SPLAT CAM: Mapping plankton distributions with bioluminescent road-kill

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Abstract-**The most common sources of planktonic bioluminescence are dinoflagellates, copepods, euphausiids, ostracods and gelatinous zooplankton. Each of these has very distinctive flash characteristics that make them easy to distinguish from each other. Using an intensified video camera mounted on a mid-water submersible we have developed the Spatial PLankton Analysis Technique (SPLAT) that identifies and maps the 3-dimensional microscale distribution patterns of bioluminescent plankton. The unique temporal and spatial characteristics of luminescent displays permit identification of many sources to the species level, and the exceptional signal-to-noise ratio afforded by a self-luminous source means that even microscopic organisms, such as a 50 µm dinoflagellate, can be identified in a field of view of 1 m. Recently we have adapted the SPLAT CAM for deployment on the HIDEX-BP (High Intake Defined EXcitation BathyPhotometer). This vertical profiling system was developed for the U.S. Navy (Naval Oceanographic Office - NAVOCEANO) for routine monitoring of bioluminescence in the oceans. The high pumping rate of this BP (18 l/s) assures a high statistical significance and a high-resolution profile of bioluminescence potential in the water column. By combining this capability with the plankton identification afforded by the SPLAT CAM, the utility of both systems is greatly enhanced. The resulting data should prove valuable for a wide range of applications such as defining the geographical boundaries of dinoflagellate blooms, tracking movement patterns of bioluminescent vertical migrators, monitoring temporal changes in the abundance of grazers as a function of environmental variables and primary production, assessing the production of primary sources of nutrition for commercially important fish species and providing data needed for NSW nowcasts and forecasts.**

I. INTRODUCTION

Remote sensing with radiometers lowered from ships has demonstrated "the occurrence of luminescent organisms in nearly every cubic meter of the ocean, from coast to coast and surface to bottom"[1]. Organisms from 14 marine phyla ranging from bacteria to fish are known to be luminescent. Included among these are some of the most abundant of the ocean's inhabitants, such as dinoflagellate species that form red tides and illuminate phosphorescent bays, copepods including all of the Metridinidae (*Metridia*, *Pleuromamma* and *Gaussia*), ostracods (e.g. *Cypridina*, *Vargula*, and *Conchoecia*) almost all euphausiids and ctenophores and many medusae and siphonophores [2,3] (Fig. 1).

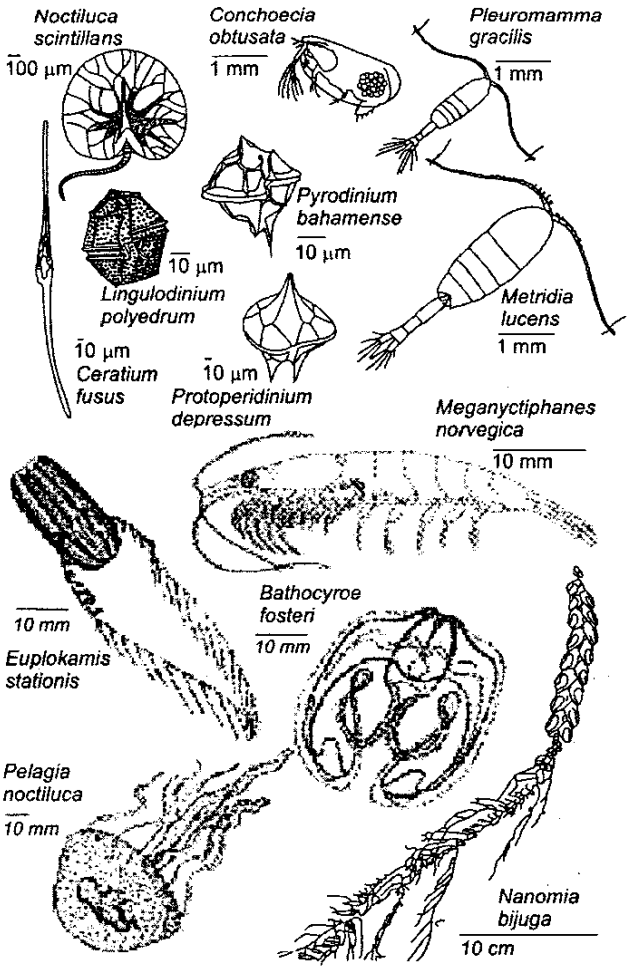

Fig. 1. Some common sources of planktonic bioluminescence.

II. BIOLUMINESCENCE MAPPING TECHNIQUES

A. Identification of bioluminescent signatures

The temporal and spatial patterns of bioluminescent emissions from these different organisms are highly distinctive and can be used as a means of identifying and mapping their distributions. We have demonstrated this using an intensified video camera, focused on a large mesh transect screen, mounted on a mid-water submersible. During horizontal transects as organisms contact the screen they are stimulated to bioluminesce in the plane of focus of the camera. The exceptional signal-to-noise ratio of a self-luminous source, viewed against a black background, permits identification of the luminescent signature of even microscopic organisms (e.g. a 50 µm dinoflagellate) in a field of view of one meter, making this both a high-resolution and a high-frequency sampling (30 fps) protocol [4,5,6] (Fig. 2).

B. Spatial plankton analysis technique

Analysis of the video recordings of stimulated displays is carried out using a computer image recognition program that first classifies the displays and then reconstructs them in three-dimensional space [7] (Fig. 3). Displays are binned according

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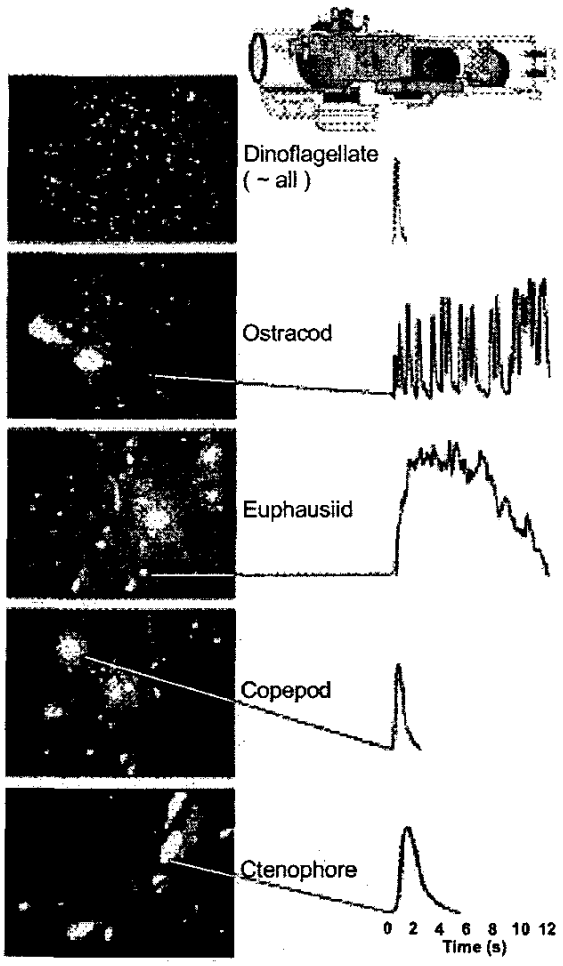

Fig. 2. A 1 m diameter screen mounted in front of the Johnson-Sea-Link submersible mechanically stimulates bioluminescence during horizontal transects. Identifications of intensified video recordings of the bioluminescent displays are based on the spatial and temporal patterns of the emissions. The primary identification characteristics for the displays shown here are the size (shown on the left where field of view is 1 m across) and temporal persistence (shown as graphs on the right).

to the spatial and temporal properties of the recorded events. Bins are labeled based on correlations with the abundance of identified bioluminescent organisms collected by a suction sampling pump during each transect. The x and y values and the frame number (converted to z value based on the forward speed of the submersible) of the initial impact point are then used for 3D reconstruction and statistical analysis of the original transect volume. The reconstructed spatial distributions are compared with Monte Carlo simulations of random distributions. Cumulative histograms of the nearest neighbor distances (NNDs) generated by the Monte Carlo simulations are used as a model of complete spatial randomness and compared with the NNDs calculated from the transects [8] (Fig. 4). Known as the Spatial PLantkon Analysis Technique (SPLAT), this method provides valuable information about the composition and organization of bioluminescent aggregations. By contrast, the standard method of mapping the distribution of bioluminescence, with bathyphotometers, does not provide such information.

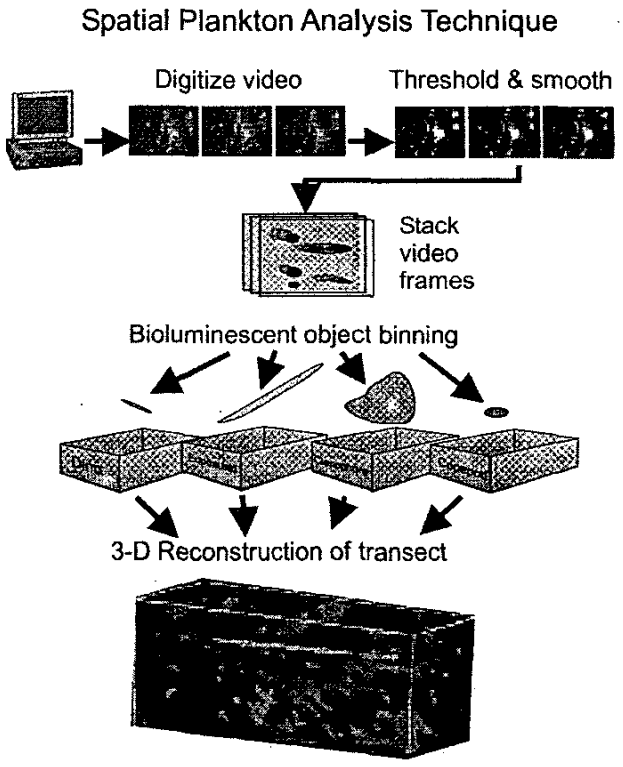

Fig. 3 Bioluminescent displays are identified and their 3D spatial distributions are analyzed using an object oriented image analysis routine. Video frames are digitized and then set to threshold and smoothed to remove camera noise. Frames are then stacked to reconstruct the complete bioluminescent event. Bioluminescent objects are binned and classified based on length, volume and maximum area. The classified blobs along with the x,y,z values of the initial impact point are used for 3D reconstruction and statistical analysis of the original transect volume.

C. *HIDEX Bathyphotometer*

In bathyphotometers, water, containing organisms, is drawn through a light-tight chamber where a light detector measures the bioluminescence stimulated by some turbulence generating mechanism (Fig. 5). Since some bioluminescent organisms produce only a single flash while others produce multiple flashes and flash durations vary from less than 100 ms to more than a second, the values measured by different bathyphotometers are generally instrument specific. Such factors as the detection chamber volume, the flow rate through the chamber, the method of stimulation and the amount of prestimulation, which occurs due to light baffling, all combine to affect the photon flux that is measured in a given body of water. The need for a standardized bathyphotometer design was first formulated within the U.S. Navy oceanographic community. Based on the combined requirements of: 1.) defined excitation in order to quantify the stimulus, 2.) high flow rates in order to improve sampling statistics, and 3.) a long residence time capable of measuring an entire flash, the HIDEX-BP (high intake defined excitation bathyphotometer) was developed [9]. In this bathyphotometer, bioluminescence is stimulated by hydrodynamically calibrated flow through a turbulence-generating grid at the entrance to a

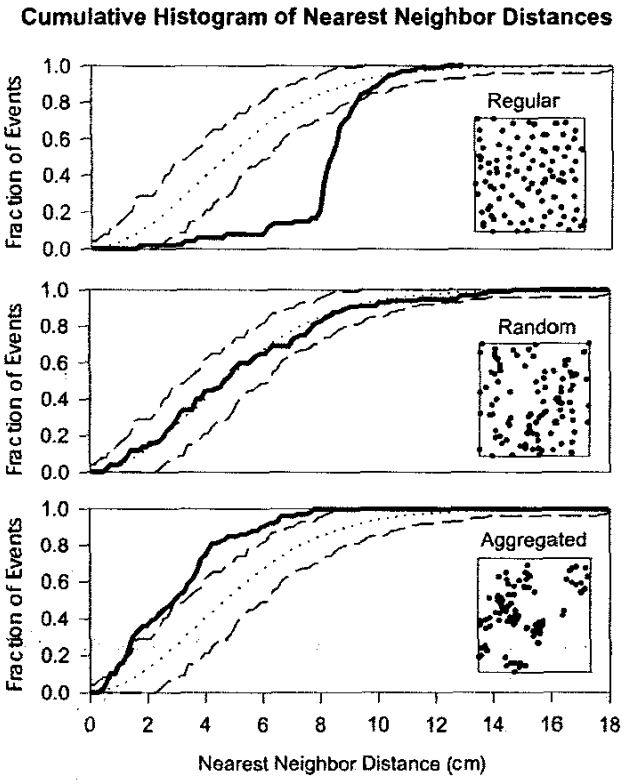

Fig. 4 Cumulative histograms of nearest neighbor distances showing examples of regular, random and aggregated distributions superimposed on cumulative histograms generated by 100 Monte Carlo simulations. The dotted line shows the average of the simulations, while the dashed lines show the upper and lower envelopes and the solid line shows the data displayed in the insets [8].

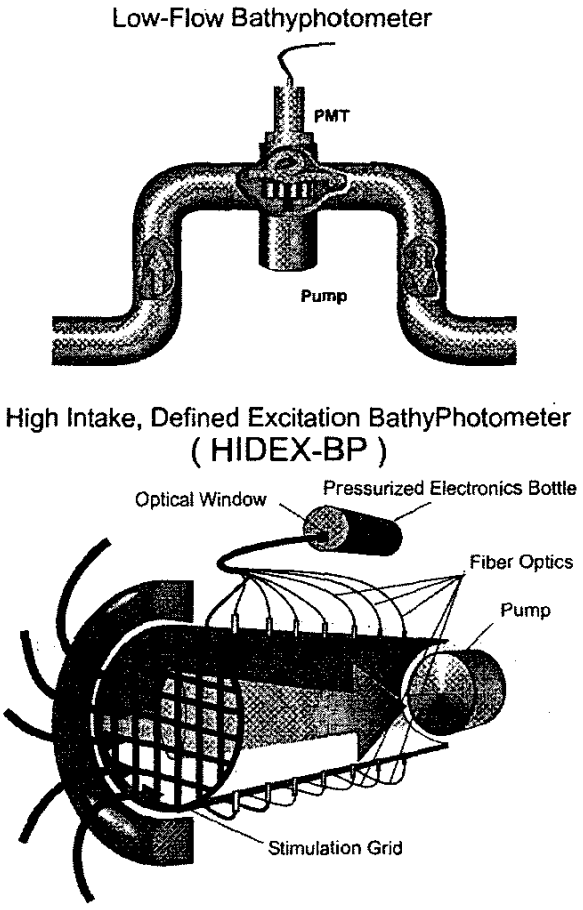

Fig. 5 Comparison of a generic low volume bathyphotometer in which excitation is provided by a pump impeller and the HIDEX-BP (high intake defined excitation bathyphotometer) in which bioluminescence is stimulated by hydrodynamically calibrated flow through a turbulence generating grid.

large cylindrical detection chamber. An array of optical fibers embedded in the wall of the detection chamber collect light and direct it to a photomultiplier tube. Water is pumped at 18 l s^{-1} as compared to the 1 l s^{-1} or less used by most traditional BPs. With this flow rate, sampling statistics are good even during rapid profiles (~15 m/min) and pump avoidance by fast swimmers such as euphausiids and copepods is reduced. In addition, thanks to the length of the detection chamber (130 cm), the residence time (820 ms) is sufficient to measure total photon flux in most bioluminescent flashes.

D. Combining HIDEX-BP profiles with SPLAT CAM recordings

In order to determine the taxonomic composition of light-emitting assemblages we have been using the Johnson-Sea-Link submersible to make discrete collections and SPLAT CAM recordings at depths of interest that were selected based on HIDEX-BP profiles (Fig. 6). This protocol has proven to be a valuable tool for mapping plankton distribution patterns [7,8]. The HIDEX-BP profiles provide a measure of the bioluminescence potential throughout the water column and the Spatial Plankton Analysis Technique identifies the sources of the bioluminescence and their nearest neighbor distances. Identifying sources is important for modeling the relationship between bioluminescence potential and environmental parameters. For example, knowing whether a given high bioluminescence potential is due to high densities of luminescent dinoflagellates or much lower densities of euphausiids will greatly impact forecasts of the persistence of high bioluminescence in a particular area. In addition, identifying the planktonic sources of bioluminescence and mapping their nearest neighbor distances is necessary for calculating how detectable an organism, such as a fish, might be while swimming through a luminescent "minefield". Luminescent plankton, which respond to even slight mechanical disturbances with a flash of light, are a threat to any animal using darkness as a means to escape detection. To determine how detectable an organism is to the eyes of visually orienting predators and prey, taxonomic identification of the light emitters is essential.

Because combining HIDEX-BP profiles with SPLAT CAM recordings has proven to be such a useful technique, we are now in the process of streamlining this methodology by mounting a SPLAT CAM and plankton collector directly on the HIDEX-BP (Fig. 7). A miniature SPLAT CAM system is mounted inside the HIDEX-BP frame, next to the detection chamber. Rather than running horizontal transects from the submersible at a forward speed of 18 m/min, we can use the drop

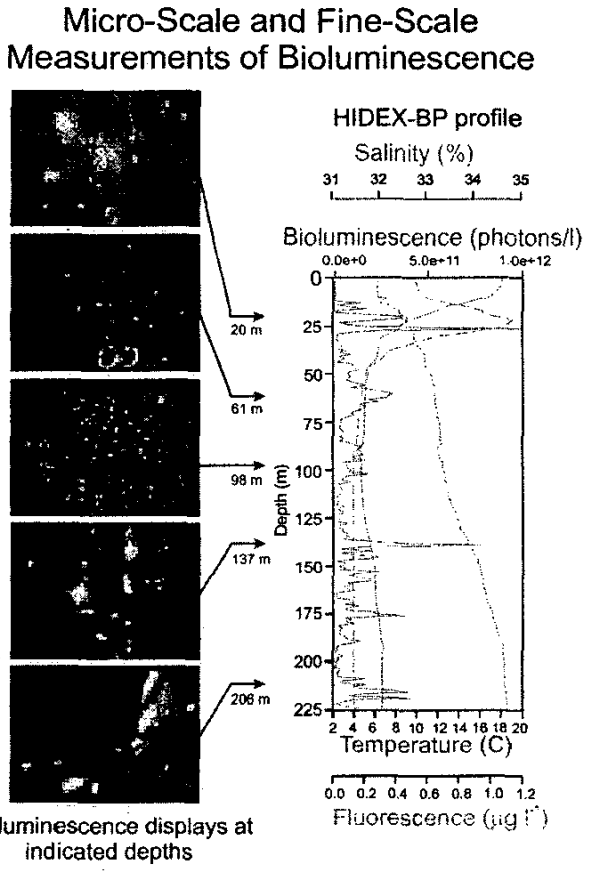

Fig. 6 A HIDEX-BP profile made in the Gulf of Maine was used to select depths for submersible transects with the SPLAT CAM. Single video frames indicate the different character of the bioluminescence associated with the different peaks of bioluminescence. Field of view for each frame is 1 m across [10].

speed of the profiler (~15 m/min) to run vertical transects. This then serves as a rapid means of taxonomic identification, mapping nearest neighbor distances and event counting. In order to "ocean truth" the data on this prototype system we are constructing a plankton "Critter-Getter" similar to the carousel system currently used on the JSL submersible. In this way we will be able to correlate the binned displays with the abundance of identified bioluminescent organisms in quantitative plankton samples collected at depths of interest. This arrangement has the additional advantage of eliminating any time delay between the measurements of stimulated bioluminescence and direct sampling of the plankton. The resulting data will provide a rapid means of mapping bioluminescent plankton distributions relative to physical and chemical variables in the environment and should prove valuable for a wide range of applications such as defining the extent of dinoflagellate blooms, tracking movement patterns of bioluminescent vertical migrators, monitoring temporal changes in the abundance of grazers as a function of environmental variables and primary production, assessing the production of primary sources of nutrition for commercially important fish species and providing data needed for NSW nowcasts and forecasts.

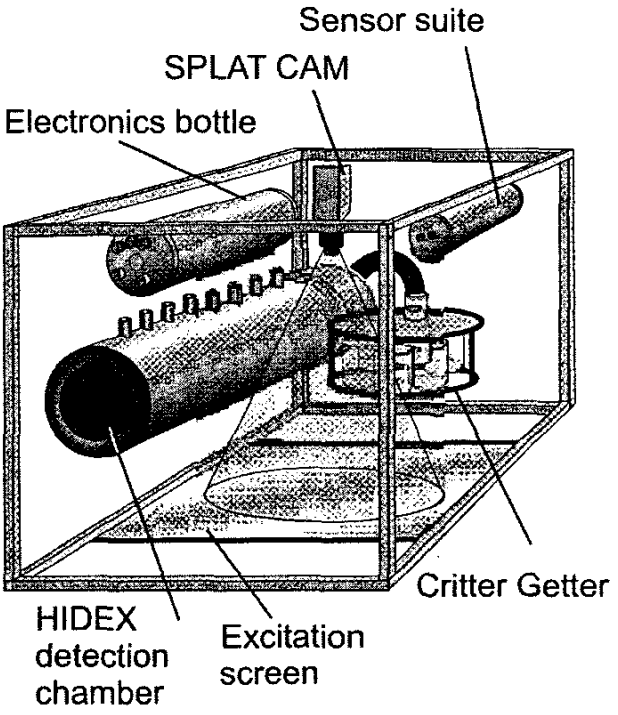

Fig. 7 The HIDEX-BP with SPLAT CAM and "Critter-Getter".

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