AD-A143 049 PROPERTIES OF THE DEEP SCATTERING LAYER ANALYZED IN TERMS OF BIOLUMINESCE..(U) CALIFORNIA UNIV SANTA BARBARA DEPT OF BIOLOGICAL SCIENCES J F CASE 28 FEB 84 1/1

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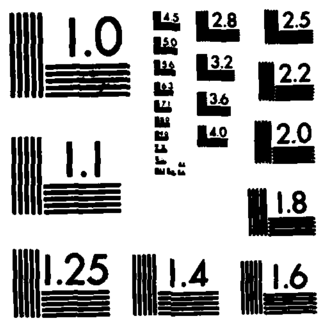

MICROCOPY RESOLUTION TEST CHART
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Properties of the Deep Scattering Layer Analyzed in Terms of Bioluminescent Behavior of Its Components.

Contract Number: N00014-69-A-0200-8006
Contract Period: March 1, 1969 to February 28, 1984
Office of Naval Research

Principal Investigator:

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Introduction

For several reasons the phenomenon of bioluminescence has, since at least 1957, been of interest to the P.I. In most bioluminescent animals and in certain dinoflagellates light emission is under control of the central nervous system, or in the case of the dinoflagellates that we have studied, controlled by an excitable surface-bounding cell membrane with many electrical properties in common with neuronal membranes. Thus bioluminescence provides and avenue to study cellular mechanisms of effector organ excitation and, at a higher lever of integration, may be used as a tracer of complex behavior, one which is especially valuable in the non-obtrusive study of behavior of nocturnal and deep-sea organisms. Our interest in bioluminescence began with the classical organism of this field of research, the firefly, which, after my introduction to marine biology, has had to share mv interest with the infinitely rich and profoundly unknown (relative to terrestrial bioluminescence) bioluminescence of the oceans.

ONR initially supported both my work on terrestrial and marine bioluminescence, recognizing, I believe, that terrestrial systems, being so well known, might serve as guides to the study of more difficultly accessible marine systems. Thus, included in this report are investigations on fireflies which are still in progress. Although funding has shifted to other sources, I think it appropriate that this work be cited here because ONR provided the spark to initiate it and because certain long-lived items of equipment originally provided by ONR are still in use both for our firefly and our marine studies. However, the preponderance of the research reported here is marine, and I believe that it images the development of our research group into a major contributor to the field, both in terms of concepts and instrumentation.

While our interests in bioluminescence are rooted in pure science, the wisdom of ONR's long-term support of such research is now becoming strongly apparent in applied problems directly applicable to the primary goals of the Navy. I am personally most gratified that we are in a position to continue to contribute to these goals. I must also express, at this particular milepost of my relationship with ONR, equal gratification with the thoughtful and interactive way my contract has been handled. Every scientific administrator with whom I have worked on this project has virtually been an enthusiastic collaborator.

Program Results

Since virtually all of the work conducted has been published in the refereed literature, the backbone of this report is the attached reprint collection. In addition, manuscripts of articles in press and submitted are included. This material is prefaced by a brief statement of major accomplishments and certain other peripheral "milestones".

Summary of Research Accomplishments

Work on bioluminescence began with a collaboration with John Buck on the electrophysiology of the firefly light organ (Buck and Case, 1961; Case and Buck, 1963; Buck *et al.*, 1963). Intermittently after that, and until the present, Buck and I have collaborated. Initially, I was interested only in the electrophysiology of these remarkable effector organs but in recent years have gotten interested in firefly behavior as related to the use of the light organ in courtship and mating. However, the interest in physiology has been maintained and has been expanded into ultrastructural and pharmacological studies of the differentiating and dedifferentiating light organs during development (Case and Trinkle, 1968; Hanson *et al.*, 1971; Oertel *et al.*, 1975; Oertel and Case, 1976; Strause *et al.*, 1979; Case and Strause, 1979; Strause and Case, 1980). the goal of the developmental studies is, of course, to capitalize on the sequential appearance of functional components of the light organ (or, similarly, the loss of components in the dedifferentiating larval light organs) to determine the physiological activity of the components of the functional adult organ.

Arrival in Santa Barbara provided a splendid opportunity to work on marine organisms since the oceans contain by far the greatest variety of luminescent organisms. Our first studies (Barnes and Case, 1970; Baguet and Case, 1971) were virtually direct translations of technology from firefly to marine organisms with the fundamental question of how nerves control systems that make light still paramount. While aspects of this question are still with us, I have recently become somewhat more interested in broader aspects of marine bioluminescence.

Among our most significant recent studies are:

a. Demonstration of a dietary requirement for luciferin in the diet of the fish, *Porichthys*. This work began with studies on the normally non-luminescent Puget Sound fish (Tsuji *et al*., 1972; Barnes *et al*., 1973) and concluded with a study that I consider highly significant in that it shows that the normally luminescent southern population probably depends on a crustacean, *Vargula tsujii*, as a dietary source of luciferin (Warner and Case, 1980).

b. Demonstration of the adrenergic mechanisms of photophore control in *Porichthys*. This work comes as close to any in demonstrating the transmitter mechanism between nerve ending and photocyte for any bioluminescent animal (Anctil and Case, 1976).

c. The first *quantitative* demonstration of the use of ventrally directed bioluminescence in counter illumination by midwater animals (Case *et al*., 1970; Warner *et al*., 1979).

d. Development of a system for investigation of cellular mechanisms of bioluminescence control in a single-celled marine organism, *Pyrocystis fusiformis*. New methods were developed to permit handling single cells without inadvertently stimulating them and to permit precise stimulation of single cells while observing electrical potentials with penetrating electrodes and observing light emission with a newly developed image intensifier system. The first work from this system (Widder and Case, 1980) shows a surprising phenomenon, namely that a "first-flash" from a cell hitherto unstimulated has kinetics represented by the precisely temporally superimposed kinetics of the microsources of light within the cell and that later flashes have kinetics described by asynchronous triggering of microsources. These observations, first obtained with photomultiplier techniques (non-imaging) have been directly confirmed and expanded with the image intensifier (Widder and Case, 1981; Widder and Case, 1982a,b).

e. Luminescence spectroscopy. We have recently developed a fast spectrometer capable of obtaining highly accurate luminescent spectra from dim, briefly and unpredictably emitting sources. The equipment is portable enough to be used at sea allowing hitherto unobtainable precision spectra to be obtained from delicate mid-water organisms. We have already amassed the most extensive collection of marine bioluminescent spectra yet obtained (Widder *et al.*, 1983) and are developing a monograph on the subject to include many as yet unpublished spectra. The system has also just recently been used to identify an infra-red emitting organism (Widder *et al.*, in press) and to assist in determining the dietary source of luciferin for the mid-water mysid, *Gnathophausia*.

f. Sensory components of the counterillumination system in *Sergestes*. Our study of counterillumination showed that this decapod crustacean does not counterilluminate to light projected from below. The question then arises as to how the animals know which way is up. Fortunately, the light emitting organs in this animal rotate under neuromuscular control so it is possible to tell by the rotating the whole animal under various experimental conditions if it is compensating. If the organs remain pointing downwards, then the animal still has a gravitational sense. Using the low-light camera it is possible to precisely record such data. Mike Latz, as part of his thesis, has shown that the statocysts control both the orientation of the light organ and of the eyes by the technique of removing the statoliths from the statocysts, which renders them functionless with little damage to the nervous system. When the system is intact there is compensation up to 90° from horizontal and none at all with the gravitational sense eliminated (Latz and Case, 1982).

Other Accomplishments of the Research Group

During the contract period the P.I. has been a Senior Queen's Fellow in Marine Science (Australia) and has served two terms as a member of the Executive Committee of the Marine Biological Laboratory, Woods Hole. He was elected a Fellow of the AAAS and of the Royal Entomological Society of London. He has been External Examiner at the University of Malaysia and the Australian National University. He provided extensive footage of bioluminescence and served as a consultant for four TV documentaries on the BBC and French National Television.

Students and post-doctorals employed on the project are currently in tenured posts at U.C. Riverside, State University of New York (Albany and Stonybrook), University of Lovvain (Belgium), University of Montreal, Australian National University, Arizona State University, and University of Florida (Whitney Marine Laboratory).

Publications Generated by the Contract

I. Published papers in refereed journals or books.

1971 Baguet, F. & Case, J.F. Luminescence control in *Porichthys* (Teleostei): excitation of isolated photophores. *Biol. Bull.* 140:15-27.

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Widder, E.A., Latz, M.I. & Case, J.F. Marine bioluminescence spectra measured with an optical multichannel detection system. Biol. Bull. 165:719-810.

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II. Publications in Press

Widder, E.A., Latz, M.I., Herring, P.J. and Case, J.F. Far red bioluminescence from two deep-sea fishes. Science.

Case, J.F. Vision in Mating Behavior of Fireflies. Royal Entomol. Soc., Centenary Symposium. Academic Press.

Frank, T., Widder, E.A., Latz, M.I. & Case, J.F. Dietary maintenance of bioluminecence in a deep-sea mysid. J. Exp. Biol.

III. Publications Submitted

Hiller-Adams, P. and Case, J.F. Optical parameters of the eyes of some benthic decapods as a function of habitat depth. J. Comp. Physiol., submitted.

Hiller-Adams, P. and Case, J.F. Eye size and relative growth rates of eyes of some pelagic crustaceans as a function of habitat depth and possession of photophores. Biol. Bull., submitted.

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