

UC San Diego

UC San Diego Previously Published Works

Title

Bathypotometer bioluminescence potential measurements: A framework for characterizing flow agitators and predicting flow-stimulated bioluminescence intensity

Permalink

<https://escholarship.org/uc/item/09h9j1v7>

Journal

Continental Shelf Research, 61

ISSN

0278-4343

Authors

Latz, Michael I

Rohr, Jim

Publication Date

2013-07-01

DOI

10.1016/j.csr.2013.04.033

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed



Research papers

Bathypotometer bioluminescence potential measurements: A framework for characterizing flow agitators and predicting flow-stimulated bioluminescence intensity

Michael I. Latz^{a,*,1}, Jim Rohr^{b,1}^a Scripps Institution of Oceanography, University of California San Diego, CA 92093-0202, USA^b SSC Pacific, 53560 Hull Street, San Diego, CA 92152-5000, USA

ARTICLE INFO

Article history:

Received 12 October 2012

Received in revised form

15 April 2013

Accepted 18 April 2013

Available online 30 April 2013

Keywords:

Dinoflagellate

Pipe flow

Turbulence

Pyrocystis

Pyrodinium

Lingulodinium

ABSTRACT

Bathypotometer measurements of bioluminescence are used as a proxy for the abundance of luminescent organisms for studying population dynamics; the interaction of luminescent organisms with physical, chemical, and biological oceanographic processes; and spatial complexity especially in coastal areas. However, the usefulness of bioluminescence measurements has been limited by the inability to compare results from different bathypotometer designs, or even the same bathypotometer operating at different volume flow rates. The primary objective of this study was to compare measurements of stimulated bioluminescence of four species of cultured dinoflagellates, the most common source of bioluminescence in coastal waters, using two different bathypotometer flow agitators as a function of bathypotometer volume flow rate and dinoflagellate concentration. For both the NOSC and BIOLITE flow agitators and each species of dinoflagellate tested, there was a critical volume flow rate, above which average bioluminescence intensity, designated as bathypotometer bioluminescence potential (BBP), remained relatively constant and scaled directly with dinoflagellate cell concentration. At supra-critical volume flow rates, the ratio of BIOLITE to NOSC BBP was nearly constant for the same species studied, but varied between species. The spatial pattern and residence time of flash trajectories within the NOSC flow agitator indicated the presence of dominant secondary recirculating flows, where most of the bioluminescence was detected. A secondary objective (appearing in the Appendix) was to study the feasibility of using NOSC BBP to scale flow-stimulated bioluminescence intensity across similar flow fields, where the contributing composition of luminescent species remained the same. Fully developed turbulent pipe flow was chosen because it is hydrodynamically well characterized. Average bioluminescence intensity in a 2.54-cm i.d. pipe was highly correlated with wall shear stress and BBP. This correlation, when further scaled by pipe diameter, effectively predicted bioluminescence intensity in fully developed turbulent flow in a 0.83-cm i.d. pipe. Determining similar correlations between other bathypotometer flow agitators and flow fields will allow bioluminescence potential measurements to become a more powerful tool for the oceanographic community.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Bioluminescent organisms are ubiquitous in the ocean, being found at all depths and in all regions. Under suitable conditions, dramatic displays of ocean bioluminescence have been associated with the flow fields produced by ship wakes (Harvey, 1952, 1957; Rohr et al., 1998; Staples, 1966; Tarasov, 1956), breaking waves

(Staples, 1966; Stokes et al., 2004; Turner, 1965), surge (Rohr et al., 1994), and swimming animals (Hobson, 1966; Rohr et al., 1998; Williams and Kooyman, 1985). The oceans can be considered a luminescent “minefield” (Widder, 2006) where bioluminescence is stimulated by sufficiently high hydrodynamic stresses. The bioluminescence displays of some swimming fish are distinct enough to differentiate species (Morin, 1983; Roithmayr, 1970) and nocturnally foraging predators can use luminescent wakes to locate prey (Fleisher and Case, 1995; Hobson, 1966; Mensinger and Case, 1992). The probability of observing flow-stimulated bioluminescence depends on the volume and level of stimulatory flow; the bioluminescence potential, which is an index of species abundance of the luminescent organisms and their ability to flash; and the

Abbreviations: BBP, bathypotometer bioluminescence potential; PMT, photomultiplier.

* Corresponding author. Tel./fax: +1 858 534 6579.

E-mail addresses: mlatz@ucsd.edu (M.I. Latz), james.rohr@navy.mil (J. Rohr).

¹ Authors share authorship equally.

radiative transfer of the light from source to receiver, as well as ambient light conditions (Moline et al., 2007; Oliver et al., 2007; Rohr et al., 1999).

Bioluminescence potential is measured by bathyphotometers that use turbulent flow to stimulate bioluminescence of organisms passing through a flow agitator (Herren et al., 2005). Measurements of bathyphotometer bioluminescence potential, hereafter referred to as BBP, have been used to provide accurate values of average cell concentrations and insight into circulation patterns within a bay (Seliger et al., 1969b), as a proxy for luminescent organism abundance (Lapota, 1998; Losee et al., 1985; McManus et al., 2003; Piontkovski et al., 1997; Swift et al., 1995; Widder et al., 1999), plankton biomass (Lapota, 1998; Moline et al., 2009; Piontkovski et al., 1997), spatial complexity in the coastal zone (Blackwell et al., 2008; Herren et al., 2005), environmental quality (Paerl, 1988), and other biological, physical, and chemical parameters of the ocean (Kushnir et al., 1997; McManus et al., 2003). Together with images of flow-stimulated bioluminescence, BBP can also be used to provide both qualitative (Latz et al., 2004; Rohr et al., 1998) and quantitative (Stokes et al., 2004) information regarding hydrodynamic properties of the stimulating flow field. However, an unknown dependence on flow rate, cell concentration, flow agitator volume, and geometry confound using BBP as a predictive tool (Widder et al., 1993). The overall objective of the present study is to begin to establish a foundation for interpreting and extrapolating BBP so that it can both provide additional insight into oceanographic flow fields and become a more useful proxy for assessing organism concentration and related oceanographic properties. The first step entails performing flow agitator studies using single dinoflagellate species of known concentrations over a range of volume flow rates. The luminescent response of four species of dinoflagellates was characterized as a function of volume flow rate and organism concentration in the NOSC (*Lingulodinium polyedrum*, formerly known as *Gonyaulax polyedra*; *Pyrocystis fusiformis*; *Ceratium fusus*) and BIOLITE (*L. polyedrum*, *P. fusiformis*, *Pyrodinium bahamense*) bathyphotometer flow agitators. Bioluminescence intensity measurements obtained from these flow agitators have been frequently used in ship-based oceanographic surveys of bioluminescence potential (Bivens et al., 2002a, 2002b; Geiger, 1996; Lapota and Losee, 1984; Lieberman et al., 1987; Losee and Lapota, 1981; Losee et al., 1985; McDuffey and Bird, 2002; Rohr et al., 2002, 1994). Individual flashes of *P. fusiformis* were imaged to provide a two-dimensional projection of flash trajectories within both flow agitators. Because flow in the NOSC flow agitator is approximately two-dimensional, it can be directly determined through observation whether flash trajectories mostly reside within the central flow core or the adjacent regions of relatively slow recirculating flow. Predictions of the effect of flash advection on BBP were explored within the context of a previously used one-dimensional flow stimulus model (Rohr et al., 1994; Seliger et al., 1969b; Widder et al., 1993).

Although bioluminescence intensity measurements have historically been collected within a marine biology context, they have also appeared in the study of hydrodynamic flow fields (Rohr et al. 1998; Stokes et al., 2004). Within this relatively new context it would be of interest to be able to predict the bioluminescence “signature” of generic flow fields, e.g., a school of fish (Altinagac et al., 2010; Roithmayr, 1970; Squire and Krumboltz, 1981) given the associated BBP and some relevant flow parameter such as the speed of the school. As discussed in the Appendix, a first step towards realizing this objective is to study the response of luminescent dinoflagellates in fully developed turbulent pipe flow. An empirically derived relationship is obtained for a 2.54-cm i.d. pipe, relating the average bioluminescence intensity within the pipe, pipe wall shear and the corresponding NOSC BBP. Given the

corresponding NOSC BBP, predictions of bioluminescence intensity in a 0.85-cm i.d. pipe are made for a range of flow rates and compared with measurements obtained from freshly collected water samples taken from San Diego Bay, over a period of two years.

2. Background

2.1. Luminescent organisms

Dinoflagellates are unicellular plankton that are the dominant sources of flow-stimulated bioluminescence in coastal surface waters (Kelly and Tett, 1978; Lapota, 1998; Moline et al., 2009; Morin, 1983; Seliger et al., 1961). Dinoflagellates typically respond to mechanical stimulation within 20 ms with a flash 100 ms or more in duration (Eckert, 1965; Latz et al., 2008; Latz and Lee, 1995; Widder and Case, 1981a). Dinoflagellate bioluminescence is believed to serve as an antipredator strategy to reduce grazing by interfering with predator feeding behavior (Buskey et al., 1983; Buskey and Swift, 1983). Bioluminescence can be stimulated by mechanical stress due to predator handling or fluid shear, which cause cell deformation that activates an intracellular signaling pathway, resulting in the production of light (Chen et al., 2007; von Dassow and Latz, 2002).

The dinoflagellate *L. polyedrum*, with an equivalent spherical diameter of approximately 35 μm (Kamykowski et al., 1992; Latz et al., 2004), is a coastal bloom species residing in temperate and tropical waters. Its distribution includes the Caribbean, the east and southwest coasts of the US, the eastern Atlantic from central Africa northward to Scandinavia, and the coasts of Australia and Japan (Lewis and Hallett, 1997). *L. polyedrum* is one of the most well-studied dinoflagellates (Lewis and Hallett, 1997) because of its role in red tides and the availability of laboratory cultures since the 1950s. *P. fusiformis* is a large, up to 1 mm in length, 374 μm equivalent spherical diameter (Latz et al., 2004), oligotrophic tropical dinoflagellate that is found at lower cell abundances than coastal species such as *L. polyedrum* and does not form blooms (Swift et al., 1981). *P. bahamense* var. *bahamense* is a tropical and subtropical coastal species (Badylak et al., 2004) similar in size and shape to *L. polyedrum* (Latz et al., 2008) that is best known as the source organism for the spectacular bioluminescent bays of the Caribbean (Seliger et al., 1970, 1969b; Seliger and McElroy, 1968). *C. fusus*, with a size of 320 μm long $\times$ 30 μm wide and 73 μm equivalent diameter (Latz et al., 2004), is a mostly coastal species with cosmopolitan distribution (Sullivan and Swift, 1995). *P. fusiformis* is not motile as it lacks flagella; although *L. polyedrum*, *P. bahamense*, and *C. fusus* are motile (Kamykowski et al., 1992), their swimming is weak compared to the flows considered throughout this study.

Flash characteristics of these species are markedly different. Maximum flash intensity and duration of *L. polyedrum* are 2×10^8 photons s^{-1} and 100–150 ms, respectively; for *P. fusiformis*, 7×10^{11} photons s^{-1} and 210–650 ms (Latz and Lee, 1995; Widder and Case, 1981b); and for *C. fusus*, 1×10^9 photons s^{-1} and 239 ms (Latz et al., 2004). Whereas each *L. polyedrum* and *C. fusus* cell can flash only a few times (Latz et al., 2008, 2004; Latz and Lee, 1995), *P. fusiformis* cells can flash 23 to 62 times or more depending on the frequency of stimulation (Swift et al., 1973; Widder and Case, 1981b). Moreover, the repetitive flashes of *P. fusiformis* can summate, resulting in exceptionally long flash durations. Because of its bright and sustained flash, the bioluminescence of *P. fusiformis* has been used to delineate fluid path lines in complicated three-dimensional flows (Latz et al., 1995) such as bioreactors (Chen et al., 2003) and the entrance and flow agitation chamber of a bathyphotometer (Herren et al., 2005).

2.2. Bathyphotometer bioluminescence potential measurements

The terms “bioluminescence potential” and “bathyphotometer bioluminescence potential” are not always used consistently (Herren et al., 2005; Losee et al., 1985; Rudyakov, 1968; Shulman et al., 2003; Widder et al., 1993). Here, bioluminescence potential is defined as the total light output that would be produced by mechanically stimulating, to the extinction of light emission without physical damage, all luminescent organisms in a prescribed volume of seawater. This definition of bioluminescence potential, which has units of light energy per volume (photons l^{-1}), is sometimes referred to as bioluminescence capacity, total bioluminescence capacity, or simply bioluminescence potential (Biggley et al., 1969; Bivens et al., 2002a; Herren et al., 2005). In a laboratory context, bioluminescence potential is commonly referred to as total mechanically stimutable light (TMSL), when referring to bioluminescence measurements of a known number and species of organisms that have been mechanically stimulated to exhaustion of light emission. In this study we will use TMSL as the measure of bioluminescence potential per cell (photons cell^{-1}).

We reserve the term “bathyphotometer bioluminescence potential”, BBP, to represent the average bioluminescence intensity (photons s^{-1}) stimulated by turbulent flowing seawater through a bathyphotometer flow agitator. Because typically not all organisms are stimulated to exhaustion of bioluminescence within the flow agitator and some may even avoid capture, BBP can only provide a fraction of the actual bioluminescence potential present in a volume of seawater. Bathyphotometer measurements of bioluminescence potential have been referred to as bioluminescence potential (Herren et al., 2005; Rudyakov, 1968; Widder et al., 1993), bioluminescence source strength (Losee and Lapota, 1981), mechanically stimulated bioluminescence potential (Moline et al., 2007), and bioluminescence potential index (Rohr et al., 2002). BBP has been expressed in radiometric, photometric, and quantum units (Bivens et al., 2002a; Losee et al., 1985; Widder, 1997; Widder et al., 1993). Moreover, in an attempt to estimate bioluminescence potential, BBP has been divided by either the volume of the flow agitator within the field of view of the detector, or the volume flow rate through it (Losee et al., 1985; Seliger et al., 1969b; Widder et al., 1993).

Two bathyphotometer flow agitators, the NOSC and BIOLITE, were available to us to compare. The NOSC flow agitator, developed at Space and Naval Warfare Systems Center Pacific, has measured BBP throughout the Atlantic, Pacific, Gulf of California, Acapulco Bay Mexico, Chesapeake Bay, and San Diego Bay at volume flow rates between about 0.2 to 0.7 l s^{-1} (Lapota and Losee, 1984; Lieberman et al., 1987; Losee and Lapota, 1981; Losee et al., 1985; Rohr et al., 2002, 1994). The BIOLITE flow agitator evolved from the NOSC agitator and has approximately double the flow agitator volume to allow simultaneous monitoring by four photomultiplier tube detectors. The BIOLITE flow agitator was routinely deployed on NAVO-CEANO T-AGS 60 class oceanographic vessels to obtain measurements of near-surface BBP while operating at a volume flow rate of about 1 L s^{-1} (Bivens et al., 2002a, 2002b; Geiger, 1996; McDuffey and Bird, 2002). Both the NOSC and BIOLITE flow agitators are designed to connect to 2.54-cm i.d. hose or pipe.

2.3. One-dimensional model

Seliger et al. (1969a, 1969b) developed a one-dimensional flow model relating BBP measurement, flash kinetics, and flash residency time within the flow agitator. Widder et al. (1993) applied this model to the case where flashes are assumed to be initiated at the entrance of the flow agitator and move at a constant velocity, U , throughout the length of the chamber, L . Each cell was assumed to produce a single flash that was modeled as $I_0 e^{-t/\gamma}$, where I_0 is the

maximum flash intensity (photons s^{-1}) and γ (s) is the flash decay time constant. Note the flow field in this model only transports cells through a fixed location where they are stimulated and is not itself stimulatory.

This model predicts that when the flash residency time, T (where $T=L/U$), within the flow agitator is large compared to the flash decay time constant, γ , then average BBP (photons s^{-1}) is equal to the product of cell concentration, n (cell m^{-1}), the total bioluminescence from one cell, $I_0\gamma$ (photons cell^{-1}), and the flow speed, U (m s^{-1}):

$$\text{BBP} = nI_0\gamma U \quad \text{for } T = L/U > \gamma \quad (1)$$

For cells producing a single flash in a one-dimensional flow, bioluminescence potential (photons m^{-1}) is by definition the product of the cell concentration and the total light energy per cell, $nI_0\gamma$. Consequently, within the assumptions of this model and $T > \gamma$, BBP should be divided by the flow rate (Widder et al., 1993) to determine bioluminescence potential.

When the flash residency time is suitably small compared to the decay time constant, this idealized model, which assumes all flashes are initiated at the entrance of the flow agitator, predicts that average BBP is equal to the product of cell concentration, maximum flash intensity, and detection chamber length:

$$\text{BBP} = nI_0L \quad \text{for } T = L/U < \gamma \quad (2)$$

Consequently, at suitably high flow rates BBP should be divided by the chamber length and multiplied by the characteristic decay rate to determine the bioluminescence potential, $nI_0\gamma$. Rohr et al. (1994) extended this analysis to pipe flow, assuming that each cell flashes randomly within the pipe, and found similar trends.

Note that this model assumes that the flow is one-dimensional, all cells are stimulated to flash once within the flow agitator, and flash kinetics do not change with flow rate. However, it is known that there is a response threshold for flashes to be stimulated, cells may flash more than once or not at all, and the fraction of the cell population producing bioluminescence may increase dramatically with increasing flow agitation (Latz et al., 2004). Nevertheless, the general trends predicted by the limiting cases, $T > \gamma$ and $T < \gamma$, have been verified for flow agitators using both an impeller pump (Seliger et al., 1969b) and a grid (Widder et al., 1993).

3. Experimental methods

3.1. Luminescent organisms

Cultures of *L. polyedrum* (Stein) Dodge, 1998, *P. fusiformis* Murray ex Haeckel 1890, *C. fusus* (Ehrenberg 1834) Dujardin 1841, and *P. bahamense* var. *bahamense* Plate 1906 were grown in full-strength seawater with half-strength *f/2* media minus silicate (Guillard and Ryther, 1962) as previously described (Latz and Rohr, 1999). Cell cultures were maintained in a Percival environment chamber on a 12:12 h light-dark cycle at $20 \pm 0.5^\circ\text{C}$ except for *P. bahamense*, which was maintained at $25 \pm 0.5^\circ\text{C}$. Daytime illumination level was $240 \mu\text{E m}^{-2} \text{ s}^{-1}$ as measured with a Biospherical Instruments QSL-100 scalar radiance meter (San Diego, CA USA). To achieve desired experimental concentrations, cultures were diluted with $5\text{-}\mu\text{m}$ glass-fiber-filtered (Whatman Inc., GE Healthcare Life Sciences, Piscataway, NJ, USA) seawater. Cell abundance was determined by counting samples under a dissecting microscope.

All tested species exhibit endogenous circadian rhythms in bioluminescence (Lapota et al., 1992b; Latz and Lee, 1995; Seliger et al., 1969a; Sullivan and Swift, 1994; Sweeney and Hastings, 1957). Cultured cells were loaded into the flow agitator test tank at the end of the light phase when sensitivity to mechanical stimulation is

minimal, and the tank contents were gently mixed by a slow paddle system until just before testing. An opaque cover was draped over the test tank at the beginning of the dark cycle of the cells, and experiments began 2.5 h later, when maximum levels of bioluminescence occur (Biggley et al., 1969).

3.2. Bathyphotometer bioluminescence potential (BBP) measurements

The NOSC flow agitator consists of a 25.5-cm³ disk-shaped chamber, approximately 4 cm in diameter and 2 cm thick, with quartz windows attached to its sides for viewing (Losee and Lapota, 1981; Losee et al., 1985). The axis of the inlet and outlet ports to the agitation chamber is aligned along its diameter (Fig. 1A). The inlet section preceding the flow agitator reduces from a diameter of 2.54 cm to 1 cm over a distance of 2.54 cm. The outlet section following the flow agitator increases from a diameter of 1 cm to 2.54 cm over a distance of 2.54 cm. The core flow, defined as the cylindrical volume of flow bridging the inlet and outlet ports of the agitator, is about 3.14 ml or about 12% of the total NOSC flow agitator volume. The area of the interface between the core and outer recirculating flow is 12.6 cm². Flash residence time within the recirculation zones will tend to decrease as this interfacial area increases. Bioluminescence within the NOSC flow agitator was measured from diluted cultures of *L. polyedrum*, *P. fusiformis*, and *C. fusus*. In one experiment using *L. polyedrum*, two NOSC flow agitators were placed in series, 60 cm apart, to determine the fraction of the upstream BBP that occurred in the downstream agitator.

The BIOLITE flow agitator chamber consists of a glass pipe that is about 4 cm in diameter and 4 cm long with a volume of approximately 50 ml, with its cylindrical axis aligned along the

et al., 1985; McDuffey and Bird, 2002). Together with the adjoining hose connected to the inlet and exit of the BIOLITE, this configuration forms three cylindrical sections in series, 2.54-cm i.d. to 4-cm i.d. to 2.54-cm i.d. (Fig. 1B). Core flow volume is 20.3 cm³ or 41% of the total BIOLITE flow agitator volume. The area of the interface between the core and outer recirculating flows is 31.9 cm², about 2.5 times greater than for the NOSC flow agitator. For experiments with the BIOLITE flow agitator, *L. polyedrum*, *P. fusiformis*, and *P. bahamense* were available to be tested.

For measurements of cultured dinoflagellates with the NOSC and BIOLITE flow agitators, a 1.4 m length of opaque 2.54-cm i.d. hose, attached to the inlet of each flow agitator, drew samples from a 60-L reservoir filled with a known concentration of dinoflagellates. The reservoirs were premixed by gentle stirring for several hours prior to the experiment to promote a homogeneous distribution of organisms. Cell homogeneity was monitored during each experiment by periodically repeating measurements for the same volume flow rate setting. Flow was established by a self-priming ULTRAFlo dual-head pump (SHURflo Pump Manufacturing Co., Costa Mesa, CA, USA) located downstream of the agitator and under computer control. Flow rate was measured by a mass flow meter (model D40, Micro Motion Inc., Boulder, CO, USA) located downstream of the flow agitator. A total of 52 experiments were performed from 2001 to 2004.

Bioluminescence of cultured dinoflagellates stimulated by the flow agitators was measured by an Electron Tubes model P10232 photon counting photomultiplier tube (ET Enterprise Ltd., Uxbridge, UK) fitted with a Uniblitz electronic shutter (Vincent Associates, Falmouth, MA USA) and clamped directly onto the agitator (Fig. 1C). The 2.54-cm shutter aperture of the photon counting photomultiplier tube, PMT, was located 3.5 cm from the face of the NOSC flow agitator, and 5 cm from the face of the

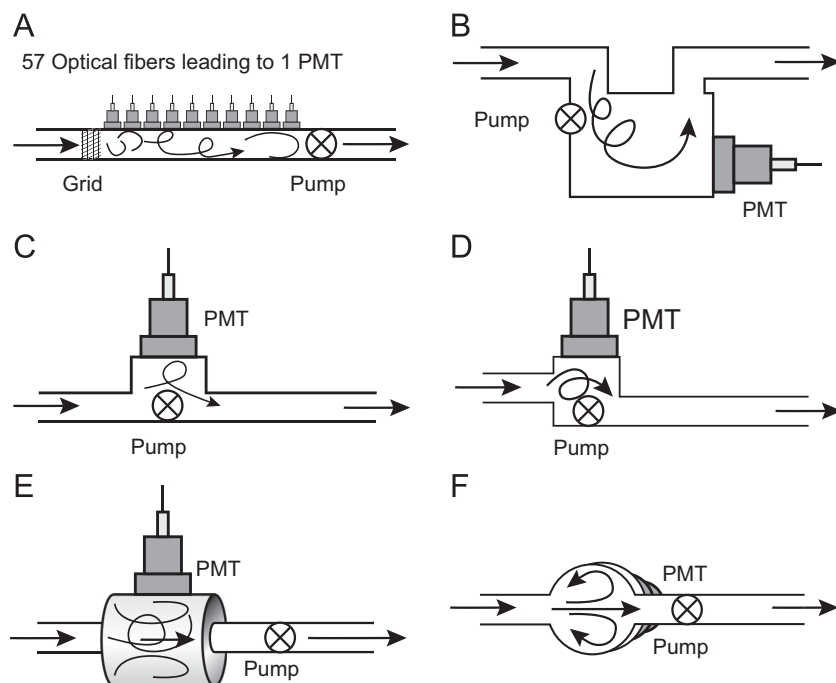


Fig. 1. Selected flow agitator systems used in bathyphotometers. Schematic diagrams are not drawn to scale and are modified in part from Case et al. (1993) with additional details in Herren et al. (2005), Table 2, and the text. (A) HIDEX bathyphotometer, which uses grid-generated turbulence to stimulate bioluminescence, which is detected by an array of optical fibers leading to a single PMT (Widder et al., 1993). (B) UBAT, similar to the MBBP prototype (Herren et al., 2005), uses an impeller pump for bioluminescence stimulation. (C) University of Rhode Island (URI) bathyphotometer, which also uses an impeller pump for stimulation (Swift et al., 1985a). (D) Seliger bathyphotometer uses an impeller pump for stimulation (Seliger et al., 1969b). (E) BIOLITE bathyphotometer, part of a photometer system for underway ship measurements, consists a cylindrical chamber with downstream pump (McDuffey and Bird, 2002). (F) NOSC bathyphotometer has a disk-shaped flow agitator with downstream pump (Losee et al., 1985).

centerline between the 2.54-cm diameter inlet and outlet (Losee

BIOLITE flow agitator, affording a field of view encompassing most

of the agitator volume. The temporal resolution of the PMT was 10 ms with the measurement duration adjusted to sample an equal flow volume, usually 1.7 L, at each volume flow rate setting. For *P. fusiformis* measurements, a 2.0 neutral density filter was used to attenuate the light signal to avoid saturating the detector. Conversion to PMT photon units was based on radiometric calibration using an Optronics Laboratories model 310 multispectral source.

Total mechanically stimutable light (TMSL) of individual cells of *L. polyedrum* and *P. fusiformis* was measured by stirring the cells to exhaustion in a 15 cm diameter light integrating collector (Lab-sphere Inc., North Sutton, USA). Light intensity was measured by an Electron Tubes model P10232 photon counting photomultiplier tube fitted with a Uniblitz electronic shutter. The temporal resolution of the PMT was 10 ms. For *P. fusiformis* measurements, a 2.0 neutral density filter was used to attenuate the light signal to avoid saturating the detector. Groups of one or five cells were placed in 1 ml filtered seawater in a 7 ml scintillation vial at the end of the light phase and stimulated by a stirrer approximately 2.5 h into the dark phase when maximal levels of bioluminescence are expressed. Bioluminescence was measured during 60 s of continuous stirring, with TMSL representing the integrated light emission over this time period, which is sufficient to capture most of the mechanically stimulated emission.

The efficiency of stimulation provides an estimate of the percent of bioluminescence potential measured in a flow agitator and is referred to as %TMSL. It is reported at the critical flow rate and calculated by dividing BBP per volume flow rate per cell concentration (units of photons cell⁻¹) by TMSL (photons cell⁻¹) and multiplying by 100%. Thus the efficiency of stimulation represents the ratio of the number of photons cell⁻¹ measured by the bathyphotometer, to the number of photons cell⁻¹ of the same species mechanically stimulated to exhaustion in an integrating light chamber. Assuming upstream bioluminescence pre-stimulation is negligible, %TMSL of 100% indicates that every luminescent organism passing through the flow agitator was mechanically stimulated to exhaustion of light emission, and that all the luminescence from each cell was measured.

3.3. Bioluminescence imaging of flash trajectories

Flash trajectories for the NOSC and BIOLITE flow agitators were visualized using *P. fusiformis* at a concentration of 0.1 cell ml⁻¹, for steady volume flow rates of 0.17, 0.33, and 0.5 l s⁻¹. Bioluminescence was imaged by a Roper Scientific Cascade:650 low-light digital camera system fitted with a Computar f/1.3 lens and operating with a 25-ms frame duration with an intensifier setting of 3400. Image acquisition was controlled by Metamorph software (Molecular Devices LLC, Sunnyvale, CA). Each flash trajectory was considered to represent the bioluminescence produced by a single dinoflagellate cell. Estimates of the residency times of the most prominent flash trajectories were determined from the number of 25-ms frames in which the trajectory occurred. Residence times of the dominant flash trajectories were compared to residence times associated with central core flow, estimated by dividing the volume of the core flow by the total volume flow rate through the flow agitator.

Two-dimensional projections of flash trajectories were created using ImageJ (National Institutes of Health) to assemble TIFF images obtained by the digital camera into a single composite image. By integrating pixel intensity of the core and recirculating flow zones of images, composed of 100 frame averages, it was possible to determine the location of the bioluminescence within the NOSC flow agitator. The geometry of the BIOLITE flow agitator precluded discriminating whether flashes originated in the core or in secondary, recirculating flow regions. This is because the

imaged volume of flow consisted of three overlapping flow layer regimes: the outermost layer composed of a portion of an annulus of recirculating, secondary flow; the inner core flow; and another annular region of recirculating, secondary flow outside of the core flow.

3.4. Statistical analysis

Unless otherwise stated, values represent means with standard deviations. Regression analysis was based on a least-squares power (log–log) relationship. Statistical significance was based on $P < 0.05$.

4. Results

4.1. Bathyphotometer bioluminescence potential measurements

Cultured dinoflagellates produced flashes in the flow agitators for all flow rates tested. Individual flash events could be resolved from the records only for the lowest cell concentrations; at higher cell concentrations, coincident flashes led to records with superimposed flashes and elevated baselines (Fig. 2).

The dependence of BBP on volume flow rate always exhibited two general trends, regardless of species, concentration, or flow agitator (Fig. 3). At lower flow rates, BBP increased sharply with volume flow rate; above a critical value of volume flow rate, henceforth referred to as Q_c , BBP was relatively independent of volume flow rate. For volume flow rates greater than Q_c , the variability in BBP with volume flow rate was expressed as the coefficient of variation, while the trend was determined by regression analysis.

For *L. polyedrum* and *P. fusiformis*, Q_c was estimated as about 0.2 l s⁻¹ in both the NOSC and BIOLITE flow agitators (Fig. 3A, B). For *L. polyedrum* the variability based on the coefficient of variation for volume flow rates $> Q_c$ (i.e., between 0.2 and 1.0 l s⁻¹) was 40.5% ($N=7$ experiments) in the NOSC flow agitator and 26.6% ($N=16$) in the BIOLITE flow agitator. Based on linear regression analysis of BBP as a function of volume flow rate values greater than Q_c for *L. polyedrum*, there was a $60 \pm 17\%$ ($N=7$ experiments) decrease in BBP in the NOSC flow agitator and a $72 \pm 50\%$ ($N=16$) decrease in the BIOLITE flow agitator. Corresponding BBP variability for *P. fusiformis* was 46.1% ($N=3$) in the NOSC flow agitator and 40.4% ($N=10$) in the BIOLITE flow agitator. For volume flow rates $> Q_c$, BBP of *P. fusiformis* increased $286 \pm 196\%$ ($N=3$) in the NOSC flow agitator and $170 \pm 74\%$ ($N=10$) in the BIOLITE flow agitator. The lower variability in the BIOLITE BBP compared to that of the NOSC BBP, 34% less for *L. polyedrum* and 12% less for *P. fusiformis*, is consistent with the larger volume of the BIOLITE flow agitator.

Critical volume flow rate, Q_c , for *C. fusus* and *P. bahamense* (Fig. 3C, D) was estimated as about 0.5 l s⁻¹. For volume flow rates $> Q_c$ (i.e., between 0.5 and 1.0 l s⁻¹), the variability in BBP for *C. fusus* was 31.9% ($N=9$) in the NOSC flow agitator and 32.6% ($N=8$) for *P. bahamense* in the BIOLITE flow agitator. Based on the linear regression analysis for BBP at volume flow rates $> Q_c$, for *C. fusus*, there was a $160 \pm 31\%$ ($N=9$) increase in BBP in the NOSC flow agitator and a $158 \pm 32\%$ ($N=8$) increase for *P. bahamense* in the BIOLITE flow agitator.

For volume flow rates $> Q_c$, NOSC and BIOLITE BBP scaled nearly linearly with cell concentration as expected (Fig. 4). For the NOSC flow agitator, BBP of *L. polyedrum* increased as the 0.92 ($N=9$ experiments; $R^2=0.845$) power of cell concentration, for *P. fusiformis* as the 0.85 ($N=3$; $R^2=0.996$) power of cell concentration, and for *C. fusus* as the 0.92 ($N=9$; $R^2=0.939$) power of cell concentration. Similarly, for the BIOLITE flow agitator BBP of *L. polyedrum* increased as the 0.90 ($N=16$; $R^2=0.956$) power of cell concentration, for *P.*

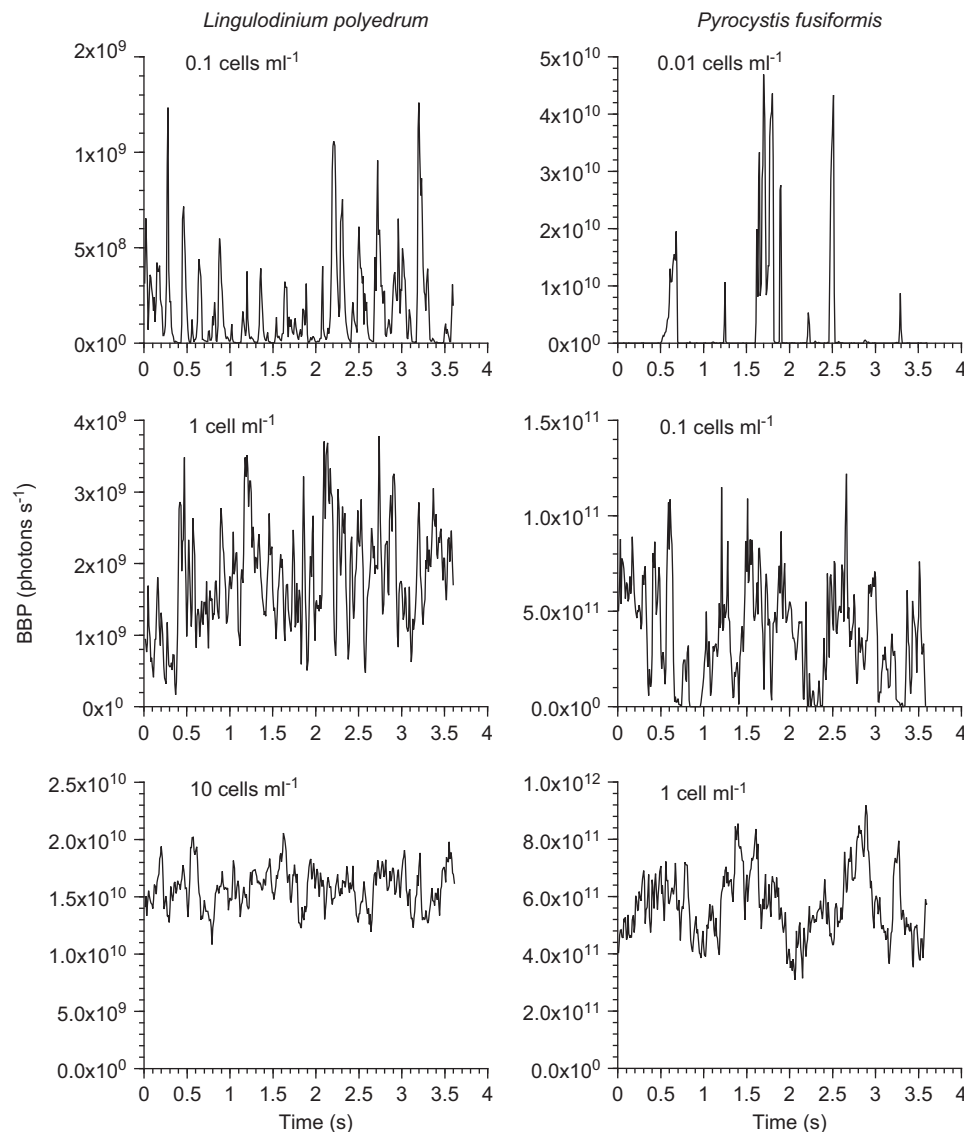


Fig. 2. Representative records of bioluminescence intensity, referred to as bathyphotometer bioluminescence potential (BBP), measured in the BIOLITE flow agitator at a volume flow rate of 0.5 l s^{-1} . Only the first 4 s of each record is shown for three cell concentrations for two dinoflagellate species. At low cell concentrations individual flash events are resolvable, while at higher cell concentrations the flashes summate.

fusiformis as the 1.00 ($N=3$, $R^2=0.957$) power of cell concentration, and for *P. bahamense* as the 1.00 ($N=10$; $R^2=0.957$) power of cell concentration. For *L. polyedrum* and *P. fusiformis* tested in both flow agitators, BIOLITE BBP was always greater than NOSC BBP at volume flow rates $> Q_c$. Based on the average difference in elevations for the calculated power regressions (Fig. 4), the ratio of BIOLITE to NOSC BBP was 1.4 for *L. polyedrum* and 3.6 for *P. fusiformis*.

NOSC BBP measurements (see Appendix for details) using freshly collected samples from San Diego, where the dominant luminescent sources were the dinoflagellates *L. polyedrum* and *Protoperidinium* spp. (Lapota, 1998), showed a similar Q_c of 0.2 l s^{-1} (data not shown) as for cultures of *L. polyedrum*. However, for volume flow rates between Q_c and 1 l s^{-1} , NOSC BBP for the two mixed plankton experiments increased by 17.6 and 23.7% as opposed to the 61% decrease in levels observed for the cultured *L. polyedrum* between about 0.2 and 1 l s^{-1} . These different dependencies between cultures of *L. polyedrum* and mixed plankton samples collected from San Diego Bay may be due to the presence of *Protoperidinium* spp.

Transit through the NOSC flow agitator did not totally exhaust bioluminescence potential. When two NOSC flow agitators were

placed in series, 0.61 m apart, downstream BBP of *L. polyedrum* at a concentration of 10 cells ml^{-1} averaged about 37% of the upstream BBP for volume flow rates greater than critical (data not shown).

Flow agitator efficiency of stimulation was based on %TMSL, calculated for the NOSC and BIOLITE flow agitators for *L. polyedrum* and *P. fusiformis* at the critical volume flow rate of 0.2 l s^{-1} . Dividing BBP by the product of volume flow rate and cell concentration results in an estimate of the average bioluminescence measured per cell (units of photons cell⁻¹) passing through the flow agitator. Dividing this number (photons cell⁻¹) by the bioluminescence potential (TMSL) of $2.4 \pm 2.0 \times 10^7$ photons cell⁻¹ ($N=9$ replicates of 5 cells) for *L. polyedrum* and $4.6 \pm 2.0 \times 10^{10}$ photons cell⁻¹ ($N=10$ replicates of 5 cells plus 4 individual cells) for *P. fusiformis*, and expressing the result as a percentage gives %TMSL. At a critical volume flow rate of 0.2 l s^{-1} , %TMSL for the NOSC flow agitator with *L. polyedrum* and *P. fusiformis* was 28% and 1.2%, respectively; and for the BIOLITE flow agitator with *L. polyedrum* and *P. fusiformis* was 42% and 1.2%, respectively. Because volume flow rate, Q , determines cell delivery rate, as BBP was nearly independent of Q for $Q > Q_c$, then the corresponding %TMSL

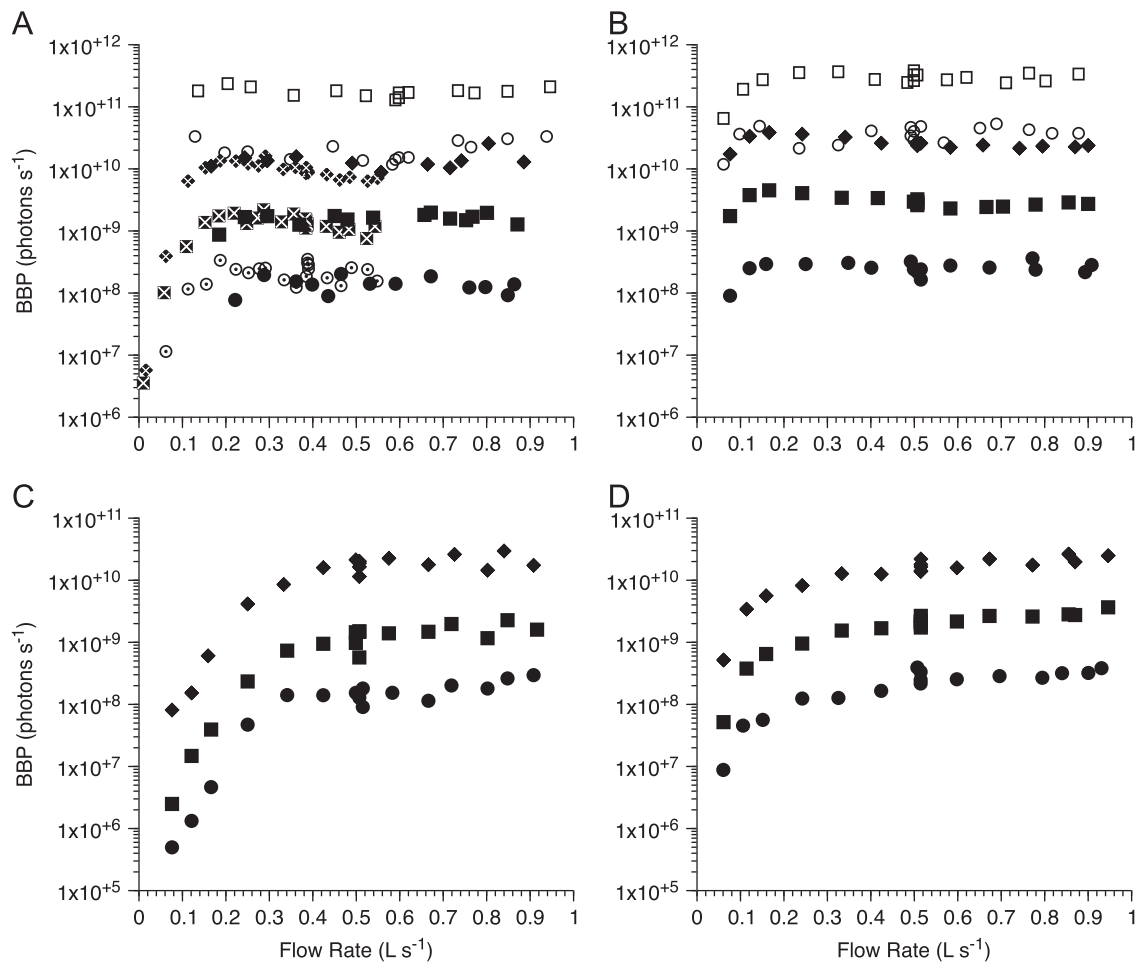


Fig. 3. Representative experiments showing the relationship between bioluminescence intensity, referred to as bathyphotometer bioluminescence potential (BBP), as a function of volume flow rate. Symbols represent the cell concentrations: circles—0.1 cell ml^{-1} , squares—1 cell ml^{-1} , diamonds—10 cell ml^{-1} . (A) NOSC flow agitator results for two concentrations of *Pyrocystis fusiformis* (open symbols) and three concentrations of *Lingulodinium polyedrum* (closed symbols). For *L. polyedrum* overlapping low and high volume flow rate experiments are shown by similar symbols. (B) BIOLITE flow agitator results for two concentrations of *P. fusiformis* (open symbols) and three concentrations of *L. polyedrum* (closed symbols). (C) NOSC flow agitator results for three concentrations of *Ceratium fusus* (D) BIOLITE flow agitator results for three concentrations of *Pyrodinium bahamense*.

should tend to decrease with increasing Q . For example, at an operational volume flow rate of 0.5 l s^{-1} , %TMSL for the NOSC flow agitator was 8% for *L. polyedrum* (vs. 28% at the critical flow rate of 0.2 l s^{-1}) and 0.4% for *P. fusiformis* (vs. 1.2% at 0.2 l s^{-1}).

Although the ratio of bioluminescence potential (TMSL) of *P. fusiformis* to *L. polyedrum* was 1966, the corresponding BBP ratios, based on average photons cell^{-1} at the critical volume flow rate of 0.2 l s^{-1} , were much lower, yielding 88 for the NOSC flow agitator and 56 for the BIOLITE flow agitator. These results demonstrate that BBP scaling of different flow agitators will depend on the species composition of the luminescent community, especially for organisms with different emission properties.

4.2. Bathyphotometer bioluminescence flow visualization

Images of bioluminescence streaks of *P. fusiformis* in the NOSC flow agitator revealed dominant recirculating, secondary flow patterns in the half cylindrical shaped chambers above and below the core flow (Fig. 5). Composite images of 100 frames showed that most bioluminescence stimulated in the NOSC flow agitator occurred in these two recirculating zones. Two different approaches were used to assess the contribution of the recirculating zones to NOSC BBP. Integrating pixel intensity over the core and secondary flow areas for the 100 frame composite image at

volume flow rates of 0.2, 0.3, and 0.5 l s^{-1} , showed that > 90% of the measured luminescence occurred in the recirculating zones. To further corroborate that most of the measured BBP was originating from the recirculating zones, an experiment was performed with *L. polyedrum* at a concentration of 10 cells ml^{-1} in which the field of view of the PMT was restricted to mainly the core flow or the recirculation zones. For volume flow rates greater than critical, BBP was reduced by at least 80% when the view of the recirculation zone was occluded, compared to only a 20% or less decrease when view of the core flow was blocked (Fig. 6). Thus both imaging and PMT measurements confirmed that most of the measured BBP was originating from the recirculating zones of the NOSC flow agitator.

If most of the BBP occurs in the recirculation zones of the NOSC flow agitator, then the flash residence time of *P. fusiformis* should be greater than the residence time of fluid particles moving relatively straight through the core flow. Based on single frame analysis of 226 prominent, longer flash trajectories for flow rates from 0.08 to 0.72 l s^{-1} , average flash residence time ranged from 233 to 79 ms, respectively, with maximum flash residence times as high as 750 ms (Table 1). The ratio of average flash to core residence times increased with volume flow rate. At the lowest volume flow rate of 0.08 l s^{-1} , the ratio of average flash to core residence time was 5.9. Between 0.17 and 0.72 l s^{-1} this ratio increased from about 12 to 18 with an average flash residence time

14.8 ± 2.5 ($N=4$ flow rates) times greater than that expected in the core. The maximum flash residence time within the NOSC flow agitator was as much as 80 times the core flow residence time at the highest volume flow rate measured (Table 1).

Single frame images showed that flash trajectories of *P. fusiformis* in the BIOLITE flow agitator were also highly convoluted and with increasing volume flow rate tended to have more complex pathlines (Fig. 7). The geometry of the BIOLITE flow agitator precluded discriminating whether flash trajectories occurred in the core or

secondary flow regions because the imaged volume of flow consisted of three overlapping flow layers. The first layer was composed of a portion of the outer annulus of secondary flow, followed by the inner core layer bridging the inlet and outlet flow, and finally the outer annulus of secondary flow on the opposite side of the flow agitator. For volume flow rates ranging from 0.09 to 0.52 l s^{-1} , the average flash residence time ranged from 108 to 56 ms, respectively, with maximum flash residence times as high as 300 ms (Table 1). The ratio of average flash to core flow residence time increased from 0.5 to 1.4 with increasing volume flow rate. Unlike the case for the NOSC flow agitator, flash residence times in the BIOLITE flow agitator at the lower flow rates were less than that calculated for the average core flow, suggesting that some cells were stimulated and entrained into the core flow near the exit. The maximum flash residence time within the BIOLITE flow agitator was as much as 13 times that of the core flow, but still much lower than for the NOSC

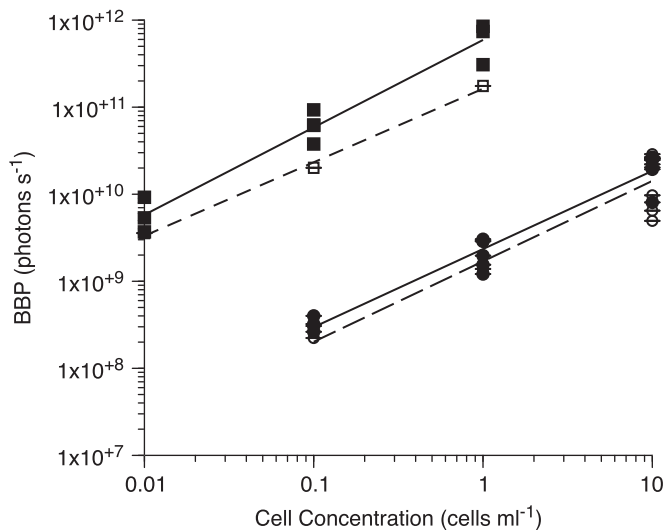


Fig. 4. Relationship between average bioluminescence intensity, referred to as bathyphotometer bioluminescence potential (BBP), for volume flow rates (Q) greater than critical, as a function of cell concentration. Closed symbols are for the BIOLITE flow agitator and open symbols for the NOSC flow agitator, for *Pyrocystis fusiformis* (squares) and *Lingulodinium polyedrum* (circles). Lines represent the least-squares power regression for each species and the BIOLITE (solid line) and NOSC (dashed line) flow agitators: *P. fusiformis* for the BIOLITE flow agitator, $\text{BBP} = 5.939 \times 10^{11} Q^{1.004}$, $R^2 = 0.957$; *P. fusiformis* for the NOSC flow agitator, $\text{BBP} = 1.635 \times 10^{11} Q^{0.845}$, $R^2 = 0.845$; *L. polyedrum* for the BIOLITE flow agitator, $\text{BBP} = 2.356 \times 10^9 Q^{0.897}$, $R^2 = 0.956$; *L. polyedrum* for the NOSC flow agitator, $\text{BBP} = 1.693 \times 10^9 Q^{0.924}$, $R^2 = 0.845$. Not shown is the relationship for *Ceratium fusus* for the NOSC flow agitator, $\text{BBP} = 3.309 \times 10^9 Q^{0.915}$, $R^2 = 0.939$; and *Pyrodinium bahamense* for the BIOLITE flow agitator, $\text{BBP} = 2.403 \times 10^9 Q^{1.004}$, $R^2 = 0.957$.

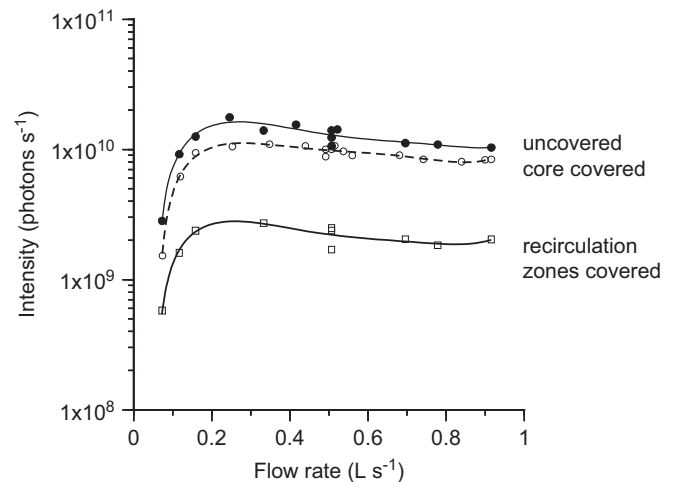


Fig. 6. Average bioluminescence intensity, referred to as bathyphotometer bioluminescence potential (BBP), of core and recirculation flow zones within the NOSC flow agitator for *Lingulodinium polyedrum* at a cell concentration of 10 cell ml^{-1} as a function of volume flow rate. Solid circles—uncovered view (control), open circles—core flow region covered, open boxes—recirculation zones covered. 80% of BBP occurred in the recirculation zones at volume flow rates greater than the critical value of 0.2 l s^{-1} .

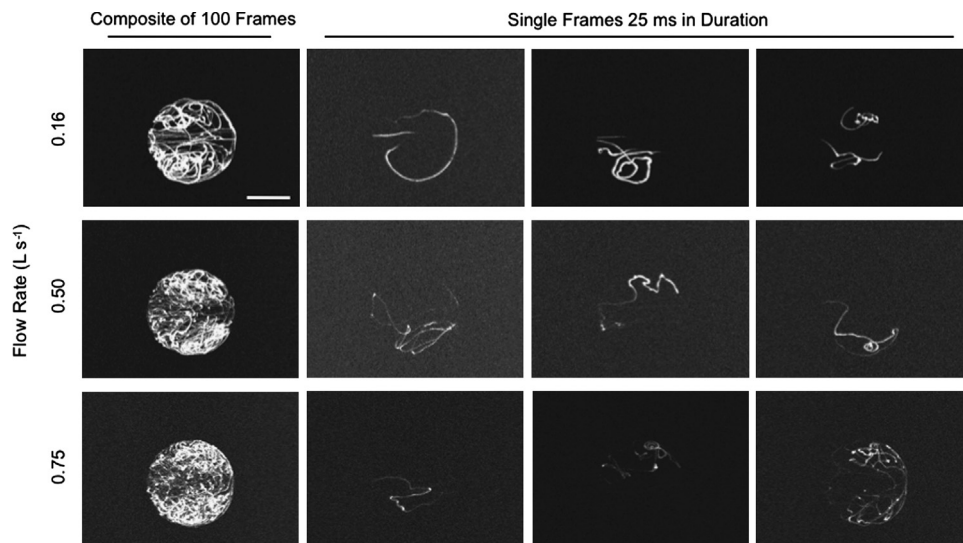


Fig. 5. Representative images of bioluminescence trajectories of *Pyrocystis fusiformis* within the NOSC flow agitator for three volume flow rates. The direction of flow is from right to left. Individual image frames have a 25-ms integration time, scale bar is 2 cm. Left column is a composite of 100 image frames that better delineate the recirculation pattern, while the other images are of single frames of one or more flash trajectories revealing their convoluted trajectories.

flow agitator at similar volume flow rates (Table 1), presumably because of the much larger interfacial area between core and secondary flow zones.

Table 1

Analysis of flash trajectories of *Pyrocystis fusiformis* in the flow agitators. Video frame duration was 25 ms, diameter of the field of view was 4 cm. Dimensions for the flow agitators and core flow in each flow agitator are given in the methods. Core flow residence time is the core flow volume divided by the volume flow rate. Values of flash residence time represent averages $\pm$ s.d. for N number of flashes.

Volume flow rate (L s^{-1})	Residence time of core flow (ms)	Flash residence time (ms)	N	Ratio of average flash to core flow residence times	Maximum residence time (ms)	Ratio of maximum flash to core flow residence times
<i>NOSC agitator</i>						
0.08	39.4	233 ± 173	9	5.9	475	12.1
0.17	18.5	223 ± 194	20	12.1	750	40.6
0.3275	9.6	134 ± 87	53	14.0	500	52.5
0.50	6.3	95 ± 53	89	15.1	300	47.7
0.72	4.4	79 ± 47	55	18.0	350	79.5
<i>BIOLITE agitator</i>						
0.09	225.2	108 ± 71	20	0.5	300	2.1
0.17	119.2	87 ± 56	24	0.7	250	6.3
0.52	39.0	56 ± 12	87	1.4	100	12.8

5. Discussion

5.1. Bathypotometer bioluminescence potential

Bioluminescence potential, used as a measure of the biomass of luminescent organisms in the ocean (Batchelder and Swift, 1989; Losee et al., 1989; Piontkovski et al., 1997; Rudyakov, 1968), is assessed from bathypotometer measurements of flow-stimulated bioluminescence, BBP. However, BBP represents only a fraction of total bioluminescence potential of the organisms during their brief transit through the flow agitation chamber (Herren et al., 2005). In practice the hydrodynamic characteristics of bathypotometers are typically overlooked in making bathypotometer comparisons of BBP (Bivens et al., 2002b). Nevertheless, the amount of flow stimulation of bioluminescence by dinoflagellates, the most common luminescent sources in coastal regions, is dependent on fluid shear stress (Blaser et al., 2002; Deane and Stokes, 2005; Latz et al., 1994, 2004; Maldonado and Latz, 2007). Therefore it is reasonable to conclude that there is no *a priori* reason that the widely different flow fields associated with different bathypotometers would provide the same amount of mechanical stimulation and flash residence times to ensure similar values of BBP when measuring an identical dinoflagellate population. In this study, direct comparison between the NOSC and BIOLITE flow agitators for identical cell concentrations showed that BBP was in fact not the same, with the BIOLITE BBP being about 1.4 times greater for *L. polyedrum* and 3.6 times greater for *P. fusiformis*. Moreover, the critical volume flow rate could differ between species for the same flow agitator. The inability to relate BBP from different bathypotometer designs has limited the utility of BBP as a routinely monitored ocean parameter (Widder et al. 1993; Bivens et al., 2002b). Developing empirical relationships between

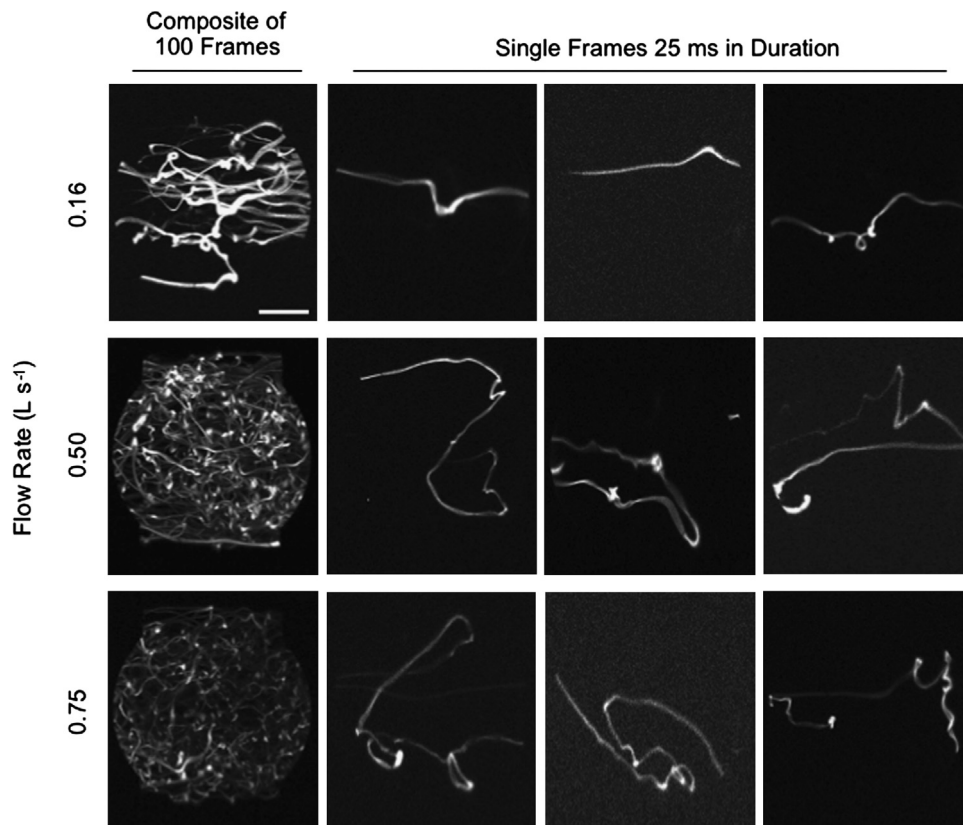


Fig. 7. Representative images of bioluminescence trajectories of *Pyrocystis fusiformis* within the BIOLITE flow agitator for three volume flow rates. The direction of flow is from right to left. Individual image frames have a 25-ms integration time; scale bar is 1 cm. Left column is a composite of 100 image frames, while the other images are of single frames of one or more flashes revealing their convoluted trajectories.

bioluminescence intensity, fluid shear stress, and BBP could provide unprecedented insight into stimulatory flow fields in both the laboratory and ocean.

The present study provides a context for measuring bathyphotometer bioluminescence potential as a basis for inter-instrument comparisons. We contend that this comparison is best done in a controlled laboratory setting under prescribed conditions of known species, cell concentration, and volume flow rate. Such an approach will help identify differences in BBP across bathyphotometer designs due to different degrees of flow stimulation and cell residency times within the flow agitator. A complete hydrodynamic characterization of the stimulatory flow is presently not possible (Herren et al., 2005; Widder et al., 1993) due to the highly turbulent nature of the flow field experienced by organisms as they pass through the flow agitator chamber. Imaging flash trajectories in the present study illustrates that secondary flows are important and can dominate the bioluminescence emission pattern. A comprehensive evaluation of each bathyphotometer should include: a coarse hydrodynamic characterization of the flow field within the agitator; estimate of %TMSL of flow agitator as a function of volume flow rate and luminescent species; laboratory tests with known species and cell concentrations to verify biomass scaling of BBP; determination of the critical volume flow rate as a guide for establishing operational range; and imaging of flash trajectories for flow visualization to determine where flashes are initiated, flash residency times, and the importance of secondary flows.

5.2. Critical volume flow rate

Although BBP is optimally measured at a single volume flow rate, under operational conditions bathyphotometers may operate at a range of flow rates. This is problematic if the flow pattern and accompanying hydrodynamic shear stresses and flash residence times that stimulate bioluminescence vary across this range. Thus bathyphotometer characterization should investigate whether there is a critical volume flow rate, Q_c , above which BBP is relatively constant. In the present study, critical volume flow rates for *P. fusiformis* and *L. polyedrum* were similar for both the NOSC and BIOLITE flow agitators, having a value of about 0.2 L s^{-1} . This finding is unexpected considering the different flow agitator designs and flash response characteristics of the organisms. Critical volume flow rates for the other species studied were higher, being 0.5 L s^{-1} for both *C. fusus* in the NOSC flow agitator and *P. bahamense* in the BIOLITE flow agitator. Most operational

flow rates of the NOSC and BIOLITE bathyphotometers are greater than these critical volume flow rates (Herren et al., 2005), so that large variations in ocean measurements of BBP due to volume flow rate for these species are not expected.

The critical flow rates of other bathyphotometers that have been similarly characterized vary with instrument design. Using a towable bathyphotometer with a 30-ml flow agitation chamber, the critical volume flow rate in Oyster Bay, Jamaica, where the dominant luminescent source is *P. bahamense*, is about 0.11 L s^{-1} (Seliger et al., 1969b), much lower than the critical value of 0.5 L s^{-1} obtained in the present study with the BIOLITE flow agitator. The Seliger study used a much different flow agitator design than the NOSC or BIOLITE, employing an impeller pump as the mode of excitation (Seliger et al., 1962). Another bathyphotometer that uses an impeller pump for bioluminescence stimulation is the UBAT bathyphotometer (Wet Labs Inc.). Based on measurements at a fixed depth, volume flow rates less than the operational value of 0.5 L s^{-1} resulted in decreased BBP for a prototype of the UBAT (Herren et al., 2005). As volume flow rates greater than the operational value were not tested, the critical volume rate could not be determined. Furthermore increased variability at the higher flow rates suggest the entrainment of larger organisms that produce higher intensity bioluminescence flashes, reinforcing the importance of using known concentrations of organisms for determining critical volume flow rates. Because differences in flow agitator design can lead to differences in flow agitation of bioluminescence, the difference in critical flow rates between the Seliger et al. (1969b), Herren et al. (2005), and present studies are not unexpected.

The difference in critical volume flow rate between flow agitators is most pronounced with the HIDEEX bathyphotometer (HIDEEX-BP), a large volume device that stimulates bioluminescence based on grid turbulence (Widder et al., 1993). For the HIDEEX-BP, the critical volume flow rate is about 22 L s^{-1} (Widder et al., 1993), based on laboratory tests with *Pyrocystis noctiluca*, which is closely related to *P. fusiformis* used in this study, and field measurements in the northern Sargasso Sea, where *P. noctiluca* as well as zooplankton are common bioluminescent sources (Batchelder and Swift, 1989; Batchelder et al., 1990; Swift et al., 1985b). A higher critical volume flow rate for the HIDEEX-BP is expected because of its large input area, which is 144 and 22 times that of the NOSC and BIOLITE flow agitators, respectively. If adjusted for the different input areas, the critical flow speeds, based on average flow speed (m s^{-1}) through the bathyphotometer when critical volume flow rate ($\text{m}^3 \text{ s}^{-1}$) is achieved, for these different flow agitators are within a factor of about 5 (Table 2). The

Table 2

Comparison of the efficiency of stimulation, critical flow properties, and residence times of different bathyphotometer flow agitation systems.

Name	Critical volume flow rate (L s^{-1})	Critical flow speed (cm s^{-1}) ^a	Residence time (ms)	Efficiency of stimulation (%TMSL)		
				<i>Lingulodinium polyedrum</i>	<i>Pyrocystis</i>	Source
HIDEEX-BP	22	195	500 ^b	94	12 ^c	Widder et al. (1993)
UBAT prototype	0.5 ^d	62	10,000 ^e	17	35 ^a	Herren et al. (2005)
URI BP	0.25		1000 ^b	–	10 ^c	Swift et al. (1985a)
NOSC	0.2	255	750 ^f	28	1.2 ^g	Present study
BIOLITE	0.2	40	250 ^f	42	1.2 ^g	Present study
Seliger BP	0.11	37	270 ^b	–	–	Seliger et al. (1969b)

^a Critical flow speed is calculated as the critical volume flow rate divided by the cross-sectional area of the inlet to the flow agitator.

^b Flow volume residence time.

^c *P. noctiluca*.

^d Operating volume flow rate; critical volume flow rate not determined.

^e Flash residence time.

^g *P. fusiformis*.

^f Maximum flash residence time.

narrow range of critical flow speeds is remarkable, considering that critical volume flow rates vary over a range of 200, and that the various flow agitator designs use different mechanisms for flow agitation. However, it is expected that average flow speed (m s^{-1}) is more descriptive than volume flow rate ($\text{m}^3 \text{s}^{-1}$) in examining critical conditions for bioluminescence stimulation. As flow-stimulated dinoflagellate bioluminescence is dependent on shear stress produced by velocity gradients within the flow (Latz and Rohr, 1999; Maldonado and Latz, 2007), then no matter how high the volume flow rate, if the shear stress is not above threshold then bioluminescence will not be stimulated. Nevertheless, adjusting for the volume of the flow agitator within the aperture of the PMT is critical for comparing bioluminescence intensity measurements (see Appendix). Until the hydrodynamic conditions of the flow agitators are fully characterized in the context of the luminescent response of the organisms, critical volume flow rates or speeds cannot be predicted and must be experimentally determined to ensure that operational volume flow rates are always greater than critical values.

Species differences in critical volume flow rate, Q_c , for the same flow agitator indicate that the bioluminescence response to flow stimulation has a biological specificity. However, there appears to be no relationship between Q_c in the flow agitators and threshold sensitivity as previously determined with quantified flow conditions using laminar pipe flow. Response threshold levels for bioluminescence stimulation expressed as values of wall shear stress are 0.09 N m^{-2} for *P. fusiformis*, 0.12 N m^{-2} for *C. fusus*, and 0.33 N m^{-2} for *L. polyedrum* (Latz et al., 2004). Thus, in terms of stimulation thresholds *P. fusiformis* and *C. fusus* can be considered more sensitive to flow stimulation than *L. polyedrum* despite *P. fusiformis* and *L. polyedrum* having the same Q_c in both the BIOLITE and NOSC flow agitators and lower Q_c than *C. fusus* in the NOSC flow agitator. However, in considering the transition from laminar to turbulent pipe flow, the average bioluminescence intensity for *P. fusiformis* and *L. polyedrum* appeared to plateau while that for *C. fusus* continued to increase with pipe wall shear stress (Latz et al., 2004). If this trend continues for the much higher shear stress levels associated with the flow field within the flow agitators, a higher critical volume flow rate for *C. fusus* would be consistent. Further studies of the population response of luminescent dinoflagellate species as a function of shear stress in turbulent flow are needed to better understand how their response to hydrodynamic stimulation in the flow agitators are related to Q_c .

With increasing volume flow rate there are several conflicting factors affecting BBP. Fluid shear stress upstream of the flow agitator increases with volume flow rate, increasing the chance of pre-stimulation (Latz and Rohr, 1999). Any upstream stimulation of bioluminescence will reduce the bioluminescence potential of the population within the flow agitator and decrease BBP. In addition, cell residence time decreases with increasing volume flow rate, potentially decreasing BBP. Conversely, BBP may increase with increasing volume flow rate because the number of cells passing through the flow agitator will increase. Furthermore the shear stress field within the flow agitator will increase with volume flow rate, and a greater proportion of the cells may experience suprathreshold levels of stimulation (Latz and Rohr, 1999).

The model of Seliger et al. (1969a, 1969b) predicts a volume flow regime that is characterized by flash residence times, T , shorter than the flash decay time, γ , where BBP will be independent of volume flow rate ($T < \gamma$; Eq. (2)). Yet for NOSC BBP essentially all the bioluminescence occurred in the recirculating zones where this approximation is poor because of the relatively long flash residence times. The prolonged residence time of fluid in the recirculation zones of the NOSC flow agitator has been previously noted (Losee et al., 1985). At volume flow rates near

critical values, 0.17 and 0.33 l s^{-1} , flash residence times of 223 and 134 ms (Table 1), respectively, were much greater than the 53 ms flash decay time of *L. polyedrum* (Latz and Lee, 1995). Moreover, conspicuously little bioluminescence was measured in the core flow, where $T < \gamma$. Similar discrepancies exist for other published BBP data. Widder et al. (1993) report a critical volume flow rate of 22 l s^{-1} for *Pyrocystis noctiluca* in the HIDE-X-BP. At this flow rate, the flash residence time is about 500 ms, which is much greater than the flash decay time of about 100 ms for *P. noctiluca* (Latz, unpublished data). Seliger et al. (1969a, 1969b) report cell residence times of about 270 ms for *P. bahamense* at the critical volume flow rate of 0.11 l s^{-1} , which again is much greater than its flash decay time of 50 ms (Seliger et al., 1961).

A possible explanation for the existence of a critical volume flow rate in the NOSC and BIOLITE flow agitators is that the flow agitator recirculation zones provide: (1) suprathreshold levels for the entire cell population, independent of volume flow rate through the agitator; (2) suitably long cell residence times, so that the flash duration is approximately equal to or less than the cell residence time; and (3) the requisite recruitment of fresh cells from the core flow to allow an approximately constant concentration of fresh cells in the recirculation zones independent of volume flow rate through the core.

For measurements at volume flow rates near and greater than critical, where BBP was relatively independent of volume flow rate, it is inappropriate to divide NOSC or BIOLITE BBP by the corresponding volume flow rate to relate to bioluminescence potential. Doing so would lead to the erroneous conclusion that bioluminescence potential was changing despite no change in cell concentration.

5.3. Comparison of BBP for the NOSC and BIOLITE flow agitators

Comparison of BBP across bathyphotometers is problematic because of their different flow agitator designs, degree of flow stimulation, and geometry of light detection. Furthermore such comparisons are typically made for ocean measurements with natural populations of organisms with spatial and temporal variability in abundance. The advantage of the present laboratory study is that the BIOLITE and NOSC flow agitators can be directly compared using the identical light detector for similar concentrations of cultured dinoflagellates. BIOLITE BBP may be expected to be greater than that of the NOSC flow agitator because of its 1.96 times greater total fluid volume. Based on the regression analysis of BBP vs. cell concentration for $Q > Q_c$, the ratio of BIOLITE to NOSC BBP averaged 1.4 for *L. polyedrum* and 3.6 for *P. fusiformis*. Although both agitator flow fields are similar in that they consist of a central jet surrounded by a cavity of recirculating flow, the geometry is not identical so the stimulating shear fields will be different. The shorter flash residence time measured in the BIOLITE flow agitator is consistent with the greater interfacial area between the core and secondary flow regions, about 2.5 times that of the NOSC flow agitator. Regardless, as expected NOSC and BIOLITE BBP were highly correlated with cell concentration, and a repeatable transfer function for each of the two dinoflagellate species relating BBP for these flow agitators was clearly evident at volume flow rates greater than critical.

5.4. Efficiency of stimulation

Given the differences in the stimulatory flow field and flash residency times for each design of bathyphotometer, one simple design comparison could be based on the percentage of bioluminescence potential (TMSL) that is measured in the flow agitator (BBP), referred to as the efficiency of stimulation and expressed as %TMSL (Herren et al., 2005; Widder et al., 1993).

In the present study, NOSC and BIOLITE flow agitator BBP constituted only a small proportion of bioluminescence potential, and was strongly dependent on luminescent species, volume flow rate for $Q < Q_c$, and flow agitator design. At the critical volume flow rate of 0.2 l s^{-1} , the %TMSL for *L. polyedrum* was 28% in the NOSC flow agitator and 42% in the BIOLITE flow agitator. The corresponding %TMSL for *P. fusiformis* at the critical volume flow rate was 1.2% for both the NOSC and BIOLITE flow agitators. The higher %TMSL for *L. polyedrum* is consistent with its producing only a few flashes (Latz and Lee, 1995; Widder et al., 1993), whereas *P. fusiformis* produces many flashes (Widder and Case, 1981b) so its %TMSL would be more dependent on residence time within the flow agitator. For the NOSC flow agitator, the low efficiency of stimulation for both species suggests that only a small proportion of cells is stimulated, and particularly for *P. fusiformis*, not all flashes are measured in their entirety. For the BIOLITE flow agitator, the efficiency of stimulation for *L. polyedrum* was 50% greater than for the NOSC agitator, suggesting that its flow field is more stimulatory. The efficiency of stimulation for *P. fusiformis* was the same for both flow agitators, despite the shorter flash residence time in the BIOLITE flow agitator, again suggesting that it is more stimulatory than the NOSC flow agitator. The very low efficiency for *P. fusiformis* in either flow agitator suggests that on average less than one flash for each cell is measured.

Approximate comparisons of efficiency of stimulation can be made with other flow agitators. The HIDEX-BP was designed to provide high levels of stimulation using a grid, long residence times, and high volume flow rates. For the HIDEX-BP at the operational volume flow rate of 22 l s^{-1} , %TMSL was 94% for *L. polyedrum* and 12% for *Pyrocystis noctiluca* (Widder et al., 1993), a species closely related to the *P. fusiformis* used in this study. Grid stimulation by the HIDEX-BP was highly effective in stimulating at least one single flash from each cell, which ensures a high efficiency of stimulation for species such as *L. polyedrum* that produce only a few flashes. For species that may produce multiple flashes before exhaustion, such as *P. noctiluca* and *P. fusiformis* (Widder and Case, 1981b), not all the bioluminescence is measured so the %TMSL is lower. The HIDEX-BP efficiency is consistent with stimulation of the first flash of *P. fusiformis*, which consists of 12% of bioluminescence potential, with each subsequent flash comprising 6% of bioluminescence potential (Widder and Case, 1981b). Placement of a second grid halfway down the chamber resulted in an additional 6% of %TMSL, consistent with the stimulation of a second flash. Even though the HIDEX-BP is highly effective in stimulating single dinoflagellate flashes, its large size ($106 \times 106 \times 235 \text{ cm}^3$) limits its use for many oceanographic applications such as fine scale oceanic measurements, deployment off small ships, and use with buoys, remotely operated underwater vehicles, and autonomous underwater vehicles.

A bathyphotometer developed at the University of Rhode Island, characterized by a relatively low volume flow rate of 0.25 l s^{-1} and bioluminescence stimulation by an impeller pump, has been widely used especially in the Sargasso Sea (Batchelder et al., 1990, 1992; Swift et al., 1985a, 1983, 1995). For this system, the efficiency of stimulation based on tests with *Pyrocystis noctiluca* yielded a %TMSL of 10% (Swift et al., 1985a), similar to that of the much larger HIDEX-BP and consistent with the stimulation of a single flash (Widder and Case, 1981b).

For a prototype of the UBAT bathyphotometer, where bioluminescence is also stimulated by an impeller pump, %TMSL was 17% for *L. polyedrum* and 35% for *Pyrocystis noctiluca* at the operational volume flow rate of 0.5 l s^{-1} (Table 2) (Herren et al., 2005). The relatively low efficiency of stimulation for *L. polyedrum* may have originated from initial stimulation by the pump impeller outside the view of the detector, as the delay from stimulation to light emission is only 15 ms (Latz et al., 2008) so that the entire first

flash by a cell may not have been measured. The relatively high %TMSL for *P. noctiluca*, compared to other flow agitator systems (Table 2), most likely results from the system's long residence time and highly turbulent flow field, which results in a higher %TMSL from organisms that produce prolonged emissions from multiple or summated flashes as produced by *Pyrocystis* spp. (Herren et al., 2005; Widder and Case, 1981b).

6. Conclusions

The flow agitators of different bathyphotometer designs are not equivalent in flow pattern, flash residence time, efficiency of stimulation, measurement geometry, and detector type. Thus any direct comparison of BBP between bathyphotometers must be made with caution if a suitable transfer function has not been established through laboratory validation studies using volume flow rates greater than critical, as done in this study for the NOSC and BIOLITE flow agitators. Only laboratory studies, as described here, would allow for an optimized flow agitator design that would entail maximizing BBP while minimizing pre-stimulation, and considering engineering constraints such as size, ease of use, and power requirements. The present investigation shows that there exists a critical volume flow rate, dependent on species and flow agitator design, above which BBP remains nearly constant. Although this general dependence of BBP on volume flow rate is consistent with the simple model of Seliger et al. (1969b), their underlying assumptions cannot be justified for the present flows where most of the bioluminescence is stimulated in recirculating secondary flows. For volume flow rates greater than critical values, NOSC and BIOLITE BBP were an excellent indicator of cell concentration, but not bioluminescence potential nor the relative bioluminescence potential between different species of luminescent dinoflagellates.

Although bioluminescence is a common ocean phenomenon that is related to a variety of environmental parameters, bathyphotometer bioluminescence potential, BBP, is often difficult to compare, interpret, and relate to the actual bioluminescence potential based on the total luminescent capacity of the organisms. Bioluminescence potential depends on the species composition and abundance of luminescent organisms, as well as their physiological state. Flash intensity among dinoflagellates may differ by several orders of magnitude (Lapota et al., 1989, 1992a; Latz et al., 2004) and dinoflagellate concentrations may vary by as much as 5–6 orders of magnitude (Lapota, 1998; Moline et al., 2009; Swift et al., 1995). Environmental factors influencing the physiological state of bioluminescent organisms such as dinoflagellates include: diurnal variability (Haxo and Sweeney, 1965; Sweeney, 1981), previous illumination levels (Sullivan and Swift, 1995; Sweeney, 1981; Swift and Meunier, 1976), temperature (Hastings and Sweeney, 1957; Sweeney, 1981), nutrition (Buskey et al., 1992; Latz and Jeong, 1996), pH (Hamman and Seliger, 1972), and flow agitation history (Latz and Rohr, 1999). Details of how the organism interacts with the stimulating turbulent flow field at relevant time and length scales are yet to be understood. BBP in turn depends on the agitation level of the stimulating flow field, flash residence time within the flow agitator, upstream pre-stimulation, and the ability of the bathyphotometer to draw in all the luminescent organisms present, as well as measurement geometry and detector type.

Despite this complexity, the present study demonstrates that robust empirical relationships between flow agitators, and between BBP and cell concentration, can be determined in a laboratory setting. Similar investigations performed with currently used flow agitators such as the HIDEX-BP and UBAT that employ much different methods of flow excitation would provide a much richer assessment of global bioluminescence potential. Such insight is needed to assess the optimal choice of detector for

oceanographic applications, especially with the availability of small, economical, low power flow agitators that record only a small proportion of the total bioluminescence potential.

Contributions

The authors shared equally in study design, data collection, data analysis, and manuscript preparation and revision.

Acknowledgments

We thank G. Anderson for technical support, J. Allen for modeling support, E. Turack for assistance with experiments, N. Moshman for image analysis of flash trajectories, A. Armendariz for the preparation of Fig. 1, and L. Hood for editing the manuscript. M. Geiger kindly provided the BIOLITE flow agitator. This material is based upon work supported by the Office of Naval Research (award numbers N00014-03-1-0449 and N00014-09-10495 to M. I. L.) and Independent Applied Research funds from Space and Naval Warfare Systems Center Pacific (to J. R.). E. Turack and N. Moshman were supported by the Naval Research Enterprise Intern Program (NREIP).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.csr.2013.04.033>.

Reference

- Altinagac, U., Özekinci, U., Ayaz, A., Ozen, O., Öztekin, A., 2010. Utilization of a bioluminescent pattern in the encircling gillnet fisheries. *Reviews in Fisheries Science* 18, 151–156.
- Badyalak, S., Kelley, K., Philips, E.J., 2004. A description of *Pyrodinium bahamense* (Dinophyceae) from the Indian River Lagoon, Florida, USA. *Phycologia* 43, 653–657.
- Batchelder, H.P., Swift, E., 1989. Estimated near-surface mesoplanktonic bioluminescence in the Western North Atlantic during July 1986. *Limnology and Oceanography* 34, 113–128.
- Batchelder, H.P., Swift, E., Van Keuren, J.R., 1990. Pattern of planktonic bioluminescence in the northern Sargasso Sea: seasonal and vertical distribution. *Marine Biology* 104, 153–164.
- Batchelder, H.P., Swift, E., Van Keuren, J.R., 1992. Diel patterns of planktonic bioluminescence in the northern Sargasso Sea. *Marine Biology* 113, 329–339.
- Biggley, W.H., Swift, E., Buchanan, R.J., Seliger, H.H., 1969. Stimulable and spontaneous bioluminescence in the marine dinoflagellates, *Pyrodinium bahamense*, *Gonyaulax polyedra*, and *Pyrocystis lunula*. *Journal of General Physiology* 54, 96–122.
- Bivens, J.R., Geiger, M.L., Bird, J.L., 2002a. Interpreting bathyphotometer signals. In: *Proceedings of the Oceans MTS/IEEE*, pp. 1705–1710.
- Bivens, J.R., Geiger, M.L., Bird, J.L., Lapota, D., 2002b. Advances in bioluminescence instrumentation. In: *Proceedings of the Oceans MTS/IEEE*, pp. 1895–1898.
- Blackwell, S.M., Moline, M.A., Schaffner, A., Garrison, T., Chang, G., 2008. Sub-kilometer length scales in coastal waters. *Continental Shelf Research* 28, 215–226.
- Blaser, S., Kurisu, F., Satoh, H., Mino, T., 2002. Hydromechanical stimulation of bioluminescent plankton. *Limnology and Oceanography* 17, 370–380.
- Buskey, E., Mills, L., Swift, E., 1983. The effects of dinoflagellate bioluminescence on the swimming behavior of a marine copepod. *Limnology and Oceanography* 28, 575–579.
- Buskey, E.J., Strom, S., Coulter, C., 1992. Bioluminescence of heterotrophic dinoflagellates from Texas coastal waters. *Journal of Experimental Marine Biology and Ecology* 159, 37–49.
- Buskey, E.J., Swift, E., 1983. Behavioral responses of the coastal copepod *Acartia hudsonica* (Pinhey) to stimulated dinoflagellate bioluminescence. *Journal of Experimental Marine Biology and Ecology* 72, 43–58.
- Case, J.F., Widder, E.A., Bernstein, S., Ferrer, K., Young, D., Latz, M.I., Geiger, M., Lapota, D., 1993. Assessment of marine bioluminescence. *Naval Research Reviews* 45, 31–41.
- Chen, A.K., Latz, M.I., Frangos, J.A., 2003. The use of dinoflagellate bioluminescence to characterize cell stimulation in bioreactors. *Biotechnology and Bioengineering* 83, 93–103.
- Chen, A.K., Latz, M.I., Sobolewski, P., Frangos, J.A., 2007. Evidence for the role of G-proteins in flow stimulation of dinoflagellate bioluminescence. *American Journal of Physiology—Regulatory, Integrative and Comparative Physiology* 292, R2020–R2027.
- Deane, G.B., Stokes, M.D., 2005. A quantitative model for flow-induced bioluminescence in dinoflagellates. *Journal of Theoretical Biology* 237, 147–169.
- Eckert, R., 1965. Bioelectric control of bioluminescence in the dinoflagellate *Noctiluca*. I. Specific nature of triggering events. *Science* 147, 1140–1142.
- Fleisher, K.J., Case, J.F., 1995. Cephalopod predation facilitated by dinoflagellate luminescence. *Biological Bulletin* 189, 263–271.
- Geiger, M.L., 1996. Bioluminescence - opportunity for passive surveillance. *Sea Technology*, 69–74.
- Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Canadian Journal of Microbiology* 8, 229–239.
- Hamman, J.P., Seliger, H.H., 1972. The mechanical triggering of bioluminescence in marine dinoflagellates: chemical basis. *Journal of Cellular Physiology* 80, 397–408.
- Harvey, E.N., 1952. *Bioluminescence*. Academic Press, New York.
- Harvey, E.N., 1957. *A History of Luminescence*. The American Philosophical Society, Philadelphia.
- Hastings, J.W., Sweeney, B.M., 1957. On the mechanism of temperature independence in a biological clock. *Proceedings of the National Academy of Sciences of the United States of America* 43, 804–811.
- Haxo, F.T., Sweeney, B.M., 1965. Bioluminescence in *Gonyaulax polyedra*. *The Luminescence of Biological Systems*. American Association for the Advancement of Science, Washington, D.C. 415–420.
- Herren, C.M., Haddock, S.H.D., Johnson, C., Orrico, C.M., Moline, M.A., Case, J.F., 2005. A multi-platform bathyphotometer for fine-scale, coastal bioluminescence research. *Limnology and Oceanography: Methods* 3, 247–262.
- Hobson, E.S., 1966. Visual orientation and feeding in seals and sea lions. *Nature* 210, 326–327.
- Kamykowski, D., Reed, R.E., Kirkpatrick, G.J., 1992. Comparison of sinking velocity, swimming velocity, rotation and path characteristics among six marine dinoflagellate species. *Marine Biology* 113, 319–328.
- Kelly, M.G., Tett, P., 1978. Bioluminescence in the ocean. In: Herring, P.J. (Ed.), *Bioluminescence in Action*. Academic Press, London, pp. 399–417.
- Kushnir, V.M., Tokarev, Y.N., Williams, R., Piontkovski, S.A., Evstigneev, P.V., 1997. Spatial heterogeneity of the bioluminescence field of the tropical Atlantic Ocean and its relationship with internal waves. *Marine Ecology Progress Series* 160, 1–11.
- Lapota, D., 1998. Long Term and Seasonal Changes in Dinoflagellate Bioluminescence in the Southern California Bight. Ph.D. Thesis, University of California Santa Barbara, 193p.
- Lapota, D., Geiger, M.L., Stiffey, A.V., Rosenberger, D.E., Young, D.K., 1989. Correlations of planktonic bioluminescence with other oceanographic parameters from a Norwegian fjord. *Marine Ecology Progress Series* 55, 217–227.
- Lapota, D., Losee, J.R., 1984. Observations of bioluminescence in marine plankton from the Sea of Cortez. *Journal of Experimental Marine Biology and Ecology* 77, 209–240.
- Lapota, D., Rosenberger, D.E., Lieberman, S.H., 1992a. Planktonic bioluminescence in the pack ice and the marginal ice zone of the Beaufort Sea. *Marine Biology* 112, 665–675.
- Lapota, D., Young, D.K., Bernstein, S.A., Geiger, M.L., Huddell, H.D., Case, J.F., 1992b. Diel bioluminescence in heterotrophic and photosynthetic marine dinoflagellates in an Arctic fjord. *Marine Biological Association of the United Kingdom* 72, 733–744.
- Latz, M.I., Bovard, M., VanDelinder, V., Segre, E., Rohr, J., Groisman, A., 2008. Bioluminescent response of individual dinoflagellate cells to hydrodynamic stress measured with millisecond resolution in a microfluidic device. *Journal of Experimental Biology* 211, 2865–2875.
- Latz, M.I., Case, J.F., Gran, R.L., 1994. Excitation of bioluminescence by laminar fluid shear associated with simple Couette flow. *Limnology and Oceanography* 39, 1424–1439.
- Latz, M.I., Jeong, H.J., 1996. Effect of red tide dinoflagellate diet and cannibalism on the bioluminescence of the heterotrophic dinoflagellates *Protoperdinium* spp. *Marine Ecology Progress Series* 132, 275–285.
- Latz, M.I., Lee, A.O., 1995. Spontaneous and stimulated bioluminescence in the dinoflagellate, *Ceratocorys horrida* (Peridinales). *Journal of Phycology* 31, 120–132.
- Latz, M.I., Nauen, J.C., Rohr, J., 2004. Bioluminescence response of four species of dinoflagellates to fully developed pipe flow. *Journal of Plankton Research* 26, 1529–1546.
- Latz, M.I., Rohr, J., 1999. Luminescent response of the red tide dinoflagellate *Lingulodinium polyedrum* to laminar and turbulent flow. *Limnology and Oceanography* 44, 1423–1435.
- Latz, M.I., Rohr, J., Hoyt, J., 1995. A novel flow visualization technique using bioluminescent marine plankton—part I: laboratory studies IEEE. *Journal of Oceanic Engineering* 20, 144–147.
- Lewis, J., Hallett, R., 1997. *Lingulodinium polyedrum* (*Gonyaulax polyedra*) a blooming dinoflagellate. *Oceanography and Marine Biology—An Annual Review* 35, 97–161.
- Lieberman, S.H., Lapota, D., Losee, J.R., Zirino, A., 1987. Planktonic bioluminescence in the surface waters of the Gulf of California. *Biology & Oceanography* 5, 25–46.
- Losee, J., Richter, K., Lieberman, S., Lapota, D., 1989. Bioluminescence: spatial statistics in the North Atlantic. *Deep-Sea Research* 36, 783–801.

- Losee, J.R., Lapota, D., 1981. Bioluminescence Measurements in the Atlantic and Pacific. In: Neelson, K.H. (Ed.), *Bioluminescence Current Perspectives*. Burgess Publishing Co., Minneapolis, pp. 143–152.
- Losee, J.R., Lapota, D., Lieberman, S.H., 1985. Bioluminescence: a new tool for oceanography. In: Zirino, A. (Ed.), *Mapping Strategies in Chemical Oceanography*. American Chemical Society, Washington, D.C., pp. 213–234.
- Maldonado, E.M., Latz, M.I., 2007. Shear-stress dependence of dinoflagellate bioluminescence. *Biological Bulletin* 212, 242–249.
- McDuffey, A., Bird, J.L., 2002. The underwater survey system of the Naval Oceanographic Office. In: *Proceedings of the Oceans MTS/IEEE*, pp. 1722–1725.
- McManus, M.A., Alldredge, A.L., Barnard, A.H., Boss, E., Case, J.F., Cowles, T.J., Donaghay, P.L., Eisner, L.B., Gifford, D.J., Greenlaw, C.F., Herren, C.M., Holliday, D.V., Johnson, D., MacIntyre, S., McGehee, D.M., Osborn, T.R., Perry, M.J., Pieper, R.E., Rines, J.E.B., Smith, D.C., Sullivan, J.M., Talbot, M.K., Twardowski, M.S., Weidemann, A., Zaneveld, J.R., 2003. Characteristics, distribution and persistence of thin layers over a 48 h period. *Marine Ecology Progress Series* 261, 1–19.
- Mensing, A.F., Case, J.F., 1992. Dinoflagellate luminescence increases susceptibility of zooplankton to teleost predation. *Marine Biology* 112, 207–210.
- Moline, M.A., Blackwell, S.M., Case, J.F., Haddock, S.H.D., Herren, C.M., Orrico, C.M., Terrill, E., 2009. Bioluminescence to reveal structure and interaction of coastal planktonic communities. *Deep Sea Research Part II: Topical Studies in Oceanography* 56, 232–245.
- Moline, M.A., Oliver, M.J., Mobley, C.D., Sundman, L., Betsky, T., Bergmann, T., Bissett, W.P., Case, J., Raymond, E.H., Schofield, O.M.E., 2007. Bioluminescence in a complex coastal environment: 1. Temporal dynamics of nighttime water-leaving radiance. *Journal of Geophysical Research* 112, C11016.
- Morin, J.G., 1983. Coastal bioluminescence: patterns and functions. *Bulletin of Marine Science* 33, 787–817.
- Oliver, M.J., Moline, M.A., Mobley, C.D., Sundman, L., Schofield, O.M.E., 2007. Bioluminescence in a complex coastal environment: 2. Prediction of bioluminescent source depth from spectral water-leaving radiance. *Journal of Geophysical Research*, 112.
- Paerl, H.W., 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography* 33, 823–847.
- Piontkovski, S.A., Tokarev, Y.N., Bitukov, E.P., Williams, R., Kiefer, D.A., 1997. The bioluminescent field of the Atlantic Ocean. *Marine Ecology Progress Series* 156, 33–41.
- Rohr, J., Hyman, M., Fallon, S., Latz, M.I., 2002. Bioluminescence flow visualization in the ocean: an initial strategy based on laboratory experiments. *Deep-Sea Research* 49, 2009–2033.
- Rohr, J., Latz, M.I., Fallon, S., Nauen, J.C., Hendricks, E., 1998. Experimental approaches towards interpreting dolphin-stimulated bioluminescence. *Journal of Experimental Biology* 201, 1447–1460.
- Rohr, J., Losee, J., Anderson, G., 1994. The Response of Bioluminescent Organisms to Fully Developed Pipe Flow. Naval Command, Control and Ocean Surveillance Center, RDT&E Div. (Tech. Rep. No. 1360).
- Rohr, J., Schoonmaker, J., Losee, J., Latz, M.I., Hyman, M., 1999. Flow visualization in the ocean—implications of laboratory bioluminescence experiments. In: *Proceedings of the Oceans '99 MTS/IEEE*, Seattle, Wash, pp. 145–156.
- Roithmayr, C.M., 1970. Airborne low-light sensor detects luminescing fish schools at night. *Commercial Fisheries Review* 32, 42–51.
- Rudakov, Y.A., 1968. Bioluminescence potential and its relation to concentration of luminescent plankton. *Oceanology* 8, 710–715.
- Seliger, H.H., Biggley, W.H., Swift, E., 1969a. Absolute values of photon emission from the marine dinoflagellates *Pyrodinium bahamense*, *Gonyaulax polyedra* and *Pyrocystis lunula*. *Photochemistry and Photobiology* 10, 227–232.
- Seliger, H.H., Carpenter, J.H., Loftus, M., McElroy, W.D., 1970. Mechanisms for the accumulation of high concentrations of dinoflagellates in a bioluminescent bay. *Limnology and Oceanography* 15, 234–245.
- Seliger, H.H., Fastie, W.G., McElroy, W.D., 1961. Bioluminescence in the Chesapeake Bay. *Science* 133, 699–700.
- Seliger, H.H., Fastie, W.G., McElroy, W.D., 1969b. Towable photometer for rapid area mapping of concentrations of bioluminescent marine dinoflagellates. *Limnology and Oceanography* 14, 806–813.
- Seliger, H.H., McElroy, W.D., 1968. Studies at Oyster Bay in Jamaica, West Indies. I. Intensity patterns of bioluminescence in a natural environment. *Journal of Marine Research* 26, 244–255.
- Seliger, H.H., Taylor, W.R., Fastie, W.G., McElroy, W.D., 1962. Bioluminescence of marine dinoflagellates. 1. An underwater photometer for day and night measurements. *Journal of General Physiology* 45, 1003–1017.
- Shulman, I., Haddock, S.H.D., McGillicuddy, D.J., Paduan, J.D., Bissett, W.P., 2003. Numerical modeling of bioluminescence distributions in the coastal ocean. *Journal of Atmospheric and Oceanic Technology* 20, 1060–1068.
- Squire Jr., J.L., Krumboltz, J., 1981. Profiling pelagic fish schools using airborne optical lasers and other remote sensing techniques. *Marine Technology Society Journal* 15, 443–448.
- Staples, R.F., 1966. The Distribution and Characteristics of Surface Bioluminescence in the Oceans/Naval Oceanography Office Technical Report TR-1841–48.
- Stokes, M.D., Deane, G.B., Latz, M.I., Rohr, J., 2004. Bioluminescence imaging of wave-induced turbulence. *Journal of Geophysical Research* 109, C01004 (01008p).
- Sullivan, J.M., Swift, E., 1994. Photoinhibition of mechanically stimuable bioluminescence in the autotrophic dinoflagellate *Ceratium fusus* (Pyrophyta). *Journal of Phycology* 30, 627–633.
- Sullivan, J.M., Swift, E., 1995. Photoenhancement of bioluminescence capacity in natural and laboratory populations of the autotrophic dinoflagellate *Ceratium fusus* (Ehrenb.) Dujardin. *Journal of Geophysical Research* 100, 6565–6574.
- Sweeney, B.M., 1981. Variations in the bioluminescence per cell in dinoflagellates. In: Neelson, K.H. (Ed.), *Bioluminescence Current Perspectives*. Burgess Publishing, Minneapolis, pp. 90–94.
- Sweeney, B.M., Hastings, J.W., 1957. Characteristics of the diurnal rhythm of luminescence in *Gonyaulax polyedra*. *Journal of Cellular and Comparative Physiology* 49, 115–128.
- Swift, E., Biggley, W.H., Lessard, E.J., 1985a. Distributions of epipelagic bioluminescence in the Sargasso and Caribbean Seas. In: Zirino, A. (Ed.), *Mapping Strategies in Chemical Oceanography*. American Chemical Society, Washington, D.C., pp. 235–258.
- Swift, E., Biggley, W.H., Seliger, H.H., 1973. Species of oceanic dinoflagellates in the genera *Dissodinium* and *Pyrocystis*: interclonal and interspecific comparisons of the color and photon yield of bioluminescence. *Journal of Phycology* 9, 420–426.
- Swift, E., Biggley, W.H., Verity, P.G., Brown, D.T., 1983. Zooplankton are major sources of epipelagic bioluminescence in the Southern Sargasso Sea. *Bulletin of Marine Science* 33, 855–863.
- Swift, E., Lessard, E.J., Biggley, W.H., 1985b. Organisms associated with stimulated epipelagic bioluminescence in the Sargasso Sea and the Gulf Stream. *Journal of Plankton Research* 7, 831–848.
- Swift, E., Meunier, V., 1976. Effects of light intensity on division rate, stimuable bioluminescence and cell size of the oceanic dinoflagellates *Dissodinium lunula*, *Pyrocystis fusiformis* and *P. noctiluca*. *Journal of Phycology* 12, 14–22.
- Swift, E., Meunier, V.A., Biggley, W.H., Hoarau, J., Barras, H., 1981. Factors affecting bioluminescent capacity in oceanic dinoflagellates. In: Neelson, K.H. (Ed.), *Bioluminescence Current Perspectives*. Burgess Publishing Co., Minneapolis, Minnesota, pp. 95–106.
- Swift, E., Sullivan, J.M., Batchelder, H.P., Van Keuren, J., Vaillancourt, R.D., Bidigare, R.R., 1995. Bioluminescent organisms and bioluminescence measurements in the North Atlantic Ocean near latitude 59.5N, longitude 21W. *Journal of Geophysical Research* 100, 6527–6547.
- Tarasov, N.I., 1956. Luminescence of the sea (Svechenie Moria), Moscow, Akademiya nauk SSSR. Institute Oceanologii. Trans. M. Slessers. 1957. U.S. Naval Oceanographic Office Report No. NOOT-21.
- Turner, R.J., 1965. Notes on the Nature and Occurrence of Marine Bioluminescent Phenomena/National Institute of Oceanography Internal Report, B4; 1–30.
- von Dassow, P., Latz, M.I., 2002. The role of Ca²⁺ in stimulated bioluminescence of the dinoflagellate *Lingulodinium polyedrum*. *Journal of Experimental Biology* 205, 2971–2986.
- Widder, E.A., 1997. Bioluminescence: Shedding some light on plankton distribution patterns. *Sea Technology*, 33–39.
- Widder, E.A., 2006. A look back at quantifying oceanic bioluminescence: seeing the light, flashes of insight and other bad puns. *Marine Technology Society Journal* 40, 137–138.
- Widder, E.A., Case, J.F., 1981a. Bioluminescence excitation in a dinoflagellate. In: Neelson, K.H. (Ed.), *Bioluminescence Current Perspectives*. Burgess Publishing Co., Minneapolis, Minnesota, pp. 125–132.
- Widder, E.A., Case, J.F., 1981b. Two flash forms in the bioluminescent dinoflagellate *Pyrocystis fusiformis*. *Journal of Comparative Physiology* 143, 43–52.
- Widder, E.A., Case, J.F., Bernstein, S.A., MacIntyre, S., Lowenstine, M.R., Bowlby, M.R., Cook, D.P., 1993. A new large volume bioluminescence bathyphotometer with defined turbulence excitation. *Deep-Sea Research* 40, 607–627.
- Widder, E.A., Johnsen, S., Bernstein, S.A., Case, J.F., Neilson, D.J., 1999. Thin layers of bioluminescent copepods found at density discontinuities in the water column. *Marine Biology* 134, 429–437.
- Williams, T.M., Kooyman, G.L., 1985. Swimming performance and hydrodynamic characteristics of harbor seals *Phoca vitulina*. *Physiological Zoology* 58, 576–589.

APPENDIX

Using Bathphotometer Bioluminescence Potential to Predict Bioluminescence Intensity in Fully Developed Pipe Flow

Michael I. Latz and Jim Rohr

A1. Background

Fully developed pipe flow has been often used to study the response of luminescent dinoflagellates to mechanical stimulation (Latz et al., 2004; Latz and Rohr, 1999; Rohr et al., 2002). The hydrodynamics of this flow field is well characterized, it offers a wide range of laminar and turbulent stimuli, provides continuous replacement of luminescent organisms, and levels of bioluminescence do not appear to be significantly degraded by the organisms' upstream transit through the pipe (Losee and Lapota, 1981). Dinoflagellate bioluminescence is stimulated by fluid shear stress rather than absolute flow velocity (Latz and Rohr, 1999; Maldonado and Latz, 2007).

Previous investigations in a 0.635-cm i.d. pipe found that the response threshold for bioluminescence stimulation occurred in laminar flow with wall shear stress, τ_{wall} , values of 0.08 N m^{-2} for *Pyrocystis fusiformis*, 0.12 N m^{-2} for *Ceratium fusus*, and 0.33 N m^{-2} for *Lingulodinium polyedrum* (Latz et al., 2004). Corresponding maximum flash intensity (i.e., the brightest 0.005 s flash event in the data record) approached nearly constant levels in laminar flow at τ_{wall} of about 1 N m^{-2} for *P. fusiformis*, between 2.5 to 6 N m^{-2} in transitional flow for *C. fusus*, and 2 N m^{-2} in laminar flow for *L. polyedrum*. Average intensity increased with flow primarily due to a greater proportion of the population responding to higher levels of shear stress (Latz et al., 2004; Latz and Rohr, 1999). At the highest laminar flow measured, with $\tau_{\text{wall}} \sim 1 \text{ N m}^{-2}$, the population response proportion was estimated as 0.036 flashes cell⁻¹ for *C. fusus*, 0.100 flashes cell⁻¹ for *L. polyedrum*, and 0.103 flashes cell⁻¹ for *P. fusiformis*. Whereas the rate of increase of average intensity of *L. polyedrum* and *P. fusiformis* with τ_{wall} decreased after transition from laminar to turbulent flow, which occurred around $\tau_{\text{wall}} \sim 2 \text{ N m}^{-2}$, the rate of increase of average intensity of *C. fusus* with τ_{wall} was similar for laminar and turbulent flows (Latz et al., 2004).

Freshly collected mixed plankton samples from San Diego Bay measured in a 0.85-cm i.d. diameter pipe repeatedly exhibited a similar bioluminescence response threshold in laminar flow of $\tau_{\text{wall}} \sim 0.1 \text{ N m}^{-2}$ (Rohr et al., 2002). Over the range of turbulent flow investigated in the 0.85-cm i.d. pipe, at $\tau_{\text{wall}} \sim 1$ to 100 N m^{-2} , average intensity for the pooled data increased nearly linearly, as the 1.1 ± 0.2 power of τ_{wall} . The most abundant luminescent organism was *Lingulodinium polyedrum*. Estimated light budgets indicate that the predominant sources of bioluminescence in San Diego Bay are *L. polyedrum* and *Protoperidinium* spp. (Lapota, 1998).

The intensity of pipe flow stimulated bioluminescence is dependent on bioluminescence potential. To compare across pipe flow experiments with mixed plankton of different bioluminescence potential, NOSC BBP was obtained periodically throughout the experiment and averaged (Rohr et al., 2002). This averaged value was applied to each pipe flow data set obtained from a single reservoir and used to scale measurements collected throughout the year. Although initially differing by about an order

of magnitude, when corrected for BBP, the spring and fall 0.85-cm i.d. pipe flow bioluminescence intensity vs. τ_{wall} data sets generally collapsed to a single power function relationship (Rohr et al., 2002). There was one exception, the 0.85-cm i.d. data set from May 1st 1994, which was considered an outlier and consequently not included in the present comparison with the 2.54-cm i.d. pipe flow data.

During this same study (Rohr et al., 2002), an experiment was conducted on May 4th 1994 to measure the variation in bioluminescence intensity in the NOSC BBP and a 0.85-cm i.d. pipe during the course of emptying the 450-L head tank. About a dozen BBP and pipe flow bioluminescence intensity measurement sets were collected alternately at nearly constant flow rates, 0.45 to 0.57 L s⁻¹ for the NOSC flow agitator and 0.14 to 0.20 L s⁻¹ for the 0.85-cm i.d. pipe.

The present study extends the 0.85-cm i.d. pipe flow study (Rohr et al., 2002) by developing an empirically derived relationship between NOSC BBP, pipe diameter, bioluminescence intensity, and wall shear stress. It is anticipated that this relationship could be used to predict bioluminescence intensity in any pipe, over the wall shear stress investigated, assuming that the luminescent species composition of the water samples do not change significantly. The previous 0.85-cm i.d. pipe flow data sets provide an opportunity to test the predictive capability of this relationship to use BBP to estimate bioluminescence intensity throughout a range of turbulent pipe flow.

A2. Experimental Methods

Using the same pipe flow apparatus and protocol as for previous 0.83-cm i.d. pipe flow bioluminescence experiments (Rohr et al., 2002), mixed plankton samples were collected from San Diego Bay. A 450-L inertly coated stainless-steel head tank attached to a 2.54-cm i.d. pipe was assembled with accompanying instrumentation within an enclosure on a pier in San Diego Bay. Water was first drawn, 2 h before sunset, into a submerged tank through a 7.6-cm diameter, hydraulically actuated valve located a few meters below the surface. The submerged tank was then gradually pressurized to slowly transfer the water sample into the head tank. The maximum shear stress during transport through the 10-cm i.d. connecting hose was estimated to be $< 0.1 \text{ N m}^{-2}$, less than the known response threshold for dinoflagellate bioluminescence (Latz et al., 2004; Latz and Rohr, 1999). Mounted to the bottom of the 450-L head tank was a 50-cm diameter elbow connected to a flange from which issued a 2.54-cm i.d. transparent acrylic pipe. Between the pipe and flange was a smooth contracting inlet with a cubic polynomial profile. In addition to NOSC BBP measurements, organism homogeneity in the reservoir was monitored during each experiment by periodically repeating pipe flow measurements for the same volume flow rate setting. Bioluminescence intensity measurements commenced 2.5 h after sunset. Variations in bioluminescence potential are primarily attributed to fluctuations in the populations of the luminescent dinoflagellates *Protoperdinium* spp. and *Lingulodinium polyedrum* (Lapota, 1998).

Pressure drop along the pipe was measured with variable reluctance differential transducers (Validyne Corporation). The volume flow rate was determined by weighing the volume flow discharge collected over a measured time. Average flow speed, U_{avg} , was calculated by dividing the volume flow rate by the pipe cross-sectional area. Pressurizing the head tank and controlling a manual valve at the end of the pipe resulted in flow rates up to 2.2 l s⁻¹, yielding average flow speeds up to 4.4 m s⁻¹. For each bioluminescence pipe flow measurement the accompanying Darcy friction factor and Reynolds

number were calculated as previously described (Latz and Rohr, 1999). Based on these data, the laminar to turbulent transition in the 2.54-cm i.d. pipe occurred between Reynolds numbers of 10,000 to 20,000. To be assured of fully developed turbulent pipe flow, 2.54-cm i.d. pipe flow data was analyzed for Reynolds numbers greater than 30,000. The range of Reynolds numbers investigated was between about 30,000 to 107,000, corresponding to average velocities of 1.3 to 4.4 m s⁻¹, volume flow rates from 0.7 to 2.2 l s⁻¹, and wall shear stress values from 5.1 to 44.2 N m⁻².

Wall shear stress, τ_{wall} , was calculated as:

$$\tau_{\text{wall}} = (\Delta p/L)(D/4) \quad (\text{A1})$$

where Δp is the pressure drop, L is the distance between pressure ports, and D is the pipe diameter. In fully developed pipe flow, regardless of whether the flow is laminar or turbulent, the shear stress profile across the pipe radius is linear, increasing from zero at centerline to a maximum at the pipe wall. For fully developed pipe flow with Reynolds numbers between 3000 and 100,000 it has been empirically established that τ_{wall} is proportional to the average velocity raised to the 1.75 power (Davies, 1972).

Bioluminescence of San Diego Bay water samples was measured using two RCA model 8575 PMTs with a 5.08-cm diameter aperture. One PMT was attached to an opaque housing clamped directly onto the 2.54-cm i.d. pipe at a position 2.4 m downstream of the pipe inlet where the turbulent pipe flow was always fully developed. The PMT field of view encompassed the entire pipe diameter and 5.0 cm of pipe length. The second PMT was attached to the NOSC flow agitator chamber fitted to the end of the same 2.54-cm i.d. pipe, 3.6 m downstream of the inlet. The flow volume viewed by both PMTs were nearly identical, 25.3 cm³ for the pipe and 25.5 cm³ for the NOSC flow agitator. The distance between the PMTs, range of average flow speeds, and the bioluminescence intensity integration times resulted in both PMTs monitoring nearly the same volume of seawater. If necessary, neutral density filters were used to attenuate the light signal to avoid saturating the detector. PMT measurements had a temporal resolution of 5 ms and duration of 20 to 100 s for each flow rate, with the duration again adjusted to provide approximately the same flow volume for each flow rate. Bioluminescence intensity (photons s⁻¹) was obtained by averaging each PMT time series and applying a previously described photon calibration procedure (Latz and Rohr, 1999). Although most of the NOSC measurements were collected at volume flow rates between 0.7 and 2.2 l s⁻¹ (assuring fully developed turbulent flow in the upstream pipe), two NOSC flow agitator experiments were performed at volume flow rates between 0.1 to 1 l s⁻¹ to compare to laboratory data obtained with cultures of *Lingulodinium polyedrum* (see Section 4.1).

A3. Results

Darcy friction factor and Reynolds numbers calculated for the 2.54-cm i.d. pipe flow experiments corroborated that the flow was fully developed and turbulent. The five San Diego mixed plankton pipe flow and downstream NOSC BBP data sets, for volume flow rates between 0.66 and 2.24 l s⁻¹, exhibited several repeatable trends as a function of τ_{wall} . For the pooled 2.54-cm pipe flow experiments ($N = 45$), bioluminescence intensity increased as $7.33 \times 10^8 \times \tau_{\text{wall}}^{0.97}$ ($R^2 = 0.66$) (Fig. A1A, open symbols), where bioluminescence intensity has units of photons s⁻¹ and τ_{wall} has units of N m⁻².

Because wall shear stress within the NOSC flow agitator was not measured, NOSC BBP is expressed as a function of the upstream pipe wall shear stress. As a function of upstream 2.54-cm pipe wall shear stress, the pooled NOSC BBP increased at a slower rate, as $8.56 \times 10^9 \times \tau_{\text{wall}}^{0.28}$ ($R^2 = 0.13$) (Fig. A1A, closed symbols). NOSC BBP was always greater than the corresponding upstream pipe flow bioluminescence intensity, but the ratio of NOSC BBP to 2.54-cm pipe flow bioluminescence intensity tended to decrease with increasing τ_{wall} because of the greater rate of increase in pipe flow bioluminescence intensity.

Differences in pipe flow bioluminescence potential among experiments were corrected by dividing by the simultaneous downstream NOSC BBP. This ratio, which represents the proportion of the downstream NOSC BBP measured in the upstream 2.54-cm i.d. pipe, was described as:

$$\text{Pipe bioluminescence intensity/NOSC BBP} = 0.0086 \times \tau_{\text{wall}}^{0.70} \quad (\text{A2})$$

($R^2 = 0.92$) (Fig. A1B). This correction resulted in greatly reduced variability across the five experiments, improving R^2 from 0.66 to 0.92 (Fig. A1B). For a similar assemblage of luminescent species, Eq. A2 can be used to predict pipe flow bioluminescence intensity through a range of τ_{wall} based on a single measurement of BBP and adjusting for different pipe diameters.

In a previous study conducted in San Diego Bay, bioluminescence intensity was measured in a 0.85-cm i.d. pipe, with NOSC BBP measured intermittently between pipe flow measurements and averaged for each experiment (Rohr et al., 2002). To compare with these results, the present NOSC BBP for each of the 2.54-cm i.d. pipe flow tests was also averaged. Using average instead of simultaneous NOSC BBP resulted in an increase in the variation of the pooled data, with R^2 decreasing from 0.92 to 0.89. The ratio of 2.54-cm i.d. pipe flow to averaged NOSC BBP bioluminescence intensity now increased as $1.16 \times 10^{-4} \times \tau_{\text{wall}}^{0.90}$ ($R^2 = 0.89$). Dividing bioluminescence intensity obtained in both the 2.54 and 0.83-cm i.d. pipe data sets, some of which were different by more than an order of magnitude, by the product of the corresponding averaged NOSC BBP and the pipe volume within each PMT aperture, equal to the ratio of the square of the pipe diameters, successfully resulted in a single relationship (Fig. A2). Pipe flow bioluminescence intensity for both pipes, corrected for average NOSC BBP and pipe volume within the PMT aperture, increased as $8.77 \times 10^{-5} \times \tau_{\text{wall}}^{1.03}$ ($R^2 = 0.80$).

The 0.85-cm i.d. pipe dataset of 12/4/94 (Rohr et al., 2002) provides another opportunity to apply the NOSC BBP correlation (Eq. A2) to predict pipe flow bioluminescence intensity in fully developed turbulent pipe flow. In this experiment, NOSC BBP and 0.85-cm i.d. pipe flow bioluminescence intensity was measured alternately at nearly constant flow rates. Bioluminescence intensity measured in the 0.85-cm i.d. pipe was compared to predictions based on the present study using the following steps: (1) For each set of three consecutive bioluminescence intensity measurements in the pipe, the NOSC BBP measured immediately before and after the pipe measurements was averaged. (2) NOSC BBP is assumed initially to be independent of upstream pipe wall shear stress, τ_{wall} . (3) Average NOSC BBP obtained from step 1 is used in Eq. A2 at the prescribed τ_{wall} to calculate the predicted bioluminescence intensity in a 2.54-cm i.d. pipe. (4) A correction factor for the different pipe diameters is applied. The result of these steps, using average NOSC BBP, which was assumed to be independent of volume flow rate, provided a reasonable approximation to the measured bioluminescence intensity in the 0.83-cm i.d. pipe (Fig. A3). Bioluminescence intensity predictions (Fig. A3, solid triangles) averaged 32% below those measured in the 0.85-cm i.d. pipe (Fig. A3, open

circles). If NOSC BBP (again averaged) is assumed to increase as $\tau_{\text{wall}}^{0.28}$ (Fig. A1), predictions (Fig. A3, solid diamonds) were a little closer, averaging 26% greater than those measured. Either way, this agreement is remarkable given that the NOSC BBP (Fig. A3, open squares) was more than an order of magnitude greater than bioluminescence intensity in the 0.85-cm i.d. pipe, was averaged, upstream conditions were not identical, and was obtained almost a year prior to the NOSC BBP measurements used to determine Eq. A2.

A4. Discussion

Greater levels of bioluminescence stimulation are expected to occur in the NOSC flow agitator positioned downstream of a pipe, compared to the upstream fully developed pipe flow. The abrupt change in the flow agitator area results in a central jet that supports strong vortices along its boundary. The loss coefficient, defined as the pressure drop across the change in pipe diameter divided by the dynamic pressure, for an abrupt 1 to 4 pipe expansion is about 0.5, and for an abrupt 4 to 1 pipe contraction is about 0.4 (Fox et al., 2004). If similar loss coefficients are approximately applicable to the NOSC flow agitator, which has an orifice diameter of 1 cm at its inlet and outlet and a maximum dimension of 4 cm at its center, then the pressure drop across it would be about an order of magnitude greater than that associated with fully developed turbulent flow through an equivalent 4-cm length of 2.54-cm i.d. pipe.

Dividing fully developed turbulent pipe flow bioluminescence intensity in the 2.54-cm i.d. pipe by the simultaneous downstream NOSC BBP compensated for the changing bioluminescence potential, providing a single relationship between wall shear stress and the proportion of NOSC BBP that occurred in the upstream pipe (Eq. A2). The ratio of simultaneous measurements of pipe flow bioluminescence intensity to NOSC BBP increased with flow rate (Fig. A1B). It is hypothesized that with increasing flow rate, the resulting increase in pipe shear stress has a greater effect on increasing the population response of the luminescent organisms (Latz and Rohr, 1999) than does any increase in flow stimulus in the NOSC flow agitator. By suitable scaling with pipe diameter and NOSC BBP, bioluminescence intensity over the range of wall shear studied should be predictable in any fully developed turbulent pipe flow, at least in San Diego Bay where the contributing luminous species are fairly constant.

Using this same relationship (Eq. A2) between pipe τ_{wall} and the proportion of NOSC BBP stimulated in the 2.54-cm i.d. pipe flow, good agreement was found between predictions and measurements of bioluminescence intensity obtained in a 0.85-cm i.d. pipe, given the corresponding NOSC BBP (Rohr et al., 2002). Based on the present study with cultures of dinoflagellates, different assemblages of luminescent organisms may result in different transfer functions. Nevertheless, the present success has a powerful implication – the ability, based on BBP, to estimate bioluminescence intensity associated with a flow signature through simple transfer functions that could be empirically determined from laboratory studies. BBP varies with species abundance and composition; the present capability can be expanded to include other representative source organisms, but needs to be validated for mixed ocean assemblages that can vary temporally and spatially. Furthermore similar investigations with other generic flow fields, providing a library of transfer functions, would allow for predictions of bioluminescence displays.

Applying the one-dimensional flash model of Seliger et al. (1969) (Seliger et al., 1969) to pipe flow to account for cell advection, Rohr and Losee (1994) also found bioluminescence intensity dependence for volume flow rates less than critical, with relative independence at higher values. However, their analysis shared the previous assumptions that every cell passing through the observation flow volume must flash, and bioluminescence stimulation was independent of flow speed. Although for a grid these assumptions may be a reasonable approximation, they are not for fully developed pipe flow where flow agitation does not change along the flow direction. Consider, for example, luminescent flashes occurring in a one-dimensional pipe that is part of a large ring. Assuming that the increase in flow agitation accompanying increasing flow rate does not directly increase bioluminescence stimulation, then the increased advection of luminescent flashes past the PMT will also not increase bioluminescence stimulation. Because the total luminescent intensity from the ring is independent of flow rate, then light measurements along any segment of the ring must be independent of flow rate.

A5. Conclusions

For the first time, bioluminescence intensity has been accurately predicted for a fully characterized flow field over a range of shear stresses given the corresponding BBP measurements and the relationship between bioluminescence intensity and τ_{wall} established in a similar flow field (e.g., fully developed turbulent pipe flow) and assemblage of luminescent organisms. This link is important in relating BBP to predictions of flow-stimulated bioluminescence intensity associated with any flow field in the ocean that provides suprathreshold levels of shear stress, of the order of 0.1 N m^{-2} (Latz et al., 2004).

Literature Cited

Davies, J.T., 1972. Turbulence phenomena. Academic Press, New York.

Fox, R.W., McDonald, A.T., Pritchard, P.J., 2004. Introduction to fluid mechanics, 6th edition. John Wiley & Sons, Hoboken, NJ.

Lapota, D., 1998. Long term and seasonal changes in dinoflagellate bioluminescence in the Southern California Bight. Ph.D. Dissertation, University of California Santa Barbara, 193 pp.

Latz, M.I., Nauen, J.C., Rohr, J., 2004. Bioluminescence response of four species of dinoflagellates to fully developed pipe flow. *Journal of Plankton Research* 26, 1529-1546.

Latz, M.I., Rohr, J., 1999. Luminescent response of the red tide dinoflagellate *Lingulodinium polyedrum* to laminar and turbulent flow. *Limnology and Oceanography* 44, 1423-1435.

Losee, J.R., Lapota, D., 1981. Bioluminescence Measurements in the Atlantic and Pacific, in: Neelson, K.H. (Ed.), *Bioluminescence Current Perspectives*. Burgess Publishing Co., Minneapolis, pp. 143-152.

Maldonado, E.M., Latz, M.I., 2007. Shear-stress dependence of dinoflagellate bioluminescence. *Biological Bulletin* 212, 242-249.

Rohr, J., Hyman, M., Fallon, S., Latz, M.I., 2002. Bioluminescence flow visualization in the ocean: an initial strategy based on laboratory experiments. *Deep-Sea Research* 49, 2009-2033.

Seliger, H.H., Fastie, W.G., McElroy, W.D., 1969. Towable photometer for rapid area mapping of concentrations of bioluminescent marine dinoflagellates. *Limnology and Oceanography*. 14, 806-813.

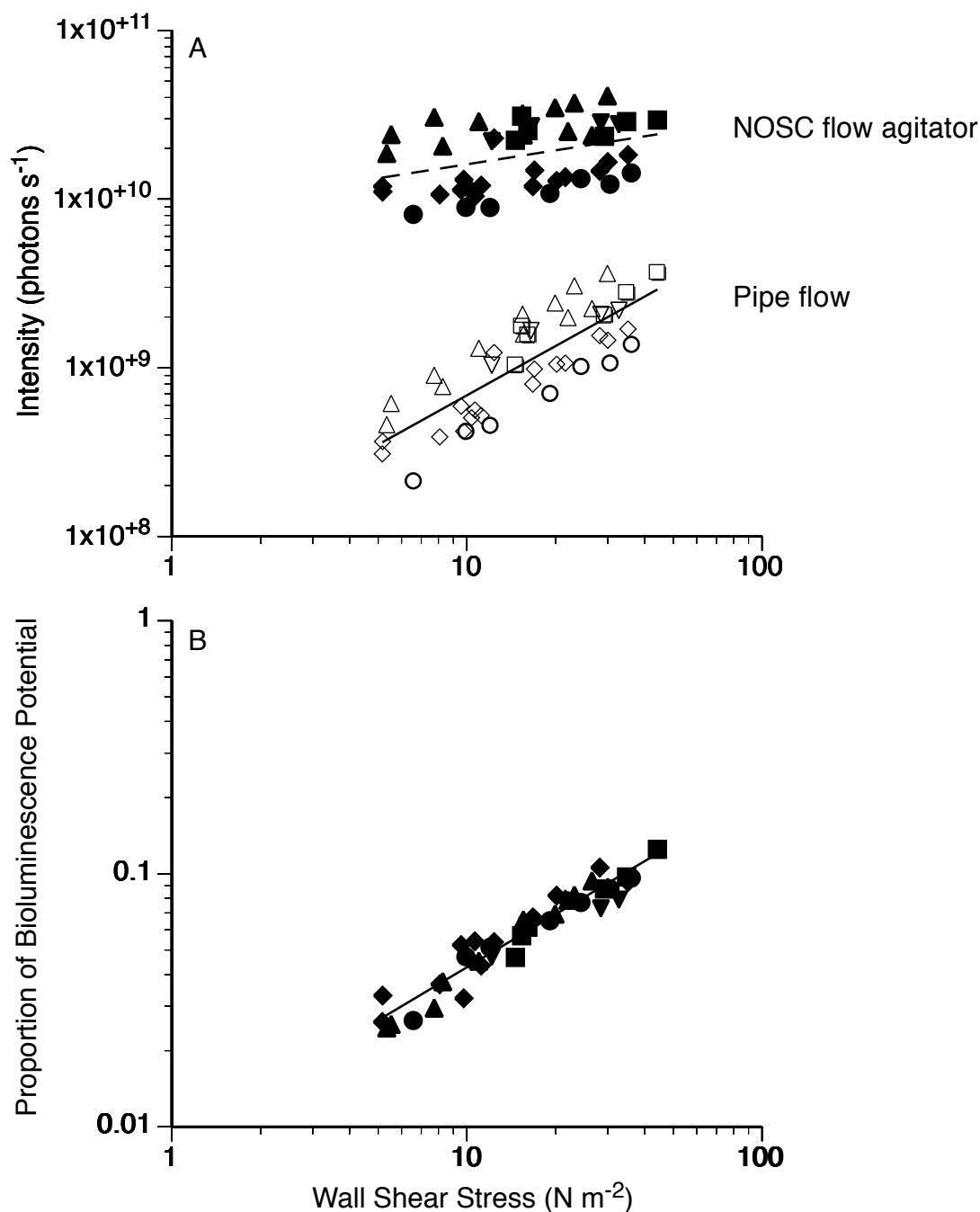


Figure A1. Stimulation of bioluminescence by flow through a 2.54-cm i.d. pipe using freshly collected plankton samples over a 9-month period in San Diego Bay. (A) Bioluminescence intensity (BI) as a function of wall shear stress (τ_{wall}) in pipe flow (open symbols, with a different symbol for each of the five experiments). The solid line represents the power regression, $\text{BI} = 7.33 \times 10^8 \tau_{\text{wall}}^{0.97}$ ($R^2 = 0.658$). Closed symbols represent the simultaneous BBP measured using the NOSC flow agitator attached to the end of the pipe. The dashed line represents the power regression, $\text{BBP} = 8.56 \times 10^9 \tau_{\text{wall}}^{0.28}$ ($R^2 = 0.131$). (B) Proportion of BBP (BBP_{prop}) stimulated by pipe flow, calculated by dividing pipe flow bioluminescence intensity by the simultaneous BBP. The proportion of BBP was highly correlated with wall shear stress ($\text{BBP}_{\text{prop}} = 0.0086 \tau_{\text{wall}}^{0.70}$, $R^2 = 0.924$).

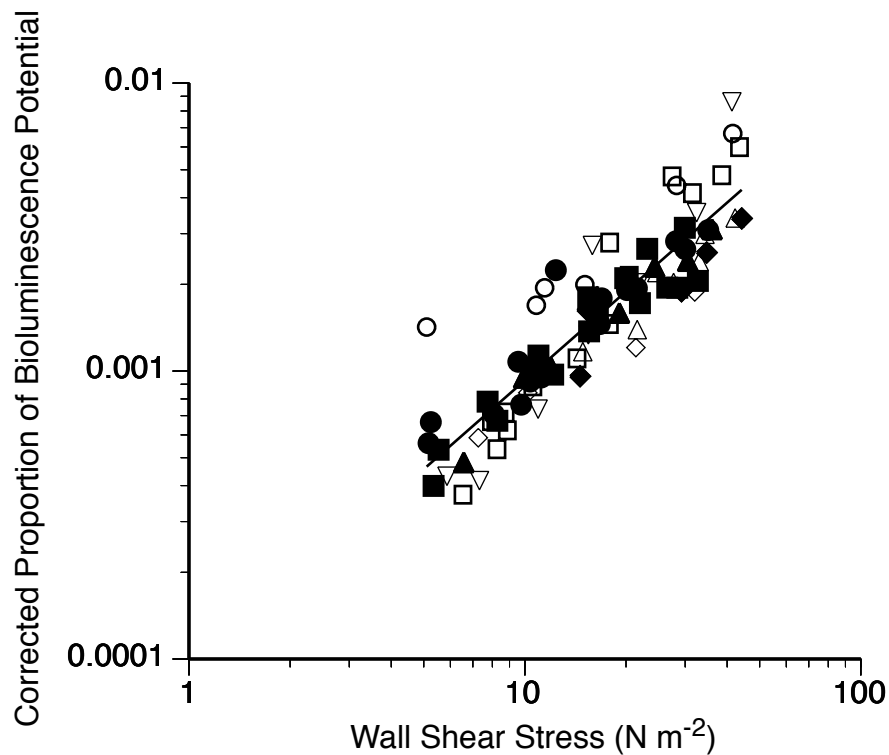


Figure A2. Proportion of BBP (BBP_{prop}), corrected by the square of the ratio of pipe diameters, for mixed plankton samples from San Diego Bay as a function of wall shear stress for two pipe diameters. Present results (closed symbols) were measured using a 2.54-cm i.d. pipe. Previous results (open symbols) are for a 0.85-cm i.d. pipe (Rohr et al. 2002). When corrected for NOSC BBP and pipe diameter, the two data sets, which initially differed by an order of magnitude, reduced to a highly correlated single relationship ($\text{BBP}_{\text{prop}} = 8.77 \times 10^{-5} \tau_{\text{wall}}^{1.03}$, $R^2 = 0.799$).

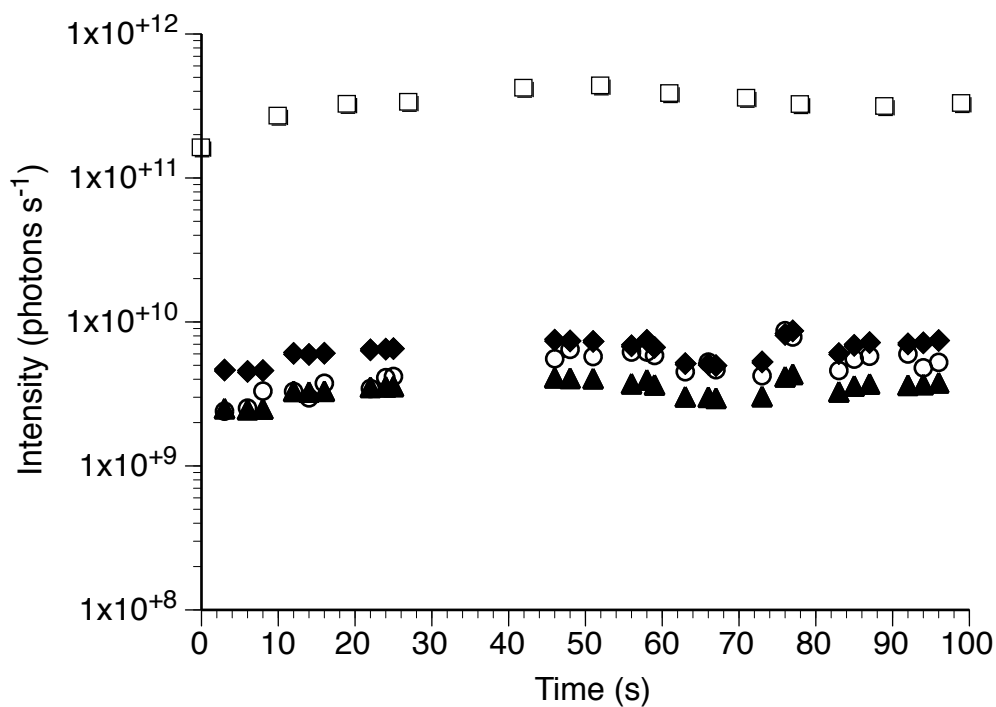


Figure A3. Measured (open circles) and predicted (solid symbols) bioluminescence intensity in a 0.85-cm i.d. pipe. Predictions were made based on Eq. (A2), adjusting for pipe diameter and NOSC BBP (open squares) assuming that: (1) NOSC BBP was independent of flow rate (solid triangles), or (2) NOSC BBP increased as $\tau_{\text{wall}}^{0.28}$ (solid diamonds) based on flow in the upstream 2.54-cm i.d. pipe.