Marine Bioluminescence: Mechanisms and Evaluation

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LONG-TERM GOALS

My long-term goal is to continue investigation of marine bioluminescence with emphasis on its mechanisms and adaptive significance. The ubiquity of marine bioluminescence, the huge variety of its underlying molecular and physiological processes and regulatory behavior, when compared with the scarcity of knowledge in all these sub-disciplines argues that marine bioluminescence most probably has major unknown significance to life in the sea (Case *et al.*, 1995).

OBJECTIVES

During the year we focussed on an interrelated set of projects that promote this goal. These range from cellular mechanisms of luminescence excitation, to field studies of population dynamics of bioluminescent organisms and to study of the adaptive significance of bioluminescence.

APPROACH

Luminescence excitation mechanisms – Using the dinoflagellate *Pyrocystis fusiformis* as a model organism, we seek to understand what might be the simplest of "sensory" luminescence triggering processes, namely how detection of mechanical stresses causes luminescence in single-cell organisms in a fluid environment. Using new, non-toxic, fluorescent ion-specific stains we hope to visualize with confocal techniques the roles of various ions and the cytoskeleton in transduction between mechanical stress and light emission.

Vicarious luciferins – Although it is estimated that luminescence has independently evolved on the order of thirty times (Hastings, 1983) the unusual fact remains that a significant number of luminescent species, widely spread among the phyla, must obtain their luminescent substrate, luciferin, in the diet from other luminescent species, hence the term *vicarious luciferins*. We have shown that one of these species, the midshipman fish, that as far as is known obtains its luciferin from an uncommon and tiny crustacean, *Vargula tsujii*, uses its luminescence in an elegant predation avoiding process called counter-illumination (Harper and Case, 1999). In laboratory experiments we are attempting to discover how the fish obtains this evidently most vital component of its diet.

Thin layers dynamics – To test the possibility that development of marine thin layers might in some instances involve bioluminescent attraction of zooplankton predators we have used a new profiling bioluminescence detector system in an examination of the fine scale bioluminescence organization of thin layers (see Alldredge, Case and MacIntyre, this volume).

Long-term population dynamics of luminescent plankton – As a contribution to understanding seasonal and inter-year variation of coastal luminescent plankton we have used moordex

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bioluminescence moorings in a four year study of bioluminescent populations in the Southern California Bight (Case *et al.,* 1993; Lapota and Case, 1999).
New bioluminescent systems – An investigation of physiological control of a poorly known animal luminescent system, that of ophiuroid starfish (brittlestars) was initiated in a collaboration with Prof. Jerome Mallefet of the University of Louvain, and will continue this coming year.
General dissemination of bioluminescence information – The bioluminescence web page, designed as a general source of reliable information on marine bioluminescence, continues to be maintained and improved.

WORK COMPLETED

Luminescence excitation mechanisms –In the thesis research of ASSERT scholar Carrie McDougall it has been verified that the huge central vacuole characteristic of several species of luminescent dinoflagellates is acidic as measured though application of an *in vivo* acidophilic dye. In another phase of the work a protocol was developed for the quantification of filamentous actin in dinoflagellates using single cells, either from culture or from freshly collected plankton.
Vicarious luciferins – With publication of the demonstration of counterillumination in the midshipman fish, we have undertaken study of its predatory behavior in seeking prey essential to maintaining its luciferin level sufficient for bioluminescence.
Thin layer dynamics – See report by Alldredge, Case and MacIntyre, this volume.
Long-term populational dynamics of luminescent plankton
Multiyear study of luminescence in the Southern California Bight - Data analysis of this massive study continues (Lapota and Case, 1999). The two moored detectors used in this work have been rebuilt after several destructive years of sea exposure and are ready for reinstallation and proposed continuation of the project.
New bioluminescent systems
Gelatinous zooplankton - Analysis of bioluminescence spectra as a function of depth of occurrence was completed and published (Haddock and Case, 1999). Investigation of neural control of luminescence in a starfish was commenced with Prof. Jerome Mallefet.
General dissemination of bioluminescence information – The bioluminescence web page use and further development continues. New articles have been incorporated and others updated.

RESULTS

Luminescence excitation mechanisms - It was verified that several species of luminescent dinoflagellates have acidic vacuoles through the circadian cycle. Quantification of filamentous actin was accomplished throughout the cell cycle.
Vicarious luciferins – Work is in progress using pulsed blue LED probes to mimic the secreted bioluminescence of *V. tsujii.* The midshipman fish appears to be a passive nocturnal midwater predator and is probably able to harvest sufficient *V. tsujii* by visual orientation to its bioluminescent display.
Thin layers dynamics – See Alldredge, Case and MacIntyre, this volume.
Long-term population dynamics of luminescent plankton – There is a marked seasonality in per cell bioluminescence as well as cell numbers in *Pyrocystis noctiluca* (autotroph) and *Protoperidinium pellucidum* (heterotroph), two major elements of luminescent phytoplankton in the study area between San Diego Bay and San Clemente Island. Their luminescence is strongly associated with environmental events such as upwelling and storm runoff from land.

New bioluminescent systems – Progress was made in study of neurocontrol of luminescence in the brittlestar *Ophiopsila californica* using a specialized photometer system and image intensified video. A new species of luminescent brittlestar was discovered and is being described by a museum specialist in the taxonomic group.
General dissemination of bioluminescence information – The Bioluminescence Web Page appears to be well established. During the past year it had over 25,000 visitors, never less than 1000 per month.

IMPACT/APPLICATIONS

Luminescent excitation mechanisms - It has long been postulated that the final step in the luminescence transduction process involves a flux of H^+ from the vacuole into the cytoplasm to trigger bioluminescence. We were able to verify that the vacuole is acidic and shall be able to track its variation during excitation. In forms non-luminous in daylight the vacuole is still acidic so there must be other steps in the excitation sequence to be discovered. Since no mechanically triggered sensory system in any organism has been completely worked out, it is hoped that this unicellular system will assist towards this important general scientific objective owing to its simple structure and amenability to cellular and microscopical study.
Long term population dynamics of luminescent phytoplankton - Since it is desirable to be able to predict phytoplankton population levels, both for economic and military reasons, this study may prove to be of importance in providing a roster of environmental signals that might have predictive value. Particularly important in this regard is the demonstration that the chlorophyll fluorescence signal is a good surrogate measurement for bioluminescence *only* when autotrophic bioluminescence dinoflagellates are present.

RELATED PROJECTS

1 - Collaboration with Dr. Michael Latz and his group at Scripps in study of the response of dinoflagellates to very low shear forces and their effect on the actin cytoskeleton in dinoflagellates.

2 - Collaboration with Prof. Mark Moline, California Polytechnic University, in development of a bathyphotometer system for year-round profiling of bioluminescence at the LEO-15 Site and elsewhere.

3 - Collaboration with Dr. David Lapota, SPAWARS, San Diego, in analysis of long term bioluminescence data from the Southern California Bight.

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