2000; Barbee, 2002; Sullivan *et al.*, 2003) in response to changing flow conditions.

Dinoflagellate bioluminescence is a powerful model system for assessing flow sensitivity because each flash is a near-instantaneous reporter (Eckert, 1965; Widder and Case, 1981a) to suprathreshold levels of shear in the immediate fluid environment of a single cell (Hamman and Seliger, 1972; Latz *et al.*, 1994, 2004). Previous studies have shown that fluid pressure (Gooch and Vidaver, 1980; Krasnow *et al.*, 1981; Donaldson *et al.*, 1983) and acceleration (Latz *et al.*, 2004) associated with general oceanic conditions are relatively unimportant compared to shear stress for the stimulation of dinoflagellate bioluminescence (Rohr *et al.*, 2002; Latz *et al.*, 2004). Turbulent length scales have also been considered important parameters for bioluminescence stimulation (Anderson *et al.*, 1988; Widder *et al.*, 1993; Latz *et al.*, 1994; Rohr *et al.*, 1997).

The bioluminescence response of the dinoflagellates *Ceratium fusus*, *Ceratocorys horrida* and *Pyrocystis fusiformis* was investigated using fully developed laminar and turbulent pipe flow and compared to previous results with *Lingulodinium polyedrum* (Latz and Rohr, 1999), for which a representative subset of those data is included for comparison. These species offer an interesting comparison because of their interspecific range in cell size (equivalent spherical diameter = 40–340 μm), cell shape (fusiform, spherical and spined) and the possession or absence of thecae, which are rigid polysaccharide plates comprising the cytoskeleton (Fig. 1, Table I). Relative sensitivity was assessed on the basis of various bioluminescence parameters of organism and population response.

In the context of the present study, morphological features thought to affect flow sensitivity of bioluminescent dinoflagellates include cell size, the presence of spines and the possession of thecae or cell wall. Generally, organisms experience increasing levels of shear stress with increasing size (Denny, 1988). In turbulent flow, the larger the organism relative to the energetic scales of the turbulence, the more effective the turbulence is at deformation (Levich, 1962). The presence of spines may also result in increased flow sensitivity (Zirbel *et al.*, 2000) because the spines could act as levers, accentuating the fluid shear around the cell at the base of the spine. A stiff cell wall may decrease flow sensitivity by minimizing flow-induced deformation (Märkl *et al.*, 1991; Namdev and Dunlop, 1995; Joshi *et al.*, 1996) or possibly increase sensitivity by distributing external forces across the cell as observed for other cell types (Helmke and Davies, 2002). Dinoflagellate thecae may act similarly as a cell wall. Without knowledge of the mechanotransduction mechanism responsible for flow-induced bioluminescence, and because morphological features co-vary among species, it is impossible to determine conclusively which morphological features are most important to flow sensitivity. Nevertheless, this study provides a foundation for interpreting the relationship between cell morphology and flow sensitivity.

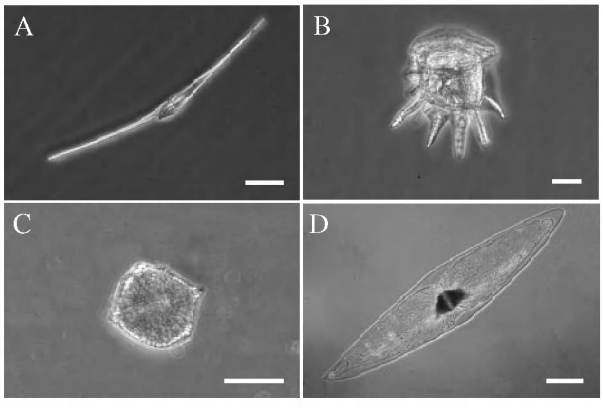

Fig. 1. Images of live cells of the bioluminescent dinoflagellates (**A**) *Ceratium fusus* (scale bar = 50 μm), (**B**) *Ceratocorys horrida* (scale bar = 25 μm), (**C**) *Lingulodinium polyedrum* (scale bar = 25 μm) and (**D**) *Pyrocystis fusiformis* (scale bar = 100 μm). Images were obtained using phase optics.

Ecologically, dinoflagellate bioluminescence is considered to serve an antipredation function, decreasing grazing pressure. Bioluminescent flashes may act as a 'startle response' to directly disrupt predator swimming behavior (Esaias and Curl, 1972; White, 1979; Buskey and Swift, 1983, 1985; Buskey *et al.*, 1983) and/or as a 'burglar alarm' to attract visual predators that prey upon the organisms (e.g. copepods) that graze upon the dinoflagellates (Burkenroad, 1943; Morin, 1983; Mensinger and Case, 1992; Abrahams and Townsend, 1993; Fleisher and Case, 1995). Regardless of the specific strategy, the range of shear sensitivity must be limited for dinoflagellate bioluminescence to have a beneficial antipredation affect. If the threshold shear stress is too low, ambient ocean flows would continually exhaust the cell's bioluminescence potential, and if too high, bioluminescence would not be stimulated by the predator. In a similar way, the response thresholds for escape behavior of copepods appear to be tuned to environmental shear levels (Fields and Yen, 1997).

Although dinoflagellate bioluminescence occurs in the vicinity of grazing copepods (Buskey *et al.*, 1985), it is unknown whether the bioluminescence of the studied dinoflagellates is stimulated by fluid shear in the feeding current of the predator or by contact with the predator. Both modes of stimulation involve mechanical deformation of the cell that if sufficient will activate a calcium signaling pathway (von Dassow, 2003), generation of a vacuole action potential (Eckert, 1966; Widder and

Table I: Morphological and bioluminescence characteristics of the dinoflagellate species studied

Species	Cell size (length × width, μm)	Equivalent spherical diameter (μm)[a]	Cell shape	Thecate	Number of flashes cell^{-1}	Flash duration (ms)	Maximum flash intensity (×10^9 photons s^{-1})
Ceratium fusus[c]	320 × 30	73	Elongate	Yes	2	239	1.1
Ceratocorys horrida[d,f]	64 × 53	70	Spherical, 6 apical spines	Yes	7	184[b]	9.2[b]
Lingulodinium polyedrum[f,g,h]	39 × 33	35	Spherical	Yes	2–3	100–150	0.19[b]
Pyrocystis fusiformis[e,i]	970 × 163	374	Fusiform	No	23–62	210[b]	690[b]

[a]Equivalent spherical diameter = (length × width^2)$^{1/3}$.
[b]Values for first flash by the cell. Subsequent flashes exhibit longer rise and decay times (Widder and Case, 1981b; Latz and Lee, 1995).
[c]For freshly collected cells from the north Atlantic during August 1991.
[d]Zirbel *et al.* (2000).
[e]Swift *et al.* (1973).
[f]Latz and Lee (1995).
[g]Biggley *et al.* (1969).
[h]Kamykowski *et al.* (1992).
[i]Widder and Case (1981b).

Case, 1981a) with proton flux into the cytoplasm and pH activation of the luminescent chemistry (Fritz *et al.*, 1990). By determining quantitative levels of shear sensitivity for several species of luminescent dinoflagellates, it is possible to calculate where flashes would occur within an idealized predator feeding current. If shear stress thresholds are higher than those associated with the feeding current, and/or if the flash delay is too long, then bioluminescence may only occur while in direct contact with the predator.

This study, which uses fully developed pipe flow, provides the first quantitative examination of the laminar flow response of *C. fusus* and *C. horrida* and the turbulent flow response of *C. fusus*, *C. horrida* and *P. fusiformis*. These results build on studies of flow-stimulated bioluminescence of cultures of *L. polyedrum* in both fully developed pipe flow (Latz and Rohr, 1999) and laminar Couette flow (Latz *et al.*, 1994) and for mixed plankton samples, where *L. polyedrum* was dominant, in fully developed pipe flow (Rohr *et al.*, 2002). Laminar flow-stimulation studies for *P. fusiformis* in Couette flow (Latz *et al.*, 1994) and *C. horrida* in converging nozzle flow (Latz *et al.*, 2004) suggest that both these species are more flow sensitive than *L. polyedrum*. In previous turbulent pipe-flow studies using *L. polyedrum* (Latz and Rohr, 1999), flow rates were not high enough to produce energetic eddies at the length scales of the organism. In the present study, the sizes of the two largest species (Table I) were on the order of the energetic length scales of the turbulence, so the effect of turbulent length scales can be explored.

METHOD

Organisms

Laboratory cultures of *C. fusus* Ehrenb, *C. horrida* Stein [strain 89A from the Sargasso Sea, see (Latz and Lee, 1995)] and *P. fusiformis* Murray were grown in seawater with f/2 additions at half strength (Guillard and Ryther, 1962) minus silicate as previously described (Latz and Rohr, 1999) on a 12:12 h light–dark cycle. To achieve desired experimental concentrations, we diluted cultures with the addition of 5-μm glass-fiber-filtered (Whatman Inc.) seawater. A target cell concentration of 15 cells mL^{-1}, as previously used with *L. polyedrum* (Latz and Rohr, 1999), was used for all species because at that concentration individual flashes could be resolved essentially throughout all laminar flow conditions. Cell-to-cell and cell-to-wall collisions do not stimulate bioluminescence (Krasnow *et al.*, 1981; Latz *et al.*, 2004). Average bioluminescence in fully developed, turbulent pipe-flow scales with cell concentration (Rohr and Latz, unpublished data), consistent with a negligible bioluminescence contribution from cell-to-cell collisions. In turbulent flow, although flashes would often overlap, preventing accurate measures of the intensity of individual flashes, general trends in maximum flash intensity as a function of wall shear stress could still be discerned.

All four species exhibit endogenous circadian rhythms in spontaneous and stimulated bioluminescence (Sweeney and Hastings, 1957; Seliger *et al.*, 1969; Sullivan and Swift,

1994; Latz and Lee, 1995). Cells were loaded into the head tank of the pipe-flow apparatus at the end of the light phase of the growth light–dark cycle when sensitivity to mechanical stimulation is minimal. Gentle stirring at levels too low to stimulate bioluminescence maintained a homogenous distribution of cells in the head tank. The identical protocol did not affect threshold levels of stimulation for bioluminescence of *L. polyedrum* (Latz and Rohr, 1999). An opaque cover was draped over the apparatus at the beginning of the dark cycle of the cells, and experiments began 2 h later, when maximum levels of bioluminescence occur (Biggley *et al.*, 1969).

Flow field

Fully developed pipe flow was chosen as the experimental flow field because (i) the flow field can be fully characterized in terms of shear stress by simple measurements of volumetric flow and pressure drop, (ii) laminar and turbulent flows with a wide range of shear stresses can be generated, (iii) new organisms are constantly entering the flow field, to minimize potential problems with depletion of bioluminescence, and (iv) the organisms experience the flow field for only a short time. By definition, fully developed pipe flow cannot determine the sensitivity of dinoflagellate cells in developing flows, an issue which must be addressed in a different flow field (von Dassow, 2003).

The pipe-flow apparatus was the identical system used by Latz and Rohr (Latz and Rohr, 1999), consisting of a 75-L acrylic tank attached through a gently constricting inlet to a 6.35-mm internal diameter clear polycarbonate pipe 1 m in length. An adjustable valve manually controlled flow through the pipe, and pressure drop was measured by two pressure ports connected to a variable reluctance differential transducer. Average flow speed was determined by dividing the mass of water collected over a measured time by the cross-sectional area of the pipe. Average flow speed and pressure drop were used to calculate Darcy friction factor (non-dimensional pressure drop) and Reynolds number (non-dimensional flow speed) to ascertain whether the flow was fully developed, laminar, turbulent or transitional (Schlichting, 1979; Latz and Rohr, 1999).

In fully developed laminar and turbulent pipe flow, shear stress is greatest at the pipe wall and decreases linearly to zero at the pipe centerline. The average shear stress across the pipe is two-third of wall shear stress (Schlichting, 1979). The range of turbulent length scales can also be estimated in fully developed pipe flow. The largest turbulent length scales are about the radius of the pipe, whereas the smallest are on the order of the Kolmogoroff scale, L_K, which is derived from dimensional analysis (Tennekes and Lumley, 1972; Gill, 1982) as:

$$L_K = \left(\frac{\nu^3}{\varepsilon}\right)^{0.25} \quad (1)$$

where ν is the kinematic viscosity and ε is the dissipation rate per unit mass, henceforth referred to as the dissipation rate. Calculations of ε at the wall (Rosenhead, 1963) were made and the corresponding L_K was determined. Kolmogorov length scales based on the wall dissipation decreased with increasing flow rate, varying from 15 to 9 μm over the range of turbulent flows investigated (Table I). If average dissipation values are used (Bakhmeteff, 1936), the associated L_K values ranged from 23 to 16 μm. In the ocean, L_K is rarely <1 mm (Thomas and Gibson, 1992; Kiørboe and Saiz, 1995; Jiménez, 1997). Nevertheless, to study the stimulatory effect of the turbulent length scales of the flow relevant to the organism, achieving such small Kolmogorov length scales is necessary.

Multiples of ×1–40 the Kolmogorov length scale are commonly used as an index for the size of the smallest energetic eddy within which smaller entrained organisms should experience laminar flow (Rohr *et al.*, 2002). As in previous studies of dinoflagellate bioluminescence (Rohr *et al.*, 2002), a conservative value of 10 L_K was chosen as representative of the smallest energetic turbulent length scales within which laminar flow presides. By this criterion, for *L. polyedrum*, *C. horrida*, *C. fusus* and *P. fusiformis* to be subjected to the small-scale spatial structure of turbulent flow, 10 L_K would have to be <50, 100, 350 and 900 μm, respectively. In this study, the largest dimension of the cell, rather than its equivalent spherical diameter, was used for comparison with turbulent length scales of the flow.

Bioluminescence measurements and analysis

The photon flux of individual flashes of *C. fusus* and *P. fusiformis* was measured to allow comparison with previous measurements for the other species. These measurements involved the identical apparatus and methods of Latz and Lee (Latz and Lee, 1995). Individual cells within an integrating light chamber were stimulated by intermittent stirring to elicit single flashes, which were measured at 0.010 s resolution using a photon-counting photomultiplier system and analysed as before (Latz and Lee, 1995).

Bioluminescence stimulated by pipe flow was measured by a RCA 8575 photon-counting photomultiplier tube detector located 0.67 m (105 pipe diameters) from the inlet. Photomultiplier measurements in units of counts s^{-1} were converted to photons s^{-1} using a previously described calibration procedure (Latz and Rohr, 1999). The detector was coupled to the pipe using a light-shielded adapter and viewed a 0.05-m length of pipe

and its entire width. Further details of data collection are found in Latz and Rohr (Latz and Rohr, 1999). A time series of the stimulated bioluminescence, composed of flash events measured by the detector, was collected for each flow rate (Fig. 2). The time series record consisted of consecutive 0.005-s signal integrations for durations of 10–100 s as done previously (Latz and Rohr, 1999). Because of gentle stirring prior to testing, sufficient cell homogeneity was achieved and no significant variation in average intensity for similar flow speeds was observed. Measurements were not made for transitional flows where flow was intermittently laminar and turbulent (wall shear stress was between 2 and 8 N m^{-2}). Previous studies of flow-stimulated bioluminescence using unialgal cultures (Latz *et al.*, 1994; Latz and Rohr, 1999) or freshly collected seawater samples in which dinoflagellates were the primary luminescent organisms (Rohr *et al.*, 1994, 2002) have shown that the response is correlated with wall shear stress. Therefore, bioluminescence was expressed as a function of wall shear stress for each flow rate.

Average intensity of bioluminescence was calculated by time averaging each time series record collected at a constant flow rate (Latz and Rohr, 1999). Maximum intensity, an index of the peak intensity of an individual flash, was determined by taking the highest 0.005 ms value in each time series record. Quantitative comparisons of the number and level of individual flash events were limited to laminar flows with wall shear stress <1.5 N m^{-2} because flashes overlapped at higher flow rates. Nevertheless, as in previous studies (Latz and Rohr, 1999; Rohr *et al.*, 2002), general trends for maximum intensity could still be determined throughout higher flow rates.

Threshold levels of wall shear stress required to stimulate bioluminescence were determined from both average and maximum intensity data. For each experiment, average intensity was expressed as a function of wall shear stress for each flow. A power regression model was fitted to laminar flow data that were above background levels. The response threshold was calculated as the wall shear stress value at which the value of the fitted regression model was equal to the average background intensity for that experiment (Latz and Rohr, 1999). For each experiment, threshold was also calculated using a modified flash criterion method (Latz and Rohr, 1999), by averaging values of the minimum wall shear stress

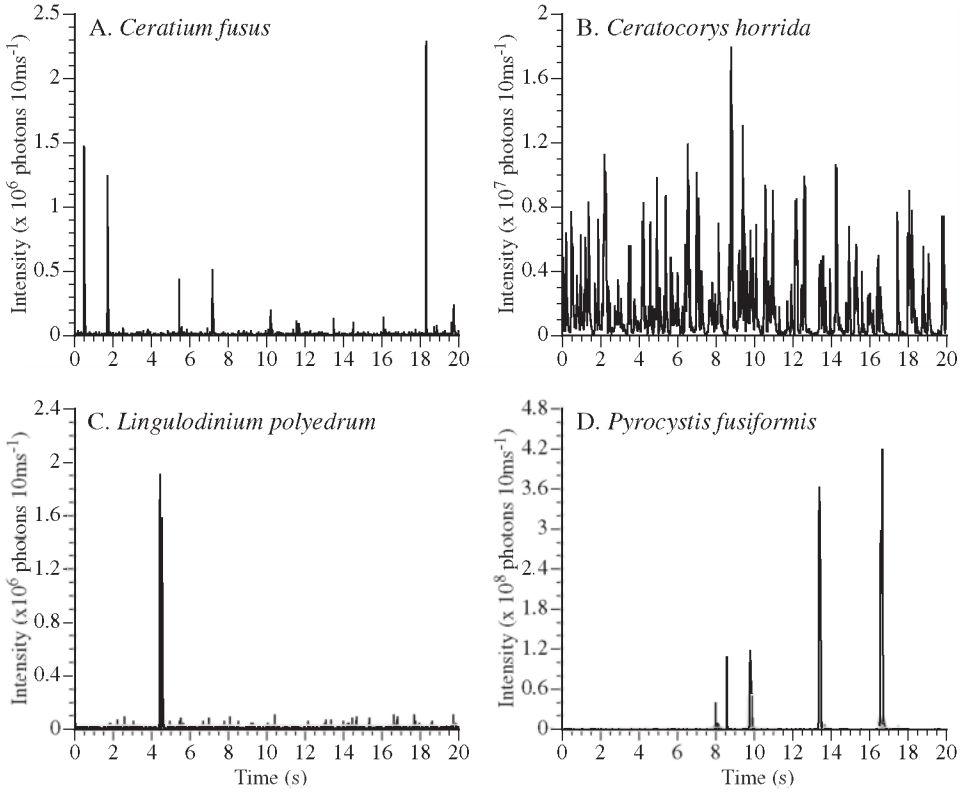

Fig. 2. Representative time series of bioluminescence of (**A**) *Ceratium fusus*, (**B**) *Ceratocorys horrida*, (**C**) *Lingulodinium polyedrum* and (**D**) *Pyrocystis fusiformis* in laminar flows with wall shear stress ≈0.4 N m^{-2}, demonstrating considerable differences in response among species. For example, *C. horrida* showed the highest response rate, while *P. fusiformis* had the brightest flashes.

where flashes occurred and the maximum wall shear stress where flashes did not occur. Average values of threshold wall shear stress were calculated for each species based on values for replicate experiments.

The population response proportion (i.e. proportion of the population flashing in view of the detector at a given flow rate) was calculated as: (flash rate/flow rate)/cell abundance (Latz and Rohr, 1999). Measurements were restricted to wall shear stresses <1.5 N m^{-2} because of the overlap of flashes at higher flow rates. It was assumed that, owing to the short residence time of cells in the field of view of the detector, each flash registered was from a different cell.

Threshold and maximum flash intensity levels for a fixed flow volume are best presented per unit time (i.e. units of photons s^{-1}) (Latz and Rohr, 1999; Rohr *et al.*, 2002). However, measurements of average intensity are often expressed per unit volume [i.e. (photons s^{-1}) (volume flow rate in units of m^3 s^{-1})$^{-1}$; (Widder *et al.*, 1993; Latz *et al.*, 1994)]. This particular representation attempts to account for advective effects that are most pronounced when the residency time of the flash within the view of the detector is long compared to its duration (Seliger *et al.*, 1969; Widder *et al.*, 1993; Widder, 1997). Average intensity of bioluminescence was expressed both as photons s^{-1}, to better identify response thresholds, and as photons m^{-3} to account for advection. Extending the mathematical analysis for bathyphotometers (Seliger *et al.*, 1969; Widder *et al.*, 1993) to fully developed pipe flow, Rohr *et al.* (Rohr *et al.*, 1994) derived a relationship between average intensity, flash kinetics and organism residency times. Throughout the present range of wall shear stress, advection accounted for a linear increase in average intensity as a function of increasing average flow rate.

The effect of advection on the relationship between average intensity and wall shear stress will be manifested differently in fully developed laminar and turbulent pipe flows. Wall shear stress increases to the first power with average flow rate in laminar flow, but as the 1.75 power in turbulent flow (Schlichting, 1979). Conversely, average flow rate increases to the first power as a function of wall shear stress in laminar flow and to the 1/1.75 power with wall shear stress in turbulent flow. Consequently, dividing bioluminescence intensity (photons s^{-1}) by the corresponding volume flow rate (m^3 s^{-1}) decreases the slope of the power law regression by 1 for laminar flow and by 1/1.75 for turbulent flow.

One-way analysis of variance (ANOVA) with post-hoc comparison of means using Fisher's Protected Least Significant Difference (PLSD) was used to test the significance of differences among species. Otherwise, paired or unpaired *t*-tests were used for pair-wise comparisons. Statistical tests were performed using Statview software (SAS Institute, Inc.). Four replicate experiments were performed for each species, except for *L. polyedrum* where data were obtained from six previous experiments (Latz and Rohr, 1999). Unless otherwise stated, values represent means with standard deviations of the mean from this data set.

RESULTS

Threshold sensitivity

The bioluminescence response threshold occurred in laminar flow for all species examined. For each experiment, there was no significant difference between threshold values calculated from the flash criterion method and threshold values calculated from the regression method (Table II; Paired *t*-test, $t = 0.21$, $P = 0.8$). Further analysis used threshold values based on the regression method because it is less sensitive to differences in the flow rate increment (Latz and Rohr, 1999).

Threshold values of wall shear stress were 0.116 ± 0.02 N m^{-2} for *C. fusus*, 0.024 ± 0.009 N m^{-2} for *C. horrida* and 0.087 ± 0.02 N m^{-2} for *P. fusiformis*.

Table II: Response threshold in fully developed laminar pipe flow, expressed as a function of wall shear stress

Species	*N*	Threshold wall shear stress (N m^{-2})	
		Based on regression method	Based on flash criterion method
Ceratium fusus	4	0.116 ± 0.025 (0.091–0.149)	0.123 ± 0.053 (0.058–0.175)
Ceratocorys horrida	4	0.024 ± 0.009 (0.011–0.033)	0.039 ± 0.011 (0.028–0.053)
Lingulodinium polyedrum	6	0.333 ± 0.104 (0.202–0.486)	0.298 ± 0.110 (0.162–0.448)
Pyrocystis fusiformis	4	0.087 ± 0.025 (0.064–0.121)	0.106 ± 0.041 (0.06–0.143)

See text and Latz and Rohr (Latz and Rohr, 1999) for a description of methods used to calculate thresholds. Values represent mean ± standard deviation; the range of values is given in parentheses. *N*, number of experiments for each species.

There was a significant difference in thresholds among species (ANOVA, $F = 19.8$, $df = 2,9$, $P < 0.0005$), with the threshold for *C. horrida* being significantly different from those of the other two species (Fisher's PLSD post-hoc test, $P \leq 0.002$ for each), which were not significantly different from each other (Fisher's PLSD, $P = 0.09$). The threshold for *L. polyedrum* of 0.333 ± 0.1 N m^{-2} (Latz and Rohr, 1999) was significantly different from that of the other three species (Fisher's PLSD, $P = 0.0001$).

Flow agitation levels in the ocean are typically characterized by the dissipation rate of kinetic energy per unit mass, ε. The corresponding bioluminescent threshold levels, calculated at the pipe wall, are $\varepsilon = 1.2 \times 10^{-2}$ m^2 s^{-3} for *C. fusus*, 5.2×10^{-4} m^2 s^{-3} for *C. horrida*, 0.1 m^2 s^{-3} for *L. polyedrum* and 6.8×10^{-3} m^2 s^{-3} for *P. fusiformis*. These dissipation levels are orders of magnitude larger than typical values found within the ocean's interior (Kunze and Sanford, 1996).

Population response proportion in laminar flow

The population response proportion response (units of flashes cell^{-1}) is an index of population sensitivity. Population response proportion measurements were restricted to laminar flows with wall shear stress values <1.5 N m^{-2}, where flash coincidence did not occur. The slope of the power regression (Fig. 3) indicated how the response proportion changed as a function of wall shear stress between threshold and the highest laminar flows measured. The response proportion for all species increased over this range. For *C. fusus*, the slope was 1.2 ± 0.6, while it was 1.6 ± 0.5 for *P. fusiformis*. *Lingulodinium polyedrum* had the largest slope, 5.1 ± 1.3, with the response proportion changing by a factor of 1000 over the range of wall shear stresses examined. *Ceratocorys horrida* exhibited the least change with a slope of 0.7 ± 0.2. For wall shear stresses >0.1 N m^{-2}, the response proportion of *C. horrida* remained nearly constant at ~0.1 flashes cell^{-1}.

One criterion for flow sensitivity in this study is the population response proportion at the shear stress response threshold. Minimum values of the population response proportion (i.e. the proportion of cells responding), calculated from the power regression for threshold wall shear stress values for pooled data from each species, varied more than two orders of magnitude among species. The population response proportion was 0.0012 flashes cell^{-1} for *C. fusus*, 0.0264 flashes cell^{-1} for *C. horrida*, 0.0003 flashes cell^{-1} for *L. polyedrum* and 0.0010 flashes cell^{-1} for *P. fusiformis* (Fig. 3). Thus, *L. polyedrum* had the lowest population response proportion at its threshold, while *C. horrida* had the highest. At the highest laminar flow rates, the population response

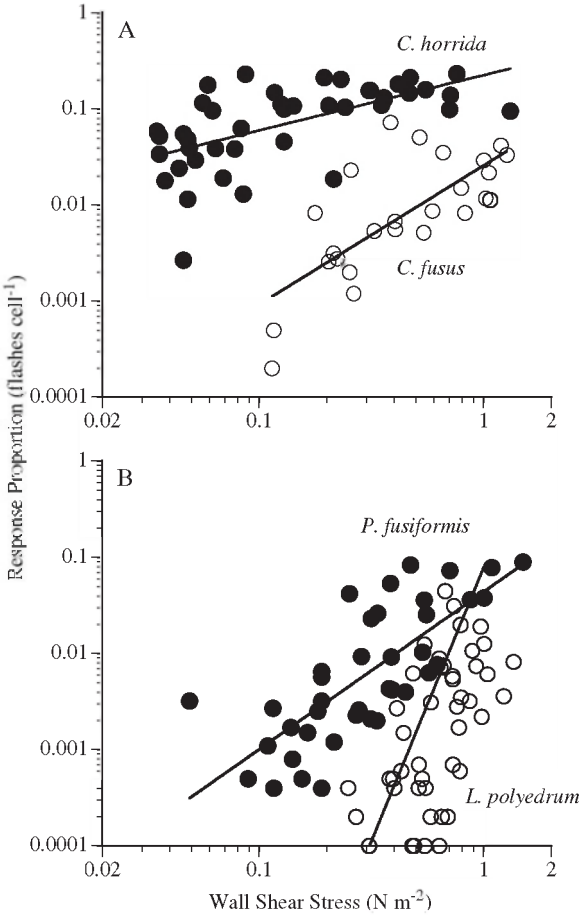

Fig. 3. The population response proportion, expressed as flashes cell^{-1}, in laminar flow as a function of wall shear stress for (**A**) *Ceratium fusus* (open circles) and *Ceratocorys horrida* (solid circles) and (**B**) *Lingulodinium polyedrum* (open circles) and *Pyrocystis fusiformis* (solid circles). Symbols represent results from single flow rates for data pooled from all experiments.

proportion varied among species by less than an order of magnitude. Maximum values of the population response proportion, based on the power regression analysis of the pooled species data, were 0.036 flashes cell^{-1} for *C. fusus*, 0.258 flashes cell^{-1} for *C. horrida*, 0.1 flashes cell^{-1} for *L. polyedrum* and 0.1029 flashes cell^{-1} for *P. fusiformis*.

Maximum intensity in laminar and turbulent flows

Maximum intensity is the brightest 0.005 s flash event in a data record obtained for a constant flow rate. For laminar flows with wall shear stress values <1.5 N m^{-2}, where coincidence of flashes did not occur, maximum intensity is representative of maximum flash intensity of an individual cell. Maximum intensity increased as a function of suprathreshold levels of wall shear stress,

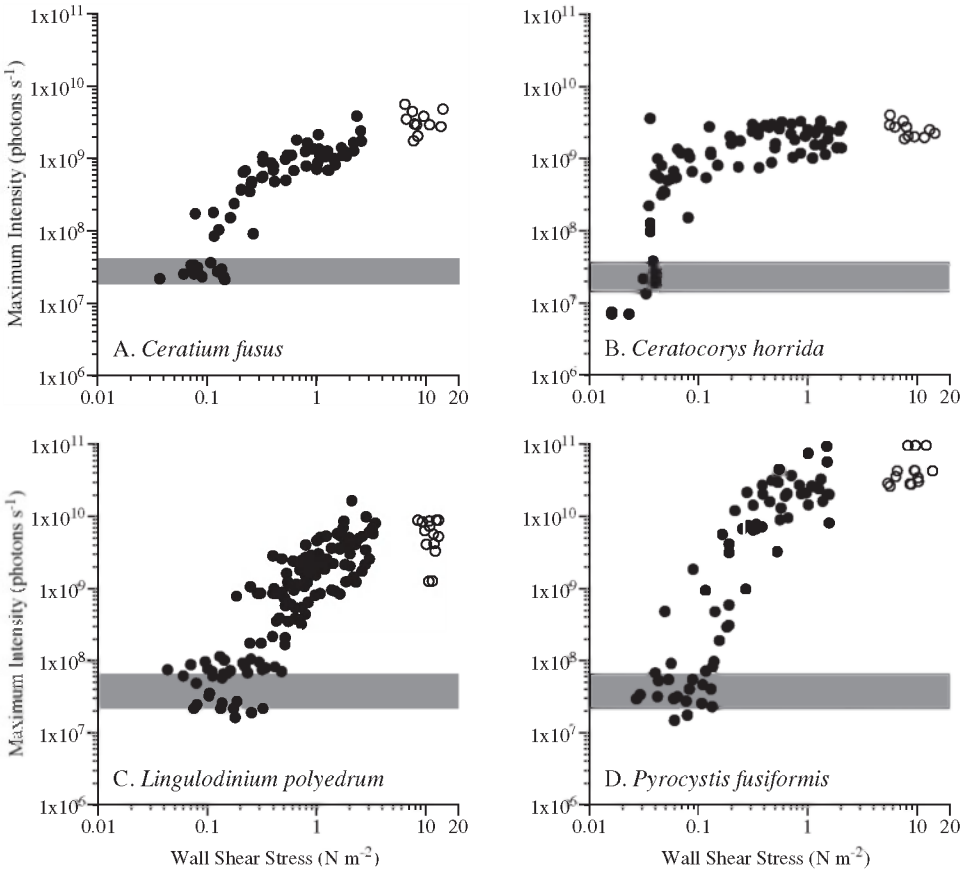

Fig. 4. Maximum intensity as a function of wall shear stress for (**A**) *Ceratium fusus*, (**B**) *Ceratocorys horrida*, (**C**) *Lingulodinium polyedrum* and (**D**) *Pyrocystis fusiformis*. Symbols represent individual values from the pooled set of experiments for each species for laminar (solid) and turbulent (open) flows. Shaded bar indicates the range of background levels for no flow for each set of experiments; at low flows, stimulation was below threshold levels and measurements were at background levels. No data were collected when the flow was transitioning between laminar and turbulent flow, for a wall shear stress of 3–7 N m^{-2}.

approaching a nearly constant level (Fig. 4). Maximum intensity values based on pooled data for each species began to plateau at wall shear stress values of approximately between 2.5 and 6 N m^{-2} for *C. fusus*, 0.1 N m^{-2} for *C. horrida*, 2 N m^{-2} for *L. polyedrum* and 1 N m^{-2} for *P. fusiformis*. The larger uncertainty for *C. fusus* is because transition to turbulence occurred when maximum intensity began to plateau. The highest value of maximum intensity for each species was $3.41 \pm 1.18 \times 10^9$ photons s^{-1} for *C. fusus*, $2.59 \pm 0.67 \times 10^9$ photons s^{-1} for *C. horrida*, $5.77 \pm 2.70 \times 10^9$ photons s^{-1} for *L. polyedrum* and $4.79 \pm 2.75 \times 10^{10}$ photons s^{-1} for *P. fusiformis*.

The general increase in maximum intensity upon transition from high laminar to turbulent flows was, regardless of species, always less than a factor of 3. Only for *C. fusus* was there a highly significant difference in maximum intensity between high laminar and turbulent flows (*t*-test, $t = 5.3$, $df = 25$, $P < 0.001$). The difference for *P. fusiformis* was barely significant ($t = 2.1$, $df = 30$, $P = 0.05$) and the differences for *L. polyedrum* and *C. horrida* were not significant ($t = 1.0$, $df = 25$, $P = 0.3$; $t = 1.7$, $df = 27$, $P = 0.1$ respectively). Given that flash coincidence occurred in turbulent flow and levels of shear stress were considerably higher, the differences in maximum intensity between laminar and turbulent flows for all species were remarkably small.

Average intensity in laminar and turbulent flows

Average intensity, expressed as photons s^{-1}, increased as a function of wall shear stress (Fig. 5). Except for *C. horrida*, this relationship for laminar flow was modeled as a simple power regression ($R^2 = 0.85$–0.87). The regression slope was 1.60 ± 0.04 for *C. fusus*, 4.27 ± 0.44 for *L. polyedrum* and 3.53 ± 0.57 for *P. fusiformis*. For *C. horrida*, there was an inflection at a wall shear stress of 0.4 N m^{-2}. The slope of the power regression for values of wall shear stress <0.4 N m^{-2} was 2.29 ± 0.45, while

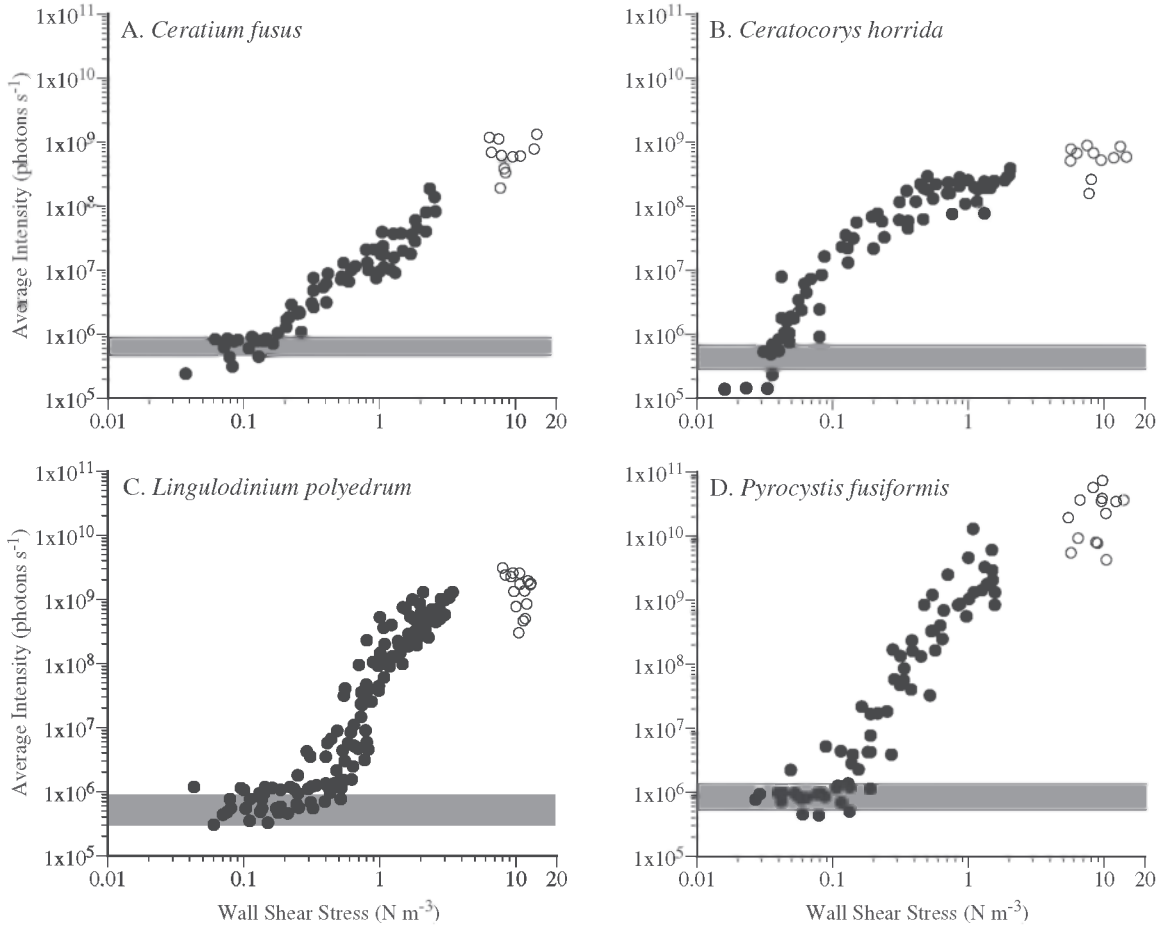

Fig. 5. Average intensity, expressed as photons s^{-1}, as a function of wall shear stress for (**A**) *Ceratium fusus*, (**B**) *Ceratocorys horrida*, (**C**) *Lingulodinium polyedrum* and (**D**) *Pyrocystis fusiformis*. Symbols represent individual values from the pooled set of experiments for each species for laminar (solid) and turbulent (open) flows. Shaded bar indicates the range of background levels for each set of experiments. No data were collected when the flow was transitioning between laminar to turbulent, for a wall shear stress range of 3–7 N m^{-2}.

the slope was 0.14 ± 0.36 for the range of higher laminar flows. For all species, the average intensity in turbulent flow was not greater than that extrapolated from trends found in laminar flow.

Average intensity, expressed as photons m^{-3} (Fig. 6) to account for advective affects in fully developed, laminar pipe flow, is expected to exhibit a decreased dependence on wall shear stress. As expected, the slope of the power regression of intensity (photons s^{-1}) as a function of wall shear stress was decreased by 1 for the photons m^{-3} regression: 0.60 ± 0.04 for *C. fusus*, 3.27 ± 0.44 for *L. polyedrum* and 2.53 ± 0.57 for *P. fusiformis*. For *C. horrida*, the slope of the power regression for values of wall shear stress <0.4 N m^{-2} was 1.29 ± 0.45, while the slope was −0.86 ± 0.36 for higher laminar flows. Except for *C. fusus*, the change in average intensity (photons m^{-3}) associated with transition from laminar to turbulent flows for pooled data was less than or equal to that expected by extrapolating the power regression found in laminar flows to higher values of wall shear stress (Fig. 6).

DISCUSSION

Response threshold

The response threshold represents the response of the most sensitive organisms, which comprise only a small fraction of the population. Threshold levels varied by approximately one order of magnitude among the four species studied. On the basis of average threshold values, the species were ranked in order of decreasing flow sensitivity as *C. horrida* > *P. fusiformis* > *C. fusus* > *L. polyedrum*. The relatively high intraspecific variation in threshold values from different experiments for *C. fusus* and *P. fusiformis* resulted in a lack of statistically significant difference between their thresholds.

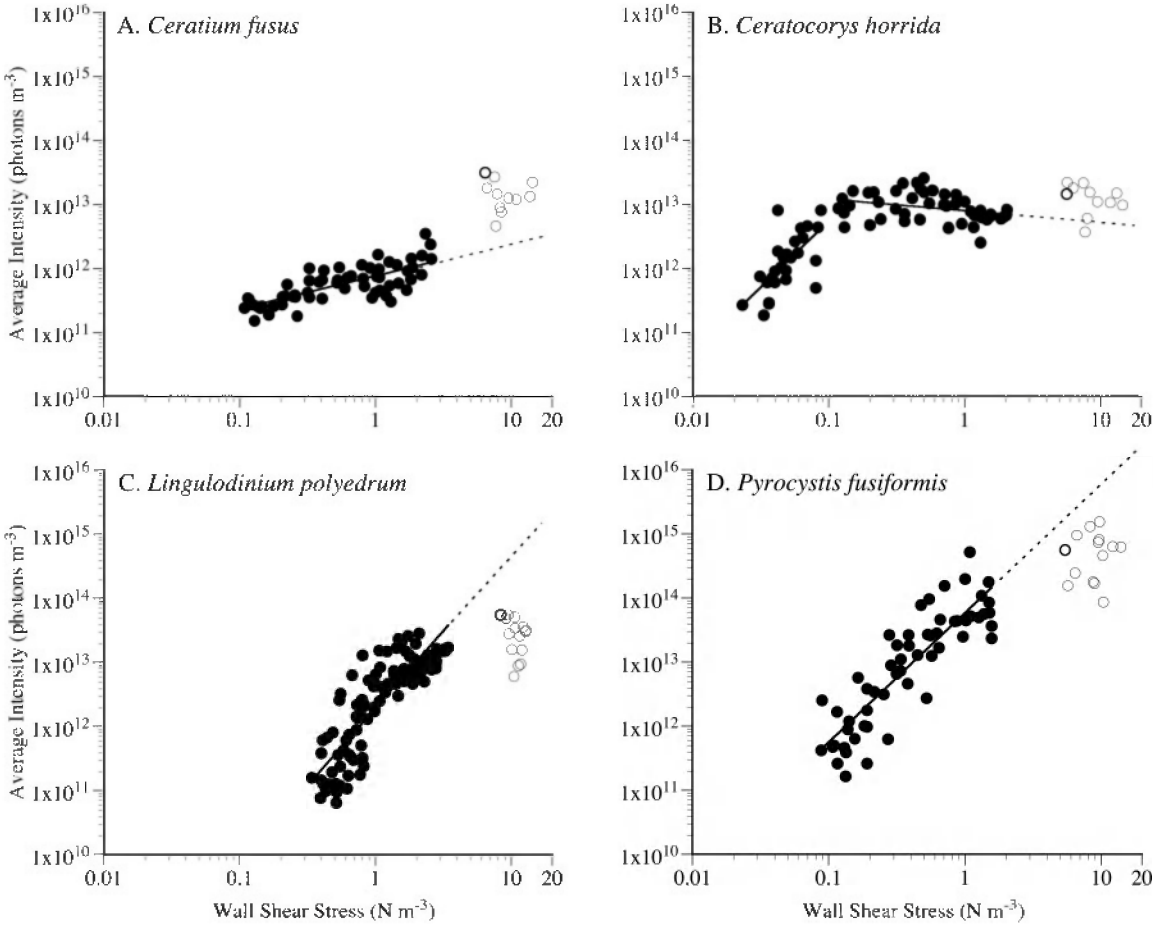

Fig. 6. Average intensity, expressed as photons m^{-3} to account for advective effects, as a function of wall shear stress for suprathreshold flows. The data of average intensity (expressed as photons s^{-1}) shown in Fig. 5 were divided by flow rate (expressed in units of $m^3 s^{-1}$). Symbols represent individual values from the pooled set of experiments for each species for laminar (solid) and turbulent (open) flows. The line represents the least-squares power regression of average intensity as a function of wall shear stress in laminar flow. (**A**) *Ceratium fusus*, $y = 7.63 \times 10^{11} x^{0.51}$, $r^2 = 0.554$, (**B**) *Ceratocorys horrida*, $y = 6.32 \times 10^{14} x^{2.10}$, $r^2 = 0.44$ for flows with wall shear stresses <0.1 N m^{-2}; $y = 7.92 \times 10^{12} x^{-0.18}$, $r^2 = 0.0944$ for flows with wall shear stresses >0.1 N m^{-2}, (**C**) *Lingulodinium polyedrum*, $y = 1.89 \times 10^{12} x^{2.40}$, $r^2 = 0.719$ and (**D**) *Pyrocystis fusiformis*, $y = 5.79 \times 10^{13} x^{2.02}$, $r^2 = 0.760$. The dotted line represents the extrapolation of the regression obtained for laminar flows to higher values of shear stress.

The response threshold for *P. fusiformis* occurred at a wall shear stress of 0.09 N m^{-2}, slightly greater than the shear stress threshold of ~0.06 N m^{-2} obtained in Couette flow (Latz *et al.*, 1994). The threshold value of wall shear stress of 0.3 N m^{-2} for *L. polyedrum* was also slightly greater than for Couette flow, in which the threshold occurred at a shear stress of 0.1 N m^{-2} (Latz *et al.*, 1994). The slightly greater threshold values obtained in pipe flow may be due to the lower cell concentration, which will result in a higher response threshold (Latz and Rohr, 1999). It may also reflect the use of wall shear stress, the maximum across the pipe radius, rather than average shear stress as the correlated parameter (Latz and Rohr, 1999). Whereas in Couette flow the shear is nearly constant as a function of radial position across the gap, in pipe flow there exists a linear gradient of shear stress, with maximum levels at the wall and zero shear stress at centerline (Schlichting, 1979). If organisms are stimulated near the wall, then the local value of shear stress will be less than the maximum value at the wall.

Previous work with *L. polyedrum* determined that cells in laminar flows with wall shear stress values <0.4 N m^{-2} are stimulated near the pipe wall where local values of shear stress were ~82% of maximum levels at the wall (Latz and Rohr, 1999). Therefore, the actual value of stimulatory shear stress for pipe flow may be less than that stated for wall shear stress and more similar to that obtained in Couette flow. The advantage of steady Couette flow is that cells experience a single value of shear stress. However, bioluminescence decreases over time because of exhaustion of luminescent capacity in the population fixed within the flow volume. The advantage of pipe flow is that organisms

are continually replenished in the flow field because of advection. Consequently, extended bioluminescence time series can be obtained during constant flow conditions, without the problem of exhausting luminescent capacity. Nevertheless, considering the different flow characteristics of fully developed pipe and Couette flow, the similarity in response thresholds for these completely independent flow fields indicates that organisms are responding to specific, quantitative aspects of the flow, regardless of the flow field. The bioluminescence response thresholds for *C. fusus* and *C. horrida* have not been previously measured. Previous work using nozzle flow (Latz *et al.*, 2004) corroborates the present quantitative finding that the response threshold for *C. horrida* was less than that for *L. polyedrum*. The range of response thresholds reported here for all species is similar to those of mixed plankton assemblages obtained for Couette and pipe flows (Latz *et al.*, 1994; Rohr *et al.*, 1998, 2002).

For the pipe-flow experiments, the shear exposure time, based on average flow rates, is between 20 s at near threshold flows and 0.4 s at the highest flow rates (Latz and Rohr, 1999). Longer shear exposure, at much lower shear stress levels, affects dinoflagellate swimming, population growth and morphology. For example, shear levels two orders of magnitude less than those associated with bioluminescence stimulation inhibit the population growth of *L. polyedrum* (Thomas and Gibson, 1990; Juhl *et al.*, 2000) at shear exposures >15 min d^{-1} (Gibson and Thomas, 1995). Changes in the morphology and swimming of *C. horrida* occur after 1 h of agitation on an orbital shaker where average shear stress values were an order of magnitude less than bioluminescence threshold values (Zirbel *et al.*, 2000). Thus, dinoflagellates exhibit a range of flow sensitivity related to growth, morphology and bioluminescence that spans at least three orders of magnitude of shear. In some plant cells cultured *in vitro*, flow-induced physiological responses span as much as six orders of magnitude of flow (Namdev and Dunlop, 1995).

Bioluminescence response in laminar flow

All of the dinoflagellate species studied exhibited a pattern of increasing maximum intensity, an index of the intensity of the brightest flash, with increasing wall shear stress in laminar flows above the response threshold. This relationship was not an artifact of residence time in front of the detector because as flow rates increased, the residence time decreased. This pattern has not been previously demonstrated for *C. fusus* and *C. horrida*. A similar pattern of flash intensity for *L. polyedrum* and *P. fusiformis* has been observed in laminar pipe and Couette flows respectively (Latz *et al.*, 1994; Latz and Rohr, 1999). *Pyrocystis fusiformis* had the greatest increase in maximum intensity (measured in photons s^{-1}), with approximately a three order of magnitude increase. This may reflect the ability of this species to respond to low-intensity stimuli with submaximal flashes localized to the area of the cell that is directly stimulated (Widder and Case, 1982).

Dinoflagellate bioluminescence capacity, commonly referred to as total mechanically stimulable luminescence (TMSL), is proportional to cell size (Buskey *et al.*, 1992). This study is the first to examine the relationship between cell size and flash intensity using a range of characterized laminar and turbulent flow conditions. *Ceratocorys fusus* and *C. horrida*, which have similar equivalent spherical diameters, produced similar maximum intensities at the highest turbulent flow rates measured. *Pyrocystis fusiformis*, the largest species tested, had the brightest flashes over the same flow range. However, the maximum intensity of *L. polyedrum* in this same flow range was twice that for *C. fusus* and *C. horrida*, despite *L. polyedrum* being about half the size. In laminar flows, maximum intensity varied with flow stimulus. For example, the maximum intensity of *C. horrida* near the response threshold of *C. fusus* was an order of magnitude greater than that for *C. fusus*, despite it being similar in turbulent flow. Thus, the maximum intensity of flow-stimulated flashes does not necessarily scale with size and can depend on the level of flow stimulus.

The order in which the pooled data of maximum intensity of each species reached a plateau with increasing wall shear stress was *C. horrida* (~0.1 N m^{-2}), *P. fusiformis* (1 N m^{-2}), *L. polyedrum* (2 N m^{-2}) and *C. fusus* (2.5–6 N m^{-2}). This order is similar to the ranking based on a response threshold criterion. The only difference is that maximum intensity for *C. fusus* appeared to level off at slightly higher wall shear stress levels than for *L. polyedrum*. Because the flow transitioned from laminar to turbulent in this same range of wall shear stress, it is difficult to discern exactly where the maximum intensity of *C. fusus* began to plateau.

The response proportion, representing the population sensitivity, varied among species both in magnitude and the rate in which it changed as a function of wall shear stress in laminar flow. On the basis of the magnitude of the response proportion near threshold values of wall shear stress, the species were ranked in order of decreasing responsivity as *C. horrida* > *P. fusiformis* > *C. fusus* > *L. polyedrum*. This is the same order for decreasing flow sensitivity as found for the threshold criterion. *Ceratocorys horrida* had the highest population sensitivity in terms of response proportion, with values near the response threshold being more than an order of magnitude higher than those of the other species.

In laminar flow, the average intensity, expressed as photons m^{-3} to account for advective effects, increased for all species but *C. horrida*, where it slightly decreased in flows with wall shear stress values >0.4 N m^{-2}. This response difference for *C. horrida* may occur because this species was

the only one whose maximum intensity and population response rate remained relatively constant throughout high laminar flows.

Bioluminescence response in turbulent flow

There are several reasons to expect increased bioluminescence stimulation upon transition from laminar to turbulent flow. The shear stress field across the pipe between the highest laminar and lowest turbulent flow increased by a factor of 2.5–3.5. Mixing is greatly enhanced in turbulent flows, so cells from areas of low shear stress around centerline can be transported to areas of high shear stress at the pipe walls (Flint *et al.*, 1960; Vames and Hanratty, 1988). Moreover, the nature of turbulent pipe flow is profoundly different than that of laminar flow. In turbulent flow, shear stresses vary in time and space.

The time scales associated with the smallest eddies in the present turbulent pipe flow study were on the order of 0.1–1 ms (Rohr *et al.*, 2002). The length scales of the turbulence ranged from the radius of the pipe (Davies, 1972) to of the order of 10 μm. As the length scales of the energetic eddies become smaller than the cell, the likelihood of greater cell deformation increases. For example, only the energy associated with turbulent length scales smaller than the size of drops or bubbles is available to cause splitting as opposed to transport (Clift *et al.*, 1978). Energetic turbulent length scales are often associated with lengths of ~10 L_K (Lazier and Mann, 1989; Rohr *et al.*, 2002). In the present study, *C. fusus* and *P. fusiformis* were the only species whose size was on the order of the energetic turbulent length scales (Fig. 7). Although *C. fusus* exhibited a conspicuous increase in average intensity (presented as photons m^{-3}) through transition to turbulent flow, *P. fusiformis*, which is larger than *C. fusus*, did not. Thus, there is no consistent evidence that average bioluminescence intensity is affected by the length and time scales of the turbulence.

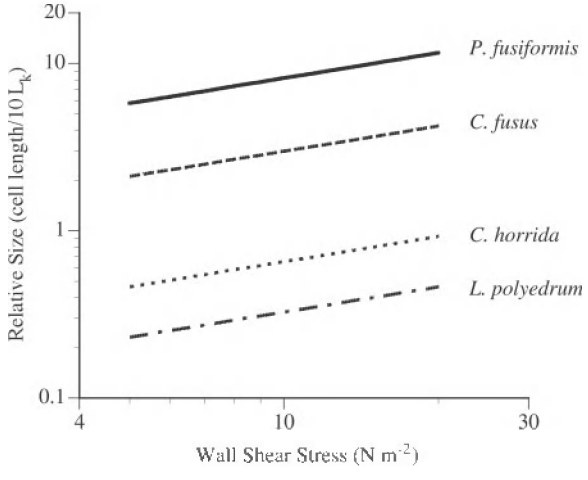

Fig. 7. Relative cell size as a function of wall shear stress for turbulent flow. Relative cell size is the ratio of cell length, the largest dimension of size, to 10 L_K at the pipe wall. Only for *Ceratium fusus* and *Pyrocystis fusiformis* was the relative size >1, indicating that turbulence may be more effective at distorting the cell membrane because the energetic length scales are about the same size or smaller than the cell length.

The maximum intensity of *C. horrida*, *P. fusiformis* and *L. polyedrum* was not noticeably affected by the transition to turbulence. Only for *C. fusus* was there a significant increase in maximum intensity. However, this change in maximum intensity in turbulent flow was no higher than that extrapolated from the trend in laminar flow, suggesting that the cause was the increase in shear stress and not the turbulent nature of the flow. Thus, there is no consistent evidence that either average or maximum intensity of bioluminescence is particularly sensitive to turbulent flow. This is true even for *C. horrida*, whose spines may increase its flow sensitivity.

Bioluminescence and morphology

Flow sensitivity is affected by the thickness and chemical composition of the cell wall (Namdev and Dunlop, 1995). All of the studied dinoflagellates had either a cell wall or thecae and polysaccharide plates deposited with vesicles located just proximal to the plasma membrane. Stiff cell components such as thecae or a cell wall could decrease flow sensitivity by minimizing local flow-induced cell deformation. Mutant cells of the alga *Chlamydomonas* lacking a cell wall are more flow sensitive than wild-type cells (Bronnenmeier and Märkl, 1982). Alternatively, in the context of bioluminescence stimulation, stiff cell components may enhance flow sensitivity by more effectively distributing fluid forces across the cell, leading to increased flow detection.

Lingulodinium polyedrum was the least shear-sensitive species in terms of response threshold, minimum shear stress level where individual flashes reach maximum intensity and population response proportion. This organism was the smallest species studied, is thecate and has a roughly spherical shape. *Ceratocorys horrida*, which is intermediate in size, spherical and thecate, had the highest sensitivity. Intermediate in sensitivity were *P. fusiformis*, which lacks thecae but has a cell wall, and *C. fusus*, which possesses thecae. Both species are considerably larger in size than *L. polyedrum* and are non-spherical. Thus, both the most and least sensitive species were thecate, and the sensitivity of *P. fusiformis*, which lacks thecae but has a cell wall, was similar to that of the thecate *C. fusus*. Based on the species selected, it is not possible to draw conclusions about the role of thecae in shear sensitivity.

Aside from *C. horrida*, sensitivity increased with increasing size. Even considering the length of its spines, the overall length of *C. horrida* cells was less than that of

P. fusiformis, which was less sensitive despite being larger. The spines of *C. horrida* may increase shear sensitivity, perhaps by acting as levers that accentuate the effect of shear flow (Zirbel *et al.*, 2000). Overall, the present data suggest that increasing cell size and the presence of spines promote flow sensitivity, although additional observations of other species will be required before a general relationship between morphology and flow sensitivity can be ascertained.

Ecological context of flow sensitivity

Dinoflagellate bioluminescence decreases grazing pressure (Esaias and Curl, 1972; White, 1979) by disrupting predator feeding behavior (Buskey *et al.*, 1983). Flashes are produced at or near the predator (Buskey *et al.*, 1985), although it is unclear whether stimulation occurs in the feeding current or by contact/handling by the predator. The distance between predator and flashing organism within a predator feeding current, modeled as siphon flow (Fields and Yen, 1997; Kiørboe *et al.*, 1999), can be calculated knowing the threshold shear levels for bioluminescent organisms and their response latency. It is assumed, for comparison sake, that the threshold bioluminescence levels determined in steady, tangential shear in pipe flow is similar to that in unsteady, extensional shear in siphon flow. In support of this assumption is the finding that the response threshold for copepod escape jumps is similar for steady tangential and spatially changing extensional shear stress (Kiørboe *et al.*, 1999). Furthermore, the bioluminescence threshold shear stress for *L. polyedrum* is similar for steady and unsteady laminar Couette flow (von Dassow, 2003).

Using siphon flow as a mimic of a predator feeding current, the associated velocity, shear stress and acceleration fields can be calculated as a function of distance from the mouth of the siphon based on the volume flow rate through the siphon (Kiørboe *et al.*, 1999). Assuming a representative feeding current flow rate of 0.279 mL s^{-1} (Table II of Jakobsen, 2001), the threshold shear stress for *C. horrida* (0.024 N m^{-2}), *P. fusiformis* (0.087 N m^{-2}), *C. fusus* (0.116 N m^{-2}) and *L. polyedrum* (0.333 N m^{-2}) occurs at r = 1.3, 0.8, 0.7 and 0.5 mm respectively. The accelerations within this range are from 0.31 to 25 m s^{-2} and are not considered to be stimulatory (Latz *et al.*, 2004). For comparison, the escape jump responses for other flagellates, ciliates and copepods are stimulated by extensional deformation rates of 0.2–10 s^{-1} (equivalent to a shear stress of 0.0002–0.01 N m^{-2}) (Haury *et al.*, 1980; Kiørboe *et al.*, 1999; Jakobsen, 2001, 2002), corresponding to r = 1.6–1.8 mm at the same flow rate. Thus, dinoflagellate bioluminescence stimulated by feeding current flow would occur considerably closer to the organism than would escape jump responses by other organisms. While three of the studied dinoflagellate species are motile, their swimming ability is limited (Kamykowski *et al.*, 1992) compared to the fluid velocity within the feeding current (Kiørboe *et al.*, 1999; Jakobsen, 2001), so it is unlikely that they can swim to avoid the siphon flow.

The critical distance $R_{critical}$ within which stimulated organisms would be advected into the mouth of the siphon before flashing was calculated based on the 20-ms response latency of dinoflagellate bioluminescence (Widder and Case, 1981a). For Q = 0.279 mL s^{-1}, $R_{critical}$ = 1.1 mm and the corresponding shear stress at this position is 0.036 N m^{-2}. Although $R_{critical}$ changed with siphon flow rate, the corresponding shear stress remained constant (Fig. 8) for a given response latency. Thus, only *C. horrida*, and in general any species having a shear stress threshold <0.036 N m^{-2} and a response latency ≤20 ms, would produce a flash prior to coming into contact with the predator. For *C. fusus*, *L. polyedrum* and *P. fusiformis* and any other dinoflagellate species with response thresholds >0.036 N m^{-2} and a response latency ≥20 ms, flashes will not occur in the siphon current, regardless of the flow rate.

Using threshold values of shear stress of *C. fusus*, *L. polyedrum* and *P. fusiformis* for determining flash position relative to the predator may be too conservative. For steady laminar pipe flow, the population response for these species near threshold is ≤0.001 flash $cell^{-1}$. A shear stress of 1 N m^{-2} may be more representative of

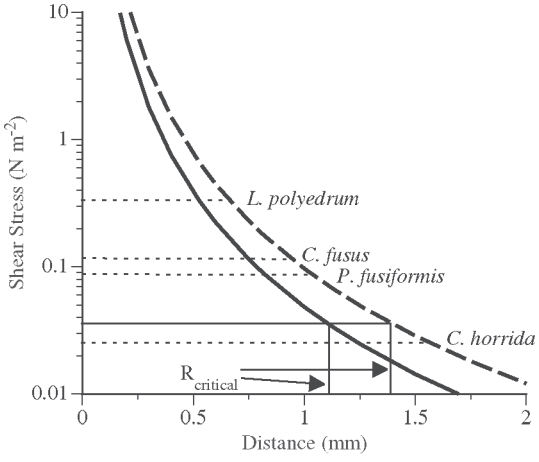

Fig. 8. Shear stress in siphon flow, representing a predator feeding current, as a function of distance from the siphon mouth. Thick solid line is for a flow rate of 0.279 mL s^{-1} (Jakobsen, 2001). For distances less than $R_{critical}$ = 1.1 mm (thin vertical line), organisms are swept into the mouth within 20 ms, the flash response latency. The corresponding level of shear stress (0.036 N m^{-2}, solid horizontal line) was less than the response thresholds for *Ceratium fusus*, *Lingulodinium polyedrum* and *Pyrocystis fusiformis* (dotted lines). At double the flow rate (0.559 cm s^{-1}, thick dashed line), $R_{critical}$ is greater but occurs at the identical shear stress level. Thus, for any siphon flow rate and a 20-ms response latency, only *Ceratocorys horrida* will flash within the siphon flow field.

the population for these species because at this level a few percentage of the cells were observed to flash. Moreover, individual flash intensity was either at or near its peak at 1 N m^{-2}. For a Q = 0.279 mL s^{-1}, a shear stress of 1 N m^{-2} occurs at r = 0.36 mm, essentially within the 0.25 mm capture volume of a copepod (Kiørboe *et al.*, 1999). For 20-ms response latency, this position is again within $R_{critical}$, indicating that flashes for these species will not occur within the feeding current. These findings indicate that the bioluminescence of *C. fusus*, *L. polyedrum* and *P. fusiformis* associated with predator interactions would occur not from flow stimulation but from contact and/or handling by the predator. However, flashes occurring at or after predator contact may yet benefit the individual cell if it is released unharmed because of a 'startle response' and/or benefit the population if the flash disrupts grazing behavior (Buskey and Swift, 1983, 1985; Buskey *et al.*, 1983).

Ceratocorys horrida, because of its lower response threshold, was the only dinoflagellate tested that could flash within the predator feeding current. *Ceratocorys horrida* differed markedly from the other species in several aspects of its bioluminescence response around threshold. Its highest maximum flash intensity occurred near the response threshold, whereas for the other species the highest maximum flash intensity occurred at wall shear stress levels an order of magnitude greater than threshold. In addition, the population response proportion of *C. horrida* near the response threshold was at least an order of magnitude greater than the other species. Whether cells with these attributes have an antipredation advantage has yet to be proven but is an attractive hypothesis. Nevertheless, even for *C. horrida* the fraction of the population that would benefit directly from flow-stimulated bioluminescence is relatively small, ~10%.

The ecological significance of dinoflagellate bioluminescence extends beyond the direct interaction of dinoflagellate and predator. Flashes serve as visible 'burglar alarms' to attract secondary predators, increasing the risk of predation to the dinoflagellate grazer (Burkenroad, 1943; Morin, 1983). The startle response observed in dinoflagellate predators in response to these light signals consists of dramatic changes in swimming speed and direction (Buskey and Swift, 1983, 1985; Buskey *et al.*, 1983) that may serve as escape behaviors; otherwise, the dinoflagellate predators may be exposed to enhanced predation from visual predators attracted by the dinoflagellate flash. Flow-stimulated dinoflagellate bioluminescence can also serve as a 'luminescent mine field' (Young, 1983), in that animals are outlined by the light stimulated by their swimming (Hobson, 1966; Rohr *et al.*, 1998), enhancing the ability of visual nocturnal predators to locate prey (Mensinger and Case, 1992; Fleisher and Case, 1995). Thus, mechanical stimulation of dinoflagellate bioluminescence is ecologically important not only in the context of predator interactions with dinoflagellate cells but also as a 'mine field' that potentially increases the risk of predation to moving animals.

While generally for the dinoflagellate species studied bioluminescence would not be stimulated by predator feeding currents, bioluminescence may still serve as an index of mechanical sensitivity to contact and handling by the predator. Suspension feeders such as copepods capture organisms by movement of the second maxillae to direct particles from the feeding current toward the mouth (Koehl and Strickler, 1981; Vanderploeg and Paffenhöfer, 1985; Price and Paffenhöfer, 1986) where they are macerated by the mandibles (Arashkevich, 1969). Copepods can reject unsuitable particles (Huntley *et al.*, 1986) apparently after handling and possibly tasting the items (Vanderploeg and Paffenhöfer, 1985). During these processes, dinoflagellates would experience mechanical agitation of an unknown magnitude. The luminescent response from cell deformation due to predator contact/handling may be similar to that caused by fluid shear deformation. Thus, dinoflagellate bioluminescence may serve as a biological reporter of the mechanical agitation generated by predators during feeding.

The response thresholds for copepod escape jumping in siphon flow appear to be tuned to environmental shear levels (Fields and Yen, 1997), with the least sensitive species occupying shallow water, more turbulent, environments. The present study allows testing of this hypothesis in the context of flow-stimulated dinoflagellate bioluminescence. The coastal species *L. polyedrum* is well known to undergo strong diel migrations (Eppley *et al.*, 1968) in which it congregates in surface waters during the day (Hasle, 1950; Sweeney, 1975; Eppley *et al.*, 1984) and would be exposed to maximal levels of wind-induced surface turbulence. Although *C. fusus* has a cosmopolitan distribution, ranging from oceanic to estuarine habitats, it is principally a coastal species (Sullivan and Swift, 1995) and has a deeper vertical distribution than *L. polyedrum* (Lapota *et al.*, 1989; Swift *et al.*, 1995). *Ceratocorys horrida* and *P. fusiformis* are oceanic species that have deeper vertical distributions, with the latter found near the base of the euphotic zone at depths of 100–200 m (Swift and Meunier, 1976; Swift *et al.*, 1981). The two oceanic species had the lowest response thresholds, whereas the two coastal species had higher thresholds, with *L. polyedrum* being the greatest. Although these results appear to support the hypothesis that flow sensitivity is tuned to ambient conditions, the response threshold for the coastal *C. fusus* was only 0.19 N m^{-2} (33%) higher than that of the oceanic species *P. fusiformis*. More importantly, it is necessary to consider response threshold levels in the context of typical background oceanic flow conditions.

Dissipation rates, ε, at the response threshold for the four species studied ranged from 5×10^{-4} to 1×10^{-1} m^2 s^{-3}, typically many orders of magnitude higher than dissipation rates commonly found in the ocean interior. For example, in the deep ocean, ε is on the order of 10^{-10} m^2 s^{-3} (Kunze and Sanford, 1996). This difference between threshold and oceanic background dissipation rates is consistent with the general lack of background bioluminescence observed in the ocean interior (Boden *et al.*, 1965; Widder *et al.*, 1989; Buskey and Swift, 1990). At the ocean boundaries, breaking surface waves and wave/tidal-forced bottom shears may provide ε levels greater than the dinoflagellate response threshold (Rohr *et al.*, 2002). It is well known that surface breaking waves stimulate bioluminescence (Staples, 1966; Latz *et al.*, 1994) as do bottom shears created by passing waves in a laboratory wave tank (M. I. Latz and J. Rohr, personal observations). In summary, the present results suggest that bioluminescence is stimulated by predator contact but not by typical oceanic flows that might continually deplete luminescent reserves.

Applications of flow-stimulated dinoflagellate bioluminescence

Aside from the ecological context of flow-stimulated dinoflagellate bioluminescence, it is useful as a flow visualization tool for regions of high shear or dissipation (Latz *et al.*, 1995; Rohr *et al.*, 1998). Cells respond nearly instantaneously to shear stress levels greater than the response threshold. Flow-stimulated bioluminescence has been used to visualize boundary layer shear associated with a moving dolphin (Rohr *et al.*, 1998), examine shear stress in bioreactors (Chen *et al.*, 2003) and quantify shear stress within breaking surface waves (Stokes *et al.*, 2004). It may be also useful for mapping highly dissipative oceanic flows (Rohr *et al.*, 2002). Moreover, different luminescent species provide varied opportunities for laboratory flow visualization. For example, the prolonged flash duration of *P. fusiformis* provides excellent streak lines, whereas the short flash duration and high concentrations of *L. polyedrum* serve as an excellent marker of relatively high flow agitation levels throughout the flow volume. Together with mathematical models coupling cell response to flow conditions (Deane and Stokes, in press), flow-stimulated dinoflagellate bioluminescence can be applied as a quantitative tool for probing complex flow fields not amenable to conventional study.

ACKNOWLEDGEMENTS

We are grateful to S. Fallon, M. Zirbel and J. Coleman for technical assistance, L. Busse for help with microscopy and A. Juhl, S. Rapoport, S. Lindsay, E. Turack, R. Shadwick, A. McCulloch, P. Franks and R. Rosenblatt for helpful comments on the manuscript. Supported by the Office of Naval Research (grant N00014-95-1-001 to M.I.L.) and the IAR and ILIR programs at SSC San Diego (to J.R.).

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