References and Notes

1. I am indebted to my wife, Jimmie, for her keen observation and unbiased interpretation that led to the discovery of territorial behavior in Uganda kob. Research during 1957 and 1958 was supported by a Fulbright appointment and financial assistance from the Uganda Administration; in 1959 a grant in aid of research from the National Science Foundation permitted concentrated study of the phenomenon. Personnel of the Uganda Department of Game and Fisheries and the Uganda National Parks assisted greatly in field operations.
2. Estimation of the population from aerial counts was made in May 1958 with Dr. William M. Longhurst, University of California, who piloted a Stinson Voyager generously loaned to us by C. D. Margach, Kinyala Estates, Misindi, Uganda.
3. H. K. Buechner, A. M. Harthoorn, J. A. Lock, *Can. J. Comp. Med. Vet. Sci.* **24**, 317 (1960).
4. The film may be rented through the Audio-Visual Center at Washington State University, Pullman.
5. F. Bourlière, verbal communication.

28 October 1960

Bioluminescence in Chesapeake Bay

Abstract. Bioluminescence measurements made by stimulation of the organisms in a jet of water directed at the face of a phototube have increased the sensitivity of data by a factor of 1000 over "spontaneous" luminescence measurements. In light-baffled cells it has been possible to map the surface bioluminescence of large areas continuously in broad daylight. Measurements of intensity versus depth during both day and night do not show any appreciable diurnal variation in maximum intensity, although there does appear to be a vertical migration of intensity.

Measurements of bioluminescence in several regions of the Chesapeake Bay indicate that light-emitting microscopic marine organisms have a wide and general distribution in these waters. The equipment was designed to measure bioluminescence independent of external incident radiation. The sample cell consisted of a defined volume of 9 by 12 by 12 inches, open to the sea above and below but sufficiently light-baffled so as to exclude the effects of incident sunlight at the water surface even in broad daylight.

Light emission by many microscopic organisms occurs only on stimulation; consequently, in order to obtain a true measure of the luminous organisms present it is necessary to stimulate the population immediately in front of the photocell. This was done by means of a miniature impeller-type pump directing a small jet of water directly toward the face of an EMI 1-inch phototube, which was mounted on the opposite side of the sample cell housing. The turbulence in the jet stream was sufficient to stimulate emission of light in those organisms within the stream. The total volume of the sample cell was large enough to replenish, by convection to the jet stream, those organisms which had not yet been stimulated.

This method of nondestructive stimulation increased the sensitivity of the bioluminescence measurements by more than a factor of 1000 over that obtainable by measuring "spontaneous" bioluminescence. These features provide what is considered to be a more precise and much more sensitive parameter for estimating the density of bioluminescent organisms than that described by G. L. Clarke and his co-workers (*1*). Further, the present measurements were made from the surface to depths of 140 feet.

The sensitivity of the technique was such that continuous measurements of bioluminescence could be made when the unit was towed behind the boat at speeds of 3 to 4 knots. In Fig. 1 are shown partial records of stimulated bioluminesmence taken with the light-baffled cell at a depth of 1 foot in bright sunlight. The speed of the boat was 6 ft/sec.

Figure 1*a* shows a representative mapping record over a distance of 2500 feet, as the boat was coming out of the channel of the Little Choptank River into Chesapeake Bay proper. The bioluminescence light intensities are not uniform and indicate the presence of "blooms" or colonies of bioluminescent organisms. Figure 1*b* is a portion of the mapping record showing the decrease in bioluminescence light intensity as the boat came into the main channel of the bay where the tide was running. The bioluminescence intensity measured in this particular mapping experiment extended over a range of 740. At the lowest level measured, at rip tide in the bay channel, the signal-to-noise ratio was still 300.

Figure 2 shows stimulated bioluminescence intensity as a function of depth made at anchor over a 140-foot hole in the bay floor. There is a shift in peak intensity with depth from about 25 feet during the day to just below the surface at night.

An important point is that there is no striking difference between the maximum light intensities measured in daylight and at night; in fact, the daylight intensities were all slightly higher. This would indicate that the light-inhibition of bioluminescence previously reported for the larger organisms, such as *Mnemiopsis* by E. N. Harvey (*2*), may not be general or else may be a secondary physiological response. Using laboratory cultures of the dinoflagellate *Gonyaulax polyhedra,* Sweeney and Hastings observed a diurnal rhythm of of luminescence. (*3*). Cells grown in daylight showed a dim luminescence during the day which increased in brightness at night. The bright luminescense observed at night could be inhibited by light. It was surprising, therefore, that such a rhythm was not observed in the bay. From the data presented in Fig. 2, it appears that the organisms migrate to a deeper region during the day and consequently maintain maximum luminescence.

A careful study of the relationships between photosynthesis and luminescence in these organisms may reveal that the type of rhythm observed by Hastings and Sweeney is offset in nature by a rhythmic vertical movement which prevents exposure to strong illumination. Additional studies on the type and number of organisms present are necessary before any definite conclusions can be made.

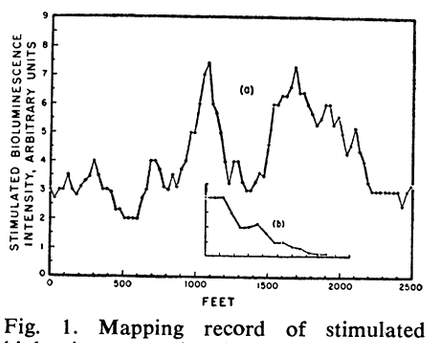

Fig. 1. Mapping record of stimulated bioluminescence in the channel of the Little Choptank.

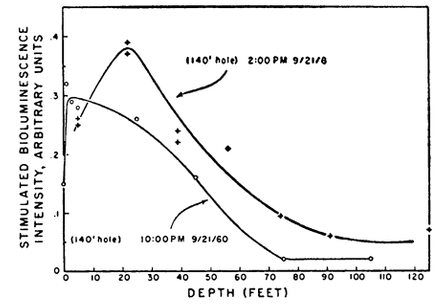

Fig. 2. Stimulated bioluminescence as a function of depth for day and night.

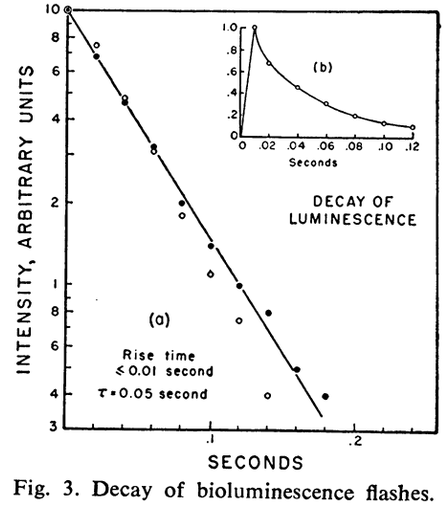

Fig. 3. Decay of bioluminescence flashes.

Individual flashes were also observed and recorded. With only one exception, which may have been a small ctenophore which penetrated the baffle and screen cover of the sample cell, all of the hundreds of flashes observed were extremely short bursts lasting only 0.1 or 0.2 second. The decay time of some representative flashes is shown in Fig. 3*a* to be first order with a mean life of 0.05 second. A complete flash is shown in Fig. 3*b*. The rise time of 0.01 second is the limit of the high frequency response of the d-c amplifier used in the equipment. The actual rise time may be much shorter (*4*).

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References and Notes

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2. E. N. Harvey, *J. Gen. Physiol.* **7**, 331 (1925).
3. B. M. Sweeney and J. W. Hastings, *J. Cellular Comp. Physiol.* **49**, 115 (1957).
4. This work was performed under a grant from the Office of Naval Research. The assistance of the Chesapeake Bay Institute of Johns Hopkins University in providing their research vessel *Maury* is gratefully acknowledged.

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Production of Biologically Active Compounds by Isolated Lichenized Fungi

Abstract. Fungi which were separated from their lichenized associations and cultured independently produced several unusual and highly pigmented compounds. Certain of these compounds exhibited marked inhibitory activity against test strains of gram-positive bacteria and free-living molds; others showed a growth stimulatory effect on selected bacterial species.

The fact that lichens form many unique and interesting chemical compounds (so-called lichenic acids) which possess strong antibiotic activity has been firmly established (*1*). Unfortunately, lichens are notoriously slow growers and are not amenable to laboratory cultivation. Thus, practical exploitation of such compounds is limited only to lichens growing naturally in sufficient abundance.

Few workers have investigated the possibility of the lichen fungal component's synthesizing such active compounds apart from the algal association. Thomas (*2*) isolated many lichen fungi, several of which produced lichen acids. Castle and Kubsch (*3*) showed the presence of several lichen acids in a culture of the mycobiont of *Cladonia cristatella*. The activity of these acids was not tested. Zehnder (*4*) demonstrated a strong inhibition of the mold *Penicillium* sp. by the mycobiont of *Lecanora subfusca*; he did not describe the active compound.

In our investigation, 11 pure cultures of mycobionts were tested for active compounds. The mycobionts were separated from the following lichens: *Acarospora fuscata, A. smaragdula, Baeomyces roseus, Cladonia cristatella, C. pleurota, C. subcariosa, Graphis* sp., *Lecanora chlarotera, Physcia stellaris, Sarcogyne simplex*, and *Stereocaulon dactylophyllum* var. *flabellatum*. The mycobionts were isolated by means of the cultivation of spores ejaculated onto the surface of a soil-extract agar medium (*5*). Small portions of the agar which were free from contamination and showed a high percentage of germinating spores were then transferred to tubes which contained a malt extract and yeast extract agar (*p*H 5.8).

After 3 to 4 months of growth in complete darkness or low light intensity (20 to 25 ft-ca) at 17° to 18°C, the fungal colonies assumed various shapes, sizes, and pigmentation, each species passing through an initial white mycelial stage. In mycobionts *Acarospora fuscata* and *A. smaragdula* a bright red, water soluble pigment, which colored both the colony and surrounding medium, was produced by the hyphal cells. This single pigment showed a high degree of variation in color, ranging from bright red and purple to yellow and brown; the color changes and the amount of pigment produced appeared to be influenced by variations of temperature, *p*H, light intensity and carbon dioxide concentration. In *Cladonia cristatella, C. pleurota, C. subcariosa*, and *Stereocaulon dactylophyllum* var. *flabellatum*, the maturing fungal colonies changed color from white to pure yellow to red to reddish-brown or dark brown. Conditions and compounds which caused the color changes of these mycobionts were not investigated. In *Cladonia pleurota* a bluish pigment also developed in some parts of the colony. The mycobiont of *Baeomyces roseus* produced an orange pigment, and the hyphal cells were filled with abundant oil droplets. The mycobiont of *Lecanora chlarotera* was bright yellow in color and produced a water-soluble, dark brown pigment.

Acetone extracts of the fungal colonies, picked directly from tubes of a malt extract and yeast extract agar, were assayed with standard paper disk-agar plate techniques. Test organisms were selected strains of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Aspergillus niger*, and *Penicillium chrysogenum*.

Results of the assay tests can be summarized as follows: Extracts of *Acarospora fuscata, A. smaragdula*, and *Cladonia cristatella* produced definite zones of inhibition against *Bacillus subtilis* and *Staphylococcus aureus*, the degree of inhibition being greater against *Bacillus subtilis*. The extract of *Acarospora smaragdula* was alone in inhibiting the two test molds. Acetone extracts of the remaining mycobionts showed no activity against the test organisms. None of the extracts showed activity against *Escherichia coli*.

The mycobiont of *Acarospora smaragdula* seemed to produce three types of compounds. During its initial growth phase, the fungus excreted large quantities of the water soluble, red-yellow-brown pigment mentioned above; this pigment was formed both on solid and in liquid media. The pigment showed strong inhibition of the two test fungi, no inhibition of the test bacteria, but an enhancement of growth of *Bacillus subtilis*. In later growth stages of the mycobiont, abundant prismatic plate-like crystals were found in the regions of agar where the pigment had diffused and also on the hyphal cells. These crystals were insoluble in water and acetone and found only on solid media. In older cultures of the mycobiont, bright yellow, plate-shaped structures appeared directly on the fungal colony and also on the agar surface in areas of the greatest pigment concentration. These yellow structures, which were produced only on solid media and which were readily soluble in acetone and insoluble in water, appeared amorphous under microscopic examination but were easily crystallized by the method of Asahina (*6*). Disks soaked in an acetone solution of such structures and then air dried to remove the solvent showed strong inhibition of *Bacillus subtilis, Staphylococcus aureus, Streptococcus fecalis, Aspergillus niger*, and *Penicillium chrysogenum*, with no activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Evidence obtained from crystallization, chromatography, color reactions, and absorption spectra showed a similarity between this compound and a sample of known usnic acid produced commercially by Nutritional Biochemicals Corporation.

Study of a few lichen fungi has given several interesting and fruitful results. If one considers the fact that some 15,000 unique and diverse lichen species occur in nature, the research possibilities on the physiology and chemistry of the mycobionts alone seem limitless. (*7*).

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