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13. ABSTRACT (Maximum 200 words)

Numerical and experimental approaches were used to investigate the effects of quantified flow stimuli on bioluminescence stimulation at the small length and time scales appropriate for individual plankton. Bioluminescence was used as a sensitive tool for examining essentially instantaneous organism response. Based on laboratory work with defined flow fields, a consistent picture of organism response emerges. There is a response threshold in laminar flow at a shear stress level of approximately 0.1 N m-2. Increasing shear stress levels lead to increased population response due to more organisms being stimulated, and to a lesser extent increases in the magnitude of the individual response, which is maximized in high laminar flow. Responsiveness is a function of shear stress, not the laminar or turbulent nature of the flow.
The boundary layers of most moving objects of Naval interest contain stimulatory levels of shear stress. The present results indicate that their bioluminescence signature will depend on boundary layer thickness and the amount of flow separation. Project findings are relevant to the concerns of nighttime covert operations, and relate to recent interest in applying hyperspectral and multispectral technologies to ocean surveillance.

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Enclosed for your records is the final technical report for the above referenced grant.

Sincerely,

Linda Ford

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Contract & Grant Assistant
SIO/UCSD

CC: Nancy Wilson, SIO OCGA
Dr. M. Latz, PI
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AN EXPERIMENTAL-NUMERICAL STUDY OF SMALL SCALE FLOW INTERACTION WITH BIOLUMINESCENT PLANKTON

N00014-95-1-0001

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Final Report August 1998

SCIENTIFIC OBJECTIVE

The goal of this study is to couple numerical and experimental approaches to investigate the effects of quantified levels of flow stimuli on bioluminescence excitation at the small length and time scales appropriate for individual plankton. This interdisciplinary project is a collaboration involving Jim Rohr at SPAWAR Systems Center San Diego (experimental fluid mechanics), and Said Elghobashi at UC Irvine (computational fluid mechanics). We consider bioluminescence the most sensitive tool for examining organism response at these scales.

APPROACHES AND RESULTS

Response to laminar and turbulent pipe flow. While it is universally accepted that plankton continually experience a dynamic fluid environment, their sensitivity to the features of the surrounding flow field at the relevant length and time scales of the organism is poorly characterized. The essentially instantaneous luminescent response of the red-tide dinoflagellate *Lingulodinium polyedrum (=Gonyaulax polyedra)* to quantified levels of hydrodynamic stimuli was studied for fully developed laminar and turbulent pipe flow. The response to hydrodynamic stimulation was correlated to pipe wall shear stress. The response threshold occurred in laminar flow at a wall shear stress of approximately 0.3 N m^{-2}. At these low flow rates, video analysis of the velocity of flash trajectories revealed that responding cells were positioned only near the pipe wall, where threshold values of local shear stress were achieved. The response threshold was independent of cell concentration, and consistent with an antipredation function for dinoflagellate bioluminescence. For laminar flows with wall shear stress values of 0.2 to 2 N m^{-2}, mean bioluminescence intensity increased as approximately the second to third power of shear stress. The increase in bioluminescence within this range was due primarily to an increasing population response rate, and to a lesser degree an increase in maximum flash intensity per cell or the increased flux of organisms with higher flow rates. At higher wall shear stress levels up to 30 N m^{-2} when the flow was turbulent, the maximum intensity per cell remained approximately constant, even though the mean intensity of bioluminescence increased because of an increase in the proportion of cells responding. Therefore the individual response intensity was maximized in laminar flow because there was no further increase in turbulent flow even when the Kolmogorov length scale of the turbulence became smaller than the average cell size. Refer to citations 12, 37.

Response to shear and acceleration. We have coupled computational and experimental approaches to investigate the response of dinoflagellates to local conditions of shear and acceleration in different flow fields. The flashing of luminescent dinoflagellates is used as an instantaneous monitor of organism response; flash location and instantaneous velocity allows us to pinpoint the location of the cell within the flow field. Two flow fields which have been investigated are fully developed pipe flow, and the developing flow in a 4:1 convergence.

The computational study can calculate all the properties of the flow field from an exact solution of the Navier-Stokes equations. Therefore the levels of shear, shear stress, acceleration, extensional stress, and pressure are known for each position in the flow field. The stimulatory properties of the flow field can be determined by correlating the computational results with the experimental data on organism responsiveness.

In fully developed pipe flow, where no mean acceleration is present, the response of the red tide dinoflagellate *Gonyaulax polyedra* is best correlated with shear. In flows near the response threshold, cells which respond are located near the pipe wall, where the shear is greatest. Threshold response occurs at shear stress levels of 0.1-0.3 N m^{-2}. In the developing flow of a convergence, cells respond only near the wall which is the only region of the flow with shear. For this flow, the response threshold occurs at a similar shear stress level of approximately 0.5 N cm^{-2}. Refer to citations 15, 16, 22.

Mechanism of the shear response. When dinoflagellates respond to fluid shear, are they sensitive to the rate of strain across the cell (shear rate), or the forces acting on them (shear stress)? Graduate student Jennifer Nauen has examined this aspect of bioluminescence stimulation by manipulating the fluid viscosity in fully developed pipe flow. Because shear stress = shear rate x viscosity, the shear stress acting on the cell can be increased by increasing the fluid viscosity while the shear rate remains constant.

The luminescent response was shifted with increasing viscosity, indicating that the response of cells was shear stress dependent. This result is similar to results for protozoa and animal cells, which also respond to mechanical stimulation via a shear stress mechanism. Nauen's results are the first for determining the mechanism of the shear response for any planktonic organism. Refer to citations 14, 28.

Bioluminescence as a flow diagnostic. Noever and Cronise (1994, *Physics Letters A*) have reported that bioluminescence can be used to resolve eddy structure in turbulent flow. In collaboration with Jeff Allen, a mathematician at SPAWAR Systems Center San Diego, we published a paper in the same journal which refutes their conclusions, and clarifies the hydrodynamic conditions (laminar and turbulent) in which plankton bioluminescence is stimulated. Results show that bioluminescence is stimulated in laminar flow, that there is no correlation between turbulent length scale and the stimulation of bioluminescence, and that power law estimates from the bioluminescence amplitude density is not an effective measure of the level of turbulence, nor an adequate discriminator between laminar and turbulent flows. Refer to citation 4.

We have applied these results to the flow field around a dolphin (described below) and other flow fields of Naval interest. For example, numerical simulations of the wake of an aircraft carrier indicate that by 20 ship lengths, the stimulatory flow volume is on the order of 10 million cubic meters.

We are beginning to determine what information can be obtained directly from the bioluminescence time series. Numerical models, with known assumptions, are being developed to generate bioluminescence time series to compare with experimental data. This will help us determine: (1) whether bioluminescence time series can be used to differentiate laminar from turbulent flow, (2) the distribution of turbulent length scales, (3) different species response to flow agitation, and (4) the best estimate of flash decay.

Interspecific variation in the response to shear. Shear acts directly on dinoflagellates by inhibiting cell division, thus affecting population growth (long-term effect), and by stimulating bioluminescence (short-term effect). Shear sensitivity has been studied in five species of cultured dinoflagellates. The instantaneous response was studied by measuring bioluminescence stimulated by fully developed pipe flow. The effect on cell division and population growth was monitored in cells grown under active hydrodynamic conditions on an orbital shaker.

Shear sensitivity is not related to cell size or the presence of thecal plates. The long-spined species, *Ceratocorys horrida*, is one order of magnitude more sensitive than the other species. The spines may act as levers to exaggerate the effect of the local shear. Unlike red tide dinoflagellates, non-red tide species are able to grow under active hydrodynamic conditions. The physiological basis of shear sensitivity is yet to be determined. Refer to citations 13, 27.

Effect of agitation on morphology. UCSD undergraduate student Marnie Zirbel has determined that the oligotrophic dinoflagellate *Ceratocorys horrida* exhibits major yet reversible changes in cell size and spine length due to turbulence. When agitated on an orbital shaker, population growth was inhibited and a short-spined cell type appeared which possessed a 49% decrease in spine length and a 53% decrease in cell volume. Short-spined cells were first observed after 1 h of agitation; after 8 to 12 d of continuous agitation, long-spined cells were no longer present. The morphological change was completely reversible; in previously agitated populations devoid of long-spined cells, cells began to revert to the long-spined morphology within 1 d after the return to calm conditions. During morphology reversal, spines on isolated cells grew up to 10 μm d^{-1}. In 30 d the population morphology had returned to original proportions, even though the overall population growth was zero during this time. The reversal presumably involves flow-induced signaling of gene activation rather than changes occurring as a result of cell division, because single-cell studies confirmed that the change occurred in the absence of cell division, and much faster than the 16 d doubling time. The ecological importance of the morphological change may be to protect the cell from mechanical damage during turbulent conditions. During turbulent conditions, the long spines may be unnecessary as a strategy to reduce sinking because enhanced mixing helps resuspend cells in the water column. This is the first demonstration for any marine planktonic organism of a rapid yet reversible change in cell morphology due to turbulence. Refer to citations 11, 19.

Flow visualization. Laboratory experiments suggest that bioluminescence will occur in boundary layers where the shear stress is greater than approximately 0.1 N m^{-2}. The light emission of individual cells increases only slightly for shear stress levels from threshold to 1 N m^{-2}. Therefore a thicker boundary layer, due to flow separation or transition from laminar to turbulent flow, results in greater bioluminescence because a greater number of cells are stimulated. From our work with hydrodynamic models, we know that Reynolds number (flow rate) and luminescent cell concentration influence the visualization of separation and transition to turbulent flow on spheres, ellipsoids, flat plates, and dolphin models. We have tested our laboratory-based predictions in the field by using bioluminescence as a tool to examine the boundary layer flow for a swimming dolphin. Luminescent flow visualization suggests that the flow remains attached over most of the body. Further study is needed to measure the thickness of the boundary layer in order to determine if flow is laminar or turbulent. Based on our current results it appears that transition to turbulent flow may occur at the blowhole. This research has been featured on the Discovery Channel show "World of Wonder", the winter 1997 video edition of SIO's Explorations Magazine, and magazine articles in *Nature*, the French edition of *Scientific American*, the French science magazine *Science & Vie*, and *Biophotonics International* magazine. Refer to citations 7, 8, 29, 33, 34, 35, 36.

Computational studies of turbulence. Even though most of the experimental work in this project has involved laminar flow fields, it is not known how the intermittency and eddy scales of oceanic turbulence are experienced by plankton. In addition, even though it is commonly known that bioluminescence is stimulated by surface breaking waves, it is not yet possible to account for the stimulatory motions of the flow. An unrealized goal is to combine experimental work with laboratory turbulent flow fields with computational studies of turbulence. The latter studies examine fundamental bubble-flow interactions; previous work shows bubbles to stimulate bioluminescence (Latz and Case, unpublished data), and bubbles may be important stimuli for generating near-surface bioluminescence.

Direct numerical simulations (DNS) of bubble-laden isotropic decaying turbulence are performed using the two-fluid approach (TF) instead of the Eulerian-Lagrangian approach (EL). The motivation for the study is that EL requires considerable computational resources especially for the case of two-way coupling where the instantaneous trajectories of a large number of individual bubbles need to be computed. The TF formulation is developed by spatially averaging the instantaneous equations of the carrier flow and bubble phase over a scale of the order of the Kolmogorov length scale which, in our case, is much larger than the bubble diameter. On that scale, the bubbles are treated as a continuum (without molecular diffusivity) characterized by the bubble phase velocity field and concentration (volume fraction). The bubble concentration is assumed small enough to neglect the bubble-bubble interactions. As a test case, direct simulation of a bubble-laden Taylor-Green vortex with one-way coupling is performed with a bubble response time of the order of the flow time scale (inverse of the mean vorticity). This simple flow allows a direct examination of the effects of the preferential accumulation of bubbles in the high-enstrophy regions of the flow on the accuracy of the two-fluid formulation. The temporal development of the maximum bubble concentration obtained from DNS agrees well with the analytical solution. DNS of the bubble-laden decaying turbulence are also performed for both cases of one-way and two-way coupling. Here, the bubble diameter and response time are much smaller than the Kolmogorov length and time scales respectively. In this case, as expected, the effects of the preferential accumulation of the bubbles are not pronounced. The results also show that the bubble-laden flow is analogous to a stratified flow with an effective density modified based on the bubble concentration. Thus, due to the two-way interaction between the bubbles and carrier flow, the turbulence decay is enhanced with stable stratification, and reduced with unstable stratification. Refer to citations 2, 3, 5, 6, 9, 10, 20, 21, 23, 24, 25, 26, 30, 31.

NAVY RELEVANCE

During 1997 a meeting on Oceanographic Support for Special Warfare was held in San Diego. One of the goals of the conference was to identify oceanographic parameters which impact the operations of Naval Special Warfare (SPECWAR) missions. One major concern of nighttime covert operations is the potential exposure from bioluminescence stimulated by swimmers or swimmer delivery vehicles. The conference draft report includes recommendations for obtaining critical data on bioluminescence which can be integrated into mission planning. Our research on the hydrodynamic stimulation of plankton bioluminescence directly addresses these needs. Moreover, with the recent interest by the Navy in applying hyperspectral and multispectral technologies to ocean surveillance, the role of flow-stimulated bioluminescence takes on new relevance. Our laboratory and at-sea tests have repeatedly shown the bioluminescence can be stimulated by flows producing shear stresses of only 0.1 N m^{-2}. Normally, oceanic flow-stimulated bioluminescence is obscured by background light from the moon or anthropomorphic sources. Because these competing sources are composed of much wider spectra, it may be possible using an intensified multispectral sensor to subtract their contribution to the relatively narrow bioluminescent band (centered on 470 nm). Consequently, when not limited by the noise and/or sensitivity of the sensor, the concept of both large- and small-scale oceanic flow visualization may take on new meaning.

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Refereed Publications

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11. Zirbel, M.J. and M.I. Latz. The reversible effect of agitation on the morphology of the dinoflagellate, *Ceratocorys horrida* (Peridiniales). *Journal of Phycology*. In revision.

12. Latz, M.I.and J. Rohr. Luminescent response of the dinoflagellate *Lingulodinium polyedrum* (=*Gonyaulax polyedra*) to laminar and turbulent flow. *Limnology and Oceanography*. To be submitted.

Manuscripts in preparation

13. Nauen, J.C., J. Rohr, and M.I. Latz. Species-specific luminescent response of dinoflagellates to short-term shear. In preparation.

14. Nauen, J.C., J. Rohr, and M.I. Latz. Mechanism of fluid shear that stimulates dinoflagellate bioluminescence. In preparation.

15. Latz, M.I., A. Juhl, S.E. Elghobashi, A.M. Ahmed, and J. Rohr. Computational and experimental study of the luminescent response of dinoflagellates to a laminar flow field. In preparation.

Invited and Contributed Presentations

16. Latz, M.I. and J. Rohr. 1998. Mechanical stimulation of dinoflagellate bioluminescence by laminar and turbulent flows: Insights and applications. 1998 Ocean Sciences Meeting, San Diego.

17. Rapoport, H.S. and M.I. Latz. 1998. *In situ* bioluminescence measurements from the Scripps pier, La Jolla, CA. 1998 Ocean Sciences Meeting, San Diego.

18. Rohr, J., J.S. Schoonmaker, M.I. Latz, and M. Hyman. 1998. Bioluminescence as a tool for oceanographic flow visualization. Ocean Optics XIV, Hawaii.

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23. Elghobashi, S. 1997. Direct numerical simulation of particle-laden homogeneous turbulent shear flows. Atomic Energy Commission - Military Applications Division, Bordeaux, France. Invited.

24. Elghobashi, S. 1997. Modification of a homogeneous turbulent shear flow by dispersed particles. Workshop on Turbulence Transport and Numerical Modeling, Center for Nonlinear Studies, Los Alamos National Laboratory. Invited.

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