Marine Bioluminescence: Mechanisms and Evaluation

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LONG -TERM GOALS

Our long-term goals are centered on helping to increase understanding of the mechanisms and adaptive significance of all forms of bioluminescence (BL). Within that broad scope, we are now focused on coastal BL. Recognizing the need for appropriate BL detectors for coastal investigations from the multiplicity of platforms likely to be utilized by various investigative groups, we have developed reliable, versatile and convenient instrumentation for study of bioluminescence in the coastal realm.

OBJECTIVES

Using this instrumentation, we are:

1) developing and contributing bioluminescence instrumentation for the California coastal environmental monitoring network (NEOCO) in the hope of developing predictive knowledge particularly of BL dinoflagellates and zooplankton over long distances;

2) participating in AOSN-II, which is testing routes to general oceanographic predictive capability based on massive adaptive sampling with many techniques and platforms, by collaborating on the BL element of that program including providing and maintaining all BL detectors used thus far; and

3) collaborating in the preliminary study of the unique localization of BL in thin layers, hoping in future years to examine the effect, if any, of BL on the population dynamics of organisms in or interacting with thin layers.

APPROACH

The approach is exhaustive iteration of in-house instrument development coupled with laboratory and field testing involving participation in ongoing research projects. There is emphasis on measurement of plankton capture efficiency of the instrument and its ability to discriminate among BL signals of major groups of coastal organisms.

WORK COMPLETED

Generation 3 of the Multipurpose Bioluminescence Bathyphotometer (G3-MBBP) was completed with the production of 7 units that have been successfully deployed from several platforms in ongoing field studies. Senior Development Engineer Cyril Johnson provided electronics design, fabrication and the mechanical design. Generation 1 was built as a mid-hull insert for an early generation REMUS AUV. A preliminary version was successfully tested as a profiler in East Sound as part of the PhD thesis work of Christen M. Herren (Dekshenicks, et al, 2003). The REMUS unit was successfully tested at

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14. ABSTRACT
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LEO-15 and in San Diego Bay (off Silver Strand) in collaboration with Mark Moline, Steven Haddock, David Lapota, Cyril Johnson, Robert Harper and the REMUS developers, C. Von Alt, et al. When REMUS design evolution eliminated mid-body inserts, a nose-cone variant was designed and built for Moline's REMUS. This has had heavy and successful use by Moline's CalPoly SLO group at LEO-15 and Monterey Bay in the SPOKES and AOSN programs (Moline, et al 2000). These ventures are believed to have been the first AUV deployments of a BL bathyphotometer and they have yielded a rich harvest of BL fine structure information.

Four Generation 2 units were built for more varied deployment modes. They have been successfully utilized in shipboard deployments, on the Donaghay and Sullivan (URI) profiling mooring in Monterey Bay and off Corpus Christi, TX, and on the MBARI Dorado AUV in Monterey Bay. These deployments were conspicuously interesting in terms of the previously unsuspected very high and localized BL signals from thin layers. Douglas J. Neilson (SIO) used them to obtain the first BL measurements ever in the CalCOFI program. He is now seeking support to make this a permanent element of that venerable coastal zone fisheries survey program.

RESULTS

Structural details of the Generation 3 MBBP are shown in Figure 1. It can be readily disassembled in the field for cleaning, vital in the typical coastal fouling environment. The impeller and flow meter actuators are flat with short vanes to reduce fouling, particularly in kelp. The entire detector chamber has a dense white Teflon lining and the PMT, in the left compartment, is baffled to admit light only indirectly by reflection off the chamber walls, giving a good approximation of the isotropic detection of an integrating sphere. Figure 2 shows the instrument rigged for plankton sampling with a rotating sample changer that can be programmed or activated on command from the surface operator.

Figure 1: The 3 sections of the Generation 3 MBBP, with impeller and flow meter actuators.

Figure 2: Generation 3 MBBP with attached plankton catcher on the instrument outflow.

Development of this instrument series involved the first capture efficiency measurements ever made for a BL bathyphotometer. This is a particularly critical parameter for any bathyphotometer processing water at low rates of flow as compared with HIDEX (ca 1 l/s *vs*. 19 l/s). The success of these at sea measurements encouraged finalization of the excitation train design as is. However, the Generation 3 units save data to flash memory and the data capture rate is raised from 2 Hz to 60 Hz. Even the 2 Hz rate showed the instrument could discriminate between typical zooplankton flashes and dinoflagellate flashes. At 60 Hz each discrete zooplankton flash is well delineated by several data points, opening the possibility of identifying some zooplankton types directly from flash duration estimates (Figure 3). This means that the G3 BP offers "on the run" discrimination of phytoplankton and zooplankton populations. A useful consequence is the possibility of adaptive, mid-cruise changes in sampling strategy without waiting for other measures of population structure. Finally, the physical design was made more compact while preserving the excitation train design. The G3 crush depth was determined to be 700m by MBARI Engineering. On this basis MBARI Engineering is considering using the G3 as a standard instrument on the DORADO class AUV, thus improving its utility in programmatic biological measurements by MBARI scientists. While the instrument component that failed can be easily strengthened, there is actually little probability that we would need to carry investigations deeper than 200 m.

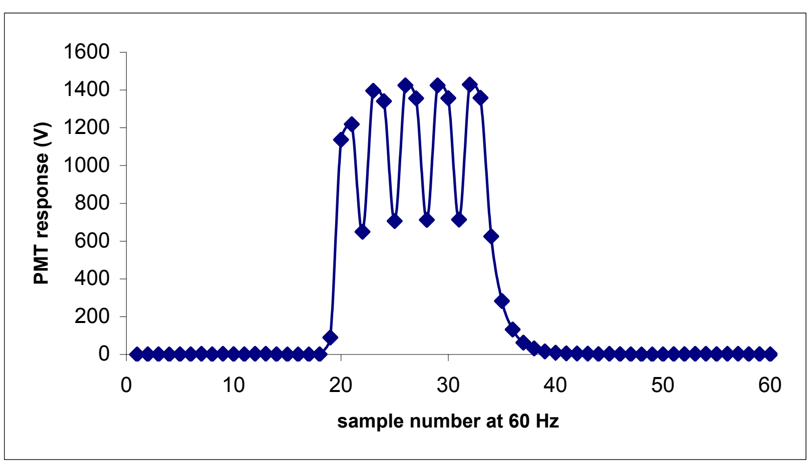

Figure 3: Flash kinetics of a 2 V, 5 ms pulsed LED captured at 60 Hz sampling frequency.

IMPACT/APPLICATIONS

The MBBP series of BPs presently appear to be the only rigorously evaluated, pumped small BP suitable for coastal operations from moorings, profiling systems, towed systems and AUVs (Case, et al, 1993). The unique design of its flow path (Figure 5) and high data rate allows “on the run” discrimination between major plankton sources. The most dramatic application to date is the fact that all BL sampling data in the August AOSN-2 field exercise (Figure 4) was obtained with 7 of these instruments in the capable hands of Cristina Orrico, Cyril Johnson and Kathryn Maynard (UCSB), Steve Haddock and Christen Herren (MBARI) and Mark Moline and his group (CALPOLY-SLO). One suspects this was the densest aggregation of BL detectors ever achieved.

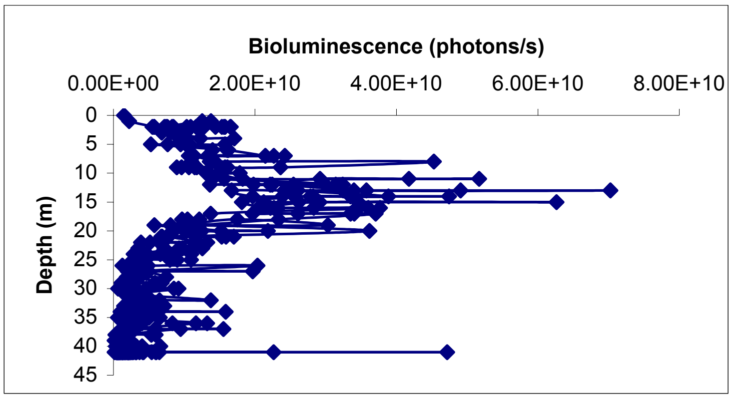

Figure 4: Example bioluminescence profile from AOSN-2 field season in Monterey Bay, CA, showing major zooplankton pulses superimposed on the low intensity dinoflagellate signal.

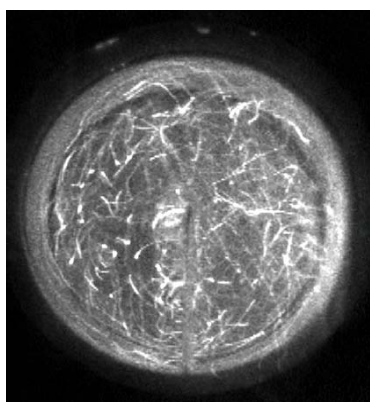

Figure 5: Intensified video of* P.fusiformis *cells luminescing in turbulent flow in the G3 detection chamber; aggregate flow 375 mL s^{-1}.

TRANSITIONS

G3-MBBPs are being distributed as follows:

1. One unit rigged for shipboard vertical profiling is being supplied to NAVO (Mark Geiger) under N00014-98-1-0202 for evaluation at sea in November 2003, in the Asian Pacific for comparison with the NAVO small BP (OTIS, developed by D. Lapota). In early 2004 a test is anticipated in

comparison with the original HIDEX and the recently completed upgrade, HIDEX-2 (developed by E. Widder, Harbor Branch).

2. Several units rigged for moored coastal operations as part of the University of California funded, Network for Environmental Observation of the Coastal Ocean (NEOCO) are being supplied as they become available in the current production run. The first will be a profiler installed at the Avila Pier node developed by Mark Moline during October 2003. The second will be supplied to Michael Latz (Latz and Case, 1994) for hydrodynamic tests and installation on the NEOCO Scripps Pier node. Other units are scheduled for the UCSB seawater inlet and UCSC (M. McManus).

3. One unit is on a MBARI offshore mooring, currently as part of the AOSN-2 program. If it survives, it will remain there if communication into the NEOCO network can be managed.

RELATED PROJECTS

At no direct expense to ONR, work continues on firefly bioluminescence using laboratory resources when free. Using our locally developed integrating sphere, normal photic communication was measured for the first time in the laboratory between tethered flying males and perched females of *Photinus pyralis,* a species in which the flying male exchanges flashes with a perched female. This work represents the first accurate measurements of light emission in the spontaneous photic exchanges in any BL communication system, marine or terrestrial (Case, 2003; Case and Hanson, 2003). The work continued with determination of the maximal light emission obtainable per photocyte during spontaneous communication behavior and during electrical excitation of the photomotor region of the brain. Completion of this part of the study awaits estimation of the number of photocytes per light organ by confocal microscopy. The investigation also revealed a hitherto unknown flight communication mode of the female that is important in understanding evolution of firefly photic communication.

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PATENTS

A patent disclosure has been made to the University of California Office of Technology Transfer.

HONORS/AWARDS/PRIZES

Co-Curator, with Edith Widder (HBOI) and Steven Haddock (MBARI) of GLOW, a traveling exhibit on all aspects of bioluminescence scheduled for such venues as the American Museum of Natural History and the Smithsonian over the next five years.