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

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ABSTRACT

Bathyphotometer 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 bathyphotometer designs, or even the same bathyphotometer 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 bathyphotometer flow agitators as a function of bathyphotometer 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 bathyphotometer 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 bathyphotometer flow agitators and flow fields will allow bioluminescence potential measurements to become a more powerful tool for the oceanographic community.

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, bathyphotometer bioluminescence potential; PMT, photomultiplier.

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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 × 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).