# Symposium on Bioluminescence in the Pacific Area of the 11th Pacific Science Congress

August 27, Saturday 1966 Chairman: Prof. Dr. Frederick I. TSUJI
Convener: Dr. Yata HANEDA

The emission of light without perceptible heat occurs in a great variety of living organisms.

Numerous aspects of this phenomenon known as bioluminescence still remain unknown.

This is especially true in the Pacific Area where many kinds of luminous organisms occur.

The synopsium on Bioluminescence in the Pacific Area of the 11th Pacific Science Congress, held at Tokyo University August 27, 1966 was quite successful, even though only one year had passed since the Conference on Luminescence held in Hakone National Park 12—16 September 1965 under the auspices of the U.S.-Japan Cooperative Science Program.

Ten papers were submitted to the symposium and the participants from abroad were the following. Dr. Frederick I. TSUJI, Professor of Pittsburgh University, Pittsburgh; Dr. Andrew THANOS of the Life Sciences, North American Aviation Inc., Los Angeles; Dr. D. E. MCALLISTER, curator of the National Museum of Canada, Ottawa, Canada; and Dr. I. V. POLUNIN, Faculty of Medicine, University of Singapore, Singapore. The Japanese participants were Dr. Yo K. OKADA, Director of the National Science Museum, Tokyo; Dr. Noboru SUGIYAMA, Professor of Chemistry, Tokyo Kyoiku University, Tokyo; Dr. Yoshimasa HIRATA, Professor of Chemistry, Nagoya University; Dr. Toshio GOTO, Professor of Chemistry, Nagoya University; Dr. Kazuo HORI of the Department of Chemistry, Tokyo Kyoiku University, Tokyo and Dr. Yata HANEDA, Director of the Yokosuka City Musium, Yokosuka, Japan.

## 1. The Significance of ventral bioluminescence in fishes[1] (Abstract)

D. E. MCALLISTER
Curator of Fishes, National Museum of Canada, Ottawa

The occurrence of complicated light producing organs in fishes has aroused great interest in biologists. Few have noticed and none have documented that these luminous organs and tissues are predominantly on the ventral surface of the fish. The present study analyzes the positioning of bioluminescence in fishes. Of the luminescent taxa at least two-thirds of the families, three-quarters of the species and four-fifths of the individuals bear their luminous organs or tissues wholly or predominantly on the underside.

[1] This paper is published in full in the Journal of the Fisheries Research Board of Canada 24 (3):...

Furthermore pigment, reflectors, and lenses assist in some groups in directing the light downwards.

A review of the specific functions attributed to photophores indicates that they are not involved in the overall selection for ventral light emission. Two general theories previously suggested to explain ventral lighting do not appear able to explain all cases. A third theory is developed. This suggests that light is emitted ventrally (for whatever special functions are involved) because this exposes them to less predation, there being more predators above than below. A further special function is suggested, to help evenly space-out individuals of a species so as to utilize more fully food or other resources. Certain counter-strategies are also discussed, demonstrating how fishes have dealt with problems created by bioluminescence.

## 2. Instrumental Bioluminescence Measurements in the Tropical Part of the Pacific[1]

I. I. GITELSON, R. I. CHUMAKOVA, V. S. FILIMONOV,
A. S. ARTEMKIN and V. F. SCHATOKHIN
(Academy of Science of the USSR)

Thoughts expressed by N. I. TARASOV and his enthusiasm played a significant role in carrying out this investigation.
This work is dedicated to the memory of N. I. TARASOV.

In 1961–1965 during an oceanographic cruise abroad the research vessel "Vitjaz" bioluminescence measurements were carried out in the central part of the Pacific.

With this aim a variant of a bathyphotometere has been designed; the instrument consists of two parts, a submerged one and above-water, connected by a cable line.

A perceptible bathyphotometre element is a photomultiplier, fed by means of a battery of silver-zink accumulators through a tension transformer 8/16000V, assembled on transistors.

The signal is sent via cable line on board the vessel and is registered by a lop-oscillograph. It is quite possible to watch the signal directly on the screen of a low frequency oscillograph too. The instrument is switched on and off from the distance by sending impulses of contrary polarity from board the vessel. The instrument is set in a cylindrical steel housing which can be submerged to 6000 m. In the front part of the housing is an illuminator made of organic glass.

The working position of the instrument is vertical, with the illuminator down; the instrument is submerged on a cable line with the help of a winch.

The calibrating of the instrument was carried out in laboratory conditions through a passport system source, its brightness being $6.1 \cdot 19^{-9}$ wt/sm$^2$ with 0.19 sm$^2$ of space. The instrument sensitivity is expressed in mkwt/sm$^2$ on a millimetre of the deviation of

[1] Presented at the Symposium on Bioluminescence in the Pacific Area, Eleventh Pacific Science Congress, August 27 to September 10, 1966 Tokyo, Japan.

the light trace of the train. Control over the process of work is done by registering a signal from the secondary photometric standard of phosphore irradiated by isotope of Tl before or after each submersion of the instrument. The estimate has shown that the instrument secures a registration of flashes with $8.7 \cdot 10^{-4}$ to $9.6 \cdot 10^{2}$ mkwt/sm$^2$ of brightness.

As laboratory experiments have shown such brightness secures the reception of signals of the intensity something like $10^{-3}$ mkwt from the cone of view, which covers the volume of abour 0.5 m$^3$ in front of the instrument illuminator.

It is necessary to note that J. CLARKE's description of the intensity of flashes in brightness units is conditional in such environment, since, while using the instrument on the sea, the space of a luminous object and the distance between the object and the receiver remains unknown. That is why the estimated value is understood as a "reduced brightness" and only means that is a luminous object were in the position of the photometric standard and had equal space with it then their brightness would correlate as the values of signals registered.

Signals quality is demonstrated in a sample of a record done in the depth of 115 m at the 40 m/sec speed of the time-scale (Table 1).

The oceanographic cruise in the Pacific was worked out so as to cross four times the areas of the northern and southern trade-wind streams and the equatorial anti-stream with their zones of maximum and minimum concentrations of plankton. The trip covered the central area of the ocean between 140°30′ W. long., 18° S. lat.—16° N. lat. Maximum depth observed was 2107 m.

At every station the bathyphotometre was submerged at several layers (from 3 to 18) and remained in each of them for 3 minutes. A sample of an entry-bathyphotogramme is shown in Table 1.

Bioluminescent flashes are registered in the form of impulses, towering over the constant background whose level is determined by the pehetration of astronomic light up to the layer under observation.

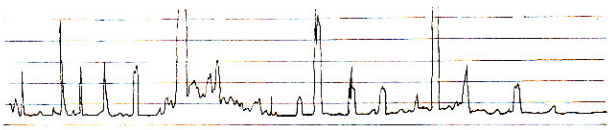


Table 1. A part of a bathyphotogramme, registered at station 5161, in 10°30′ N.lat., 160°00′ E.long. in December 3, 1961, at 7 hr 02 m～ 20 hr 05 m; 115 m depth, the time-scale was 40 mm/sec.

The most general result of the bathyphotometric observation of the central part of the central part of the Pacific is the detection of bioluminescence in the whole area under observation. Only in three layers out of 239 under observation a single impulse was not registered during three minutes standard period of observation, and all the three cases refer to the layers lower than 1500 m. In the rest of the layers impulses were registered with the frequency of some dozens to 500 in 1 m$^3$ per minute. In addition their intensity may excel the background in two and more orders.

A bathyphotogramme enables to characterize the bioluminescence by qualitative indices. Each separate flash can be characterized as follows: 1) by a relative (minimum) intensity; 2) by its duration; 3) by the impulse form.

The light regime of the water volume under observation is characterized: 1) by the level of a constant light background; 2) by the exceeding of bioluminescent signals over the background; 3) by a concentration of objects capable of luminescence; 4) by the distribution of signals according to their amplitude and duration; 5) by their distribution in time (by the degree of heterogenity).

## Intensity

The instrument ensured the measurement of flashes with $8.7 \cdot 10^{-4}$ to $9.6 \cdot 10^{-2}$ mkwt intensity. Since a bathyphotometre does not allow to determine the distance to an object, the given above signal values may be accepted only as minimal.

According to our data single flashes last to 0.4 sec; we have come across flashes with maximum frequency of 0.05~0.15 sec.

Summary duration of luminescence, which is twice larger than the background at station 5161 (160°E. long. and 10°30'N. lat.) relatively poor in plankton (0.013 $sm^3/m^3$) was, for example, 18% from the whole period of observation; at station 5157, richer in plankton (0.05 $sm^3/m^3$), situated at the same longitude, only 5° to the South, the summary duration of luminiscence was 53%, i.d. it exceeded the time of the light absence.

Signal forms are quite various and so far do not lend themselves to a certain classification. As a common rule, we may mention the steepness of fronts of the growth and slump of impulses, the absence of a flat peak, i.d. the period of a stable level of signals. It is necessary to have a receiving apparatus adequate in frequency to transmit these peculiarities.

The signal form data bears a purely illustrative character so far, they display a possibility of registering the differences in species and this enables us to suggest that they can serve as a means of recognition signals between members of species. The description of bioluminescence signals is only primary. It is absolutely evident that the quantity of the information enclosed is much larger and the aim of our work in the future is to master its extracion from this new source.

As an impulse characterizes a single flash, a bathyphotogramme as a whole characterizes the light regime of a water layer under observation.

While analyzing a bathyphotogramme the first task is to divide astronomic and biological luminescence. Such a division is quite possible due to the fact that astronomic luminescence gives a permanent signal, while bioluminescence displays itself in the form of separate impulses in this permanent background.

According to the ratio of astronomic and biological luminescence three zones may be pointed out:

1) 0~200 m—the zones of a day-sun and mixed night (astronomic and bioluminescent) illumination;

2) 2000~7000 m—the zone of a day-sun and night bioluminescent illumination;

3) deeper than 7000 m—the zones of a bioluminescence only; the value of these confines requires a more precise definition and division into districts.

In order to define the bioluminescence concentration in a layer all the bathyphotogrammes were subjected to a similar processing. The signals were divided into six classes according to their amplitude. The bathyphotogramme ordinate was uniformly divided into 5 classes (20% in each classes) and all the impulses which were out of the instrument scale referred to the sixth class. For each layer the number of impulses during the standard time of observation (3 min.) was estimated. Knowing the active instrument volume, the result may be expressed in the number of impulses at 1 $m^3$ of water per minute. The values achieved at different stations and depths varied 0~525 imp/$m^3$ min.

## Vertical Bioluminescence Distribution

Table 2 shows a typical way of vertical bioluminescent distribution.

The greatest luminescence is registered, as a rule, not on the surface, but in the zone of 50~200 m. Luminescence in this layer is 0.3~7.5 larger than that on the water

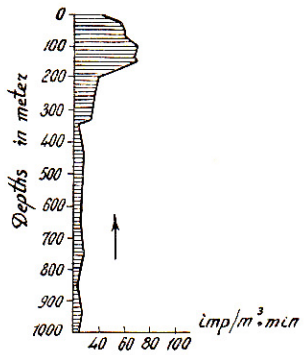



Table 2. Intensity of Luminescence

Vertical bioluminescence distribution, station 5109, coordinates: 10°12′ S.lat., 148°43′ W.long. 18 hr 30 min~21 hr 00 min, the moon in its last quarter; sparse clouds; roughness force 5; wind force −9.3m/sec, pressure 760.4 mm; air temperature +23.9°, water t. +25.2°.

The depth of a bathyphotometre submersion is plotted on a vertical line, the number of impulse at 1 $m^3$ min is plotted on a horizontal line; a vertical arrow points the direction of a bathyphotometre movement between measurements.

surface. In deeper zones (25~300 m) luminous organisms concentration sharply decreases and then diminishes gradually, but without reducing to zero even at the maximum depth of 2107 m, achieved in this cruise.

Besides the main, upper, bioluminescent layer, some deeper luminescence layers were detected, but they were less intensive. The intensity of secondary luminescent layers was from 0.15 to 0.13 of a primary maximum. In this respect our result is closer to the data given by J. CLARKE (CLARKE, HUBHARD, 1955), who detected a secondary increase in bioluminescence at the depth of 900 m and to the data of BODEN and KAMPA (1957), who registered a luminescence increaes in a migrating sonic-scattering layer.

Speaking of a vertical bioluminescence distribution we must lay a particular stress in the fact that the data about the number of light impulses given above do not mean that in an unagitated state in the water under observation there exists a similar light regime. Most likely, in calm water, not agitated by the movements of a control instrument, the number of flashes is smaller, but not equal to zero. The agitation, created by mechanical, chemical and, quite probably, by electromagnetic irritants, causes luminescence. Not only our instrument, but a great number of other natural phenomena may be an trritant like that.

Therefore quantitative data given above should be treated not as a characteristics of a light regime in an unagitated layer, but only as as estimation of a potential capacity for luminescence in it which realizes when agitated.

## Physiological luminescent Rhythm

The most common luminescent from in the surface layers of the water under observation was dinoflagellate *Pyrocystis pseudonoctiluca*. In order to investigate a day-night rhythm of their luminescence different samples were taken, consisting nearly exclusively of *P. pseudonoctiluca*; they were taken at stations 5139, 5141 from the depth of 0~30 m. The samples were diluted in 81 of sea water in a polyethylene aquarium, which was cooled from the outside by a continuous stream of the overboard water. The aquarium was kept on deck the vessel in natural light conditions. Luminescence was registered on a flowing filtrephotometre.

The tests gave the same result; *P. pseudonoctiluca* gave a light only in the dark and stops its luminescence when it is light. Our data confirmed that *P. pseudonoctiluca* possesses a day-night periodicity, as all other dinophlagellates (SWEENEY, HASTINGS, 1957).

It was of great importance to find out wheter the *Pyrocystis* luminescence rhythm was endogenous or is caused directly by outer light. With this aim a number of tests was carried out with samples taken at stations 5153 and 5159. Immediately after they had been taken all the samples were divided into two groups: the first one was put into a streamed aquarium by natural light, the second one was inder the same conditions but without light.

The tests showed that *Pyrocystis* luminescence directly depends on the outer light regime and is independent of the time of the day. In this respect out result does not coincide with the data given by SWEENEY and HASTINGS (1957) for a relative species of dinophlagellate *Gonyaulax*, where endogenous diurnal rhythm was described.

## Conclusions

1. Apparatus was designed and methods for bathyphotometric analyses were worked out. Bioluminescence measurements were carried at the depth of 2000 m.

2. In the Pacific areas, covered by the 34th and 38th cruise on RV the "Vitjaz", luminescence was detected everywhere. It varies in its intensity and from depending on the depth, time of the day, quantity and structure of bioluminescents, and other factors not yet investigated.

3. Phytoplankton (*Pyrocystis pseudonocitluca*) plays an important role in forming a luminescence layer within 0~200 m in the analyzed area.

4. Bioluminescence intensity has a tendency of increasing in the areas rich in plankton, but it does not depend directly on its quantity.

5. Bioluminescence has a vertical stratification. At night time a maximum bioluminescent concentration was registered at the depth of 50~200 m. In deeper layers bioluminescent intensity decreases, without reducing to zero at the maximum depth of 2107 m. Secondary luminescent layers were registered at the depths of 400 to 940 m.

6. Sea bioluminescence has an accurately expressed diurnal rhythm. Two gears of this rhythm were noticed:

a) luminescence of a non-migrating phytoplankter (*Pyrocystis*) stops in the daytime; the main rhythm factor is not astronomical time, but a direct inhibiting effect of outer light on luminescence;

b) a number of luminescent zooplanktons migrate to the depth in the daytime, and this fact causes a dispersion of a luminescent layer.

7. Regular detection of bioluminescence in the considerable aquatorium of the Pacific and the Indian Ocean along with the data of J. CLARKE, BODEN and KAMPA on the Pacific, the Atlantic Ocean and the Mediterranean sea allown to offer a suggestion that permanent bioluminescence is appropriate to the major part of the World Ocean and requires a study as a permanently acting specific factor of sea biology.

## References

BODEN B. F., KAMPA E. M. 1959. Pacific Sci., 2.
CLARKE G., WERTHEIM G. 1956. Deep Sea Res., 3, 189.
CLARKE J., BRESLAY L. 1959. Bull. Inst. Oceanogr. Monaco, N 1147.
CLARKE J., HUBBARD Ch. 1959. Limnol. and Oceanogr., 4 (2), 163.
CLARKE J., BRESLAU L., 1960. Bull. Inst. Oceanogr. Monaco, N 1171.
FILIMONOV V. S., ARTEMKIN A. S., GITELSON I. I. Apparatus Complex for bioluminescence investigations in sea cruise. Coll. Biogenetics and biological spectrophotometry. "Science", Moscow, 1967.
GITELSON I. I., R. I. CHUMAKOVA, O. G. BAKLANOV, A. S. ARTEMKIN, V. F. SCHATOKHIN. Sea Bioluminescence. Theses XI All-Union Lumin-Congress, 1962, Ac. Sci. USSR, Minsk.
GITELSON I. I., CHUMAKOVA R. I., FISH A. M. Bioluminescence as Sourse of Biological Process Informatian. Coll. Bionics. Ac. Sci. USSR, 1965, pp. 314–323.
SWEENEY B., HASTINGS I., 1957. J. Cell. Comp. Physiol., 49, 115.
TARASOV N. I. 1956. Sea Luminescence. Moscow, Ac. of Sc. USSR.

## 3. Luminescence in the Pacific fishes, *Apogon ellioti* and *Parapriacanthus ransonneti*[1]

F. I. TSUJI and Y. HANEDA

Department of Biophysics, University of Pittsburgh, and Veterans Administration Hospital, Pittsburgh, Pensylvania, U.S.A. and Yokosuka City Museum, Yokosuka, Japan

The blue light emitted by the Japanese shallow water fishes, *Apogon ellioti* and *Parapriacanthus ransonneti*, originates from two sets of ventral light organs (thoracic and anal luminous ducts) and is due to a classical luciferin-luciferase reaction (HANEDA and JOHNSON, 1958; HANEDA, JOHNSON, and SIE, 1958; and HANEDA, JOHNSON, and SIE, 1959). The luciferin and luciferase prepared from the photogenic organs of *Apogon* and *Parapriacanthus* have also been shown to cross react with each other and with the luciferin and luciferase of the marine ostracod crustacean, *Cypridina hilgendorfii* (JOHNSON, HANEDA, and SIE, 1960; and JOHNSON, SUGIYAMA, SHIMOMURA, SAIGA, and HANEDA, 1961). Both *Apogon* and *Parapriacanthus* belong to different taxonomic families and *Cypridina* to another phylum, but they are distributed in the same geographic area. Anatomical studies indicate that the thoracic luminous ducts are connected to the pyloric caeca in *Apogon* and *Parapriacanthus*, whereas the anal luminous duct is joined to the rectum in *Apogon* and lies alongside the rectum in *Parapriacanthus* (without apparent anatomical connection) (HANEDA and JOHNSON, 1962). Crystalline luciferin prepared from the pyloric caeca of *Parapriacanthus* has been shown to possess properties virtually identical with that of crystalline *Cypridina* luciferin (JOHNSON, SUGIYAMA, SHIMOMURA, SAIGA, and HANEDA, 1961). The similarities include absorption spectra, melting point, specific luminescence activity per unit amount of *Cypridina* luciferase, a positive SAKAGUCHI reaction, and paper chromatograms of the whole molecules and products derived by acid and alkaline hydrolysis of the molecules. A highly purified preparation of *Apogon* luciferin has also been shown to possess an absorption spectrum identical to that of crystalline *Cypridina* luciferin. The bioluminescence emission spectra of *Apogon* and *Cypridina* luciferins with either of the luciferases have also been found to be practically identical (SIE, MCELROY, JOHNSON, and HANEDA, 1961). Because of the foregoing evidence, it has been suggested that the luciferins of *Apogon* and *Parapriacanthus* are derived from the ingestion of *Cypridina* organisms and that the pyloric caeca act as the storage organ for luciferin (HANEDA and JOHNSON, 1962; and HANEDA, JOHNSON, and SHIMOMURA, 1966).

Although *Apogon*, *Parapriacanthus*, and *Cypridina* luciferins appear to be the same compound, the origin and identity of the luciferases are less certain. Recent chromatographic, immunologic, and kinetic studies have shown that *Apogon* luciferase differs from *Cypridina* luciferase in a number of respects (TSUJI and HANEDA, 1966). The most pronounced differences are observed with reference to chromatographic and immunologic properties and details of these differences are described in the present report. The preliminary purification of *Parapriacanthus* luciferase is also presented.

[1] Presented at the Symposium on Bioluminescence in the Pacific Area, Eleventh Pacific Science Congress, August 21 to September 10, 1966, Tokyo, Japan.

## Materials and Methods

*Apogon* luciferase. Except for fresh *Apogon* luciferase and *Cypridina* luciferase, all of the materials and procedures employed in the study have been previously described (TSUJI and HANEDA, 1966). For purposes of comparison, *Apogon* luciferase was also purified from 50 thoracic light organs of *Apogon* specimens frozen in dry ice, using the same procedure as that previously used for 100 dessicated organs. A white powder weighing 34 mg. was obtained by purifying on a short Sephadex G-200 column (2.5×45 cm.). The yield was considerably higher than the 5-10 mg. previously reported for 100 dessicated light organs. *Cypridina* luciferase was isolated from 100 mg. of dried, ground *Cypridina* organisms (not previously extracted with either benzene or ether) with 400 ml. of water, dialyzed against water, and freeze-dried. The freeze-dried powder was dissolved in a small volume of 0.05 M sodium phosphate buffer, pH 6.8, and chromatographed on a short Sephadex G-200 column (2.5×45 cm.), using the same buffer and conditions as employed for *Apogon* luciferase purification. Active fractions were combined, dialyed against distilled water, and freeze-dried. *Cypridina* luciferin was used throughout the study as the substrate. The light intensity of the luminescent reaction was measured with a specially designed photomultiplier photometer. The antibodies were isolated as the 7S $\gamma$-globulin fraction from the antisera of rabbits immunized against luciferase by the method of electrophoresis-convenction. About 12 ml. of antiserum was diluted with an equal volume of 0.07 M sodium phosphate buffer, pH 7.5, and the electrophoresis-convection was run at 45 v., 0.75 amp., for 24 hr. at 3°C. The electrophoretically pure top solution from the channel of the apparatus was used. The top solution containing antibody to *Cypridina* luciferase was thoroughly dialyzed against distilled water and freeze-dried. A white powder was obtained that was highly effective in inactivating *Cypridina* luciferase. In the case of antibody to *Apogon* luciferase, the top solution of the channel was used directly.

*Parapriacanthus* luciferase. A preliminary attempt was also made to purify the enzyme from whole, excised sections of pelvic tissue containing the thoracic light organs. About 100 dried sections were ground with a mortar and pestle and the powder was suspended in 60 ml. of 0.07 M sodium phosphate buffer, pH 6.8. Toluene was added as a preservative. The mixture was then dialyzed against many changes of the phosphate buffer at 4°C. When the suspension was initially examined in the dark, luminescence was readily visible. After 3.5 days of dialysis, the extract was sedimented at 105,000×g for 1 hr. One ml. of the supernatant when tested against *Cypridina* luciferin gave a readily visible luminescence. The supernatant was further dialyzed overnight against a large volume of 0.07 M sodium phosphate buffer, pH 6.8, at 4°C. The supernatant was then chromatographed on a short Sephadex G-200 column (2.5×45 cm.), using 0.07 M sodium phosphate buffer, pH 6.8, as eluant. The collected fractions were assayed (0.2 ml. aliquot) for luciferase activity with *Cypridina* luciferin, as previously done for *Apogon* luciferase (TSUJI and HANEDA, 1966). The absorbancy of each fraction was determined with a BECKMAN DU spectrophotometer. The fractions showing luciferase activity were combined, dialyzed against distilled water, and freeze-dried. A few mg. of a light, brown powder was obtained.

## Results and Discussion

*Apogon* luciferase. In the previous paper (TSUJI and HANEDA, 1966), it was shown that when partially purified *Apogon* luciferase (from a short Sephadex G-200 column) is chromatographed on a long Sephadex G-200 column (2.5×90 cm.), the result is a main activity peak and a small activity peak or shoulder. However, when *Cypridina* luciferase is chromatographed, only a single activity peak is observed. The position of the small *Apogon* peak or shoulder corresponds almost exactly with the position of the *Cypridina* peak, but the main *Apogon* peak is eluted ahead of either peak. Since it was thought possible that Millipore filtration may be responsible for the formation of the component in the main peak, e.g., by some process of surface denaturation-aggregation reaction, *Apogon* luciferase was also prepared by omitting the Millipore filtration. Three different lots of *Apogon* luciferase prepared in this manner were examined in the long Sephadex

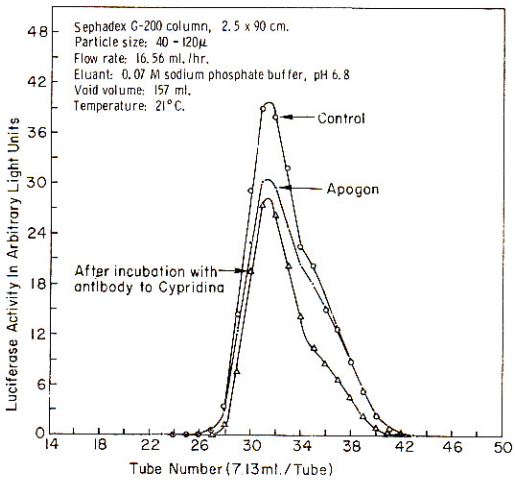
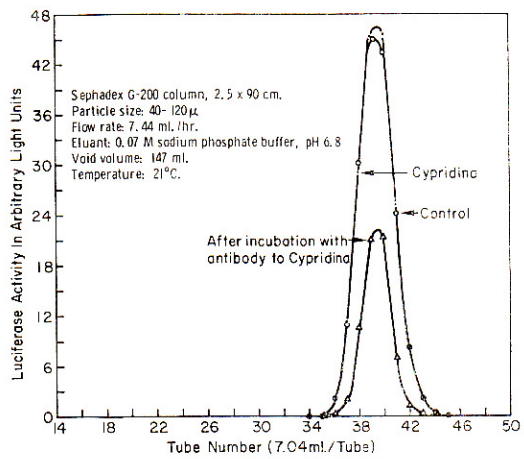



Fig. 1.




2ig. 2.

Fig. 1. Elution pattern of *Apogon* luciferase (prepared from *Apogon* specimens preserved in dry ice) on long Sephadex G-200 column and subsequent changes in activity, in presence and absence of antibody to *Cypridina* luciferase. Twenty-eight mg. of partially purified luciferase dissolved in 2.5 ml. of 0.07 M sodium phosphate buffer, pH 6.8, was placed on column. Composition of assay mixture: *Apogon*, 2.0 ml. of fraction+1.0 ml. of isotonic saline+7.0 ml. of buffer; *Apogon*+antibody, 2.0 ml of fraction+1.0 ml. of antibody solution+7.0 ml. of buffer; and *Apogon* control, 2.0 ml. of fraction+1.0 ml. of isotonic saline+7.0 ml. of buffer. Buffer=sodium phosphate, 0.07 M, pH 6.8. Concentration of antibody (rabbit #101), 0.20 mg. $\gamma$-globulin/ml. (in isotonic saline). Incubation time, 4.5 hr. for latter two curves. Substrate, *Cypridina* luciferin.

Fig. 2. Elution pattern of *Cypridina* luciferase on long Sephadex G-200 column and subsequent changes in activity, in presence and absence of antibody to *Cypridina* luciferase. Amount of luciferase placed on column: $4.5\times10^{-2}$ mg. dissolved in 2.5 ml. of 0.07 M sodium phosphate buffer, pH 6.8. Composition of assay mixture: *Cypridina*, 2.0 ml. of fraction+1.0 ml. of isotonic saline+7.0 ml. of buffer; *Cypridina*+antibody, 20 ml. of fraction+1.0 ml. of antibody solution+7.0 ml. of buffer; and *Cypridina* control, 2.0 ml. of fraction+1.0 ml. of isotonic saline+7.0 ml. of buffer. Buffer=sodium phosphate, 0.07 M, pH 6.8 Concentration on antibody (rabbit #101), 0.20 mg. $\gamma$-globulin/ml. (in isotonic saline). Incubation time, 4.5 hr. for latter two curves. Substrate, *Cypridina* luciferin.

G-200 column and they all showed elution diagram no different from those obtained by filtration.

The result of using dried instead of fresh *Apogon* light organs for luciferase purification was also investigated by repeating the chromatography using luciferase purified from light organs of *Apogon* specimens freshly preserved in dry ice. Normally, the excised organs are thoroughly dried and stored in a dessicator before being used. The data for the experiment are presented in Fig. 1. The chromatography was carried out overnight and the luciferase activity of each of the collected fractions was determined the following morning on a 2.0 ml. aliquot. Another 2.0 ml. aliquot was incubated for 4.5 hr. with 1.0 ml. of antibody solution and 7.0 ml. of phosphate buffer. A third aliquot served as a control for estimating any changes in luciferase activity during the 4.5 hr. incubation period. The *Apogon* curve is seen to exhibit a main peak and a distinct shoulder, characteristic of luciferase isolated from dessicated organs. Drying the organs, therefore, does not appear to influence the chromatographic behavior of *Apogon* luciferase. Both the main peak and shoulder are seen to be sensitive to inhibition by antibody against *Cypridina* luciferase. When luciferase was allowed to stand in phosphate buffer, there was also a gradual increase in enzyme activity. The greatest increase ia activity appeared to occur beneath the main peak, whereas only a small rise in activity occurred with the shoulder. Because of the gradual increase in luciferase activity on standing and the incomplete resolution of *Apogon* luciferase into two peaks, it was difficult to calculate the relative sensitivities of the two luciferase to inhibition by *Cypridina* luciferase antibody.

*Cypridina* luciferase, when chromatographed overnight and followed for activity changes under the same conditions as *Apogon* luciferase, gave results that were different from *Apogon* in several respects. A single homogenous elution peak was obtained, with its position shifted to the right of the main *Apogon* peak (Fig. 2). Incubation of aliquots from each fraction with antibody to *Cypridina* luciferase for 4.5 hr. resulted in a loss of activity relatively greater than in the case of *Apogon* luciferase. Moreover, there was only a slight decrease in activity in the *Cypridina* luciferase control that was allowed to stand in phosphate buffer for 4.5 hr. In contrast, Fig. 1 shows that *Apogon* luciferase gradually increases in activity on standing in phosphate buffer.

Fig. 3 shows the result of chromatographing a mixture of *Apogon* luciferase (prepared from dessicated organs) and *Cypridina* luciferase on a long Sephadex G-200 column under the same conditions as before. Measurements for luciferase activity were carried out on the collected fractions after overnight chromatography and subsequent standing in phosphate buffer, in the presence and absence of antibody to *Cypridina* luciferase. The plotted curves indicate that *Cypridina* luciferase and the shoulder formed by *Apogon* luciferase occupy virtually the same position in the elution diagram. The increase in luciferase activity that occurred on standing in phosphate buffer is relatively greater for the main peak than the shoulder and confirms the results previously presented in Fig. 1. The exact mechanism for the increase in luciferase activity is not clear, but the changes may possibly involve a gradual release of the enzyme from the action of an inhibitor.

Data on the cross-reaction of *Apogon* and *Cypridina* luciferase with antibodies against the two luciferases are given in Table 1. *Apogon* luciferase and *Cypridina* luciferase were found to be inhibited by both antibodies but each enzyme was found to be more sensitive to inhibition by its own antibody than the other. Although the *Apogon* luciferase

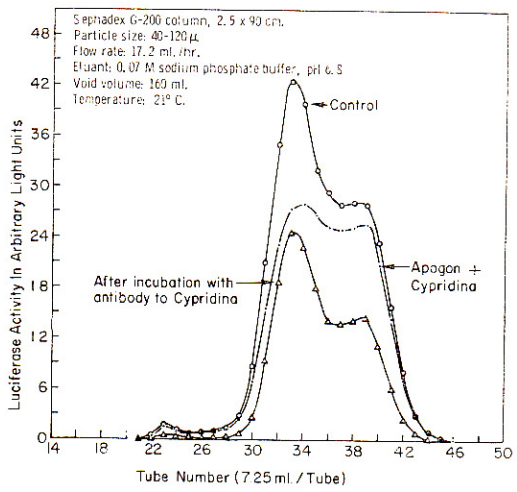



Fig. 3. Elution pattern of mixture of *Apogon* luciferase and *Cypridina* luciferase on long Sephadex G-200 column and subsequent changes in activity, in presence and absence of antibody to *Cypridina* luciferase. Amount of luciferase placed on column: $2.25\times10^{-2}$ mg. of *Cypridina* luciferase+6.55 mg. of partially purified *Apogon* luciferase, dissolved in 2.62 ml. of 0.07 M sodium phosphate buffer, pH 6.8. Composition of assay mixture: *Apogon*+*Cypridina*, 2.0 ml. of fraction+1.0 ml. of isotonic saline+7.0 ml. of buffer; *Apogon*+*Cypridina*+antibody, 2.0 ml. of fraction+1.0 ml. of antibody solution +7.0 ml. of buffer; and *Apogon*+*Cypridina* control, 2.0 ml. of fraction+1.0 ml. of isotonic saline+7.0 ml. of buffer. Buffer=sodium phosphate, 0.07 M, pH 6.8. Concentration of antibody (rabbit #101), 0.20 mg. γ-gloublin/ml. (in isotonic saline). Incubation time, 4.5 hr. for latter two curves. Substrate, *Cypridina* luciferin.

Table 1. Cross-inhibition *Apogon* and *Cypridina* luciferases by Rabbit antibody (γ-globulin)

| Antibody added[1] | *Apogon* | | *Cypridina* | |
|---|---|---|---|---|
| | Observed initial light intensity | %Control | Observed initial light intensity | %Control |
| Luciferase+0 ml. antibody solution | 28.6[2] | 100 | 57.3[3] | 100 |
| Luciferase+1.0 ml. anti-*Apogon*[4] | 4.3 | 15.0 | 33.2 | 57.9 |
| Luciferase+1.0 ml. anti-Cypridina[5] | 20.5 | 71.7 | 18.3 | 31.9 |

1. Incubated for 3 hr. 40 min. at 21°C,
2. 1.0 ml. of *Apogon* luciferase solution (1.26 mg. luciferase/ml. of 0.07 M sodium phoshate buffer, pH 6.8)+9.0 ml. of 0.07 M sodium phosphate buffer, pH 6.8. *Apogon* luciferase was purified from specimens frozen in dry ice, using short Sephadex G-200 column.
3. 1.0 ml. of *Cypridina* luciferase solution ($1.5\times10^{-3}$ mg./ml. of 0.07 M sodium phosphate buffer p.H 6.8) +9.0 ml. of 0.07 M sodium phosphate buffer, pH 6.8.
4. 1.0 ml. of γ-globulin solution (rabbit #575) from top of electrophoresis-convection cell+1.0 ml. of luciferase solution+8.0 ml. of 0.07 M sodium phosphate buffer, pH 6.8.
5. 1.0 ml. of γ-globulin solution (rabbit #101; 0.20 mg./ml. of 0.07 M sodium phosphate buffer, pH 6.8) +1.0 ml. of luciferase solution+8.0 ml. of 0.07 M sodium phoshate buffer, pH 6.8.

employed was purified from specimens frozen in dry ice, similar results were also obtained using luciferase isolated from dessicated light organs.

*Parapriacanthus* luciferase. Fig. 4 represents the result of chromatographing an extract of dried *Parapriacanthus* light organs, including the surrounding excised tissue,

on a short Sephadex G-200 column. Luciferase activity is seen to be confined to fractions 17–31. The collected fractions were turbid (milky) between 11–12, brown between 12–40, and most intense brown between 32–36. The activity and absorption curves do not appear to have any resemblance to the comparable *Apogon* curves (TSUJI and HANEDA, 1966). The activity curve, being assayed under different conditions, is not comparable to the activity curves for *Apogon* luciferase in Figs. 1 and 3. However, a rough calculation shows that the amount of activity present in 100 *Parapriacanthus* light organs is

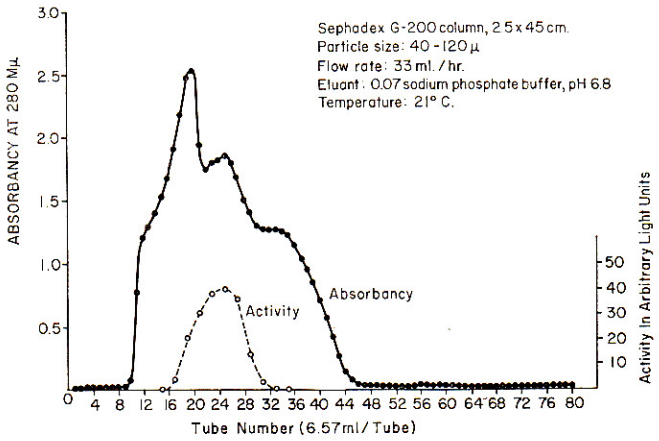



Fig. 4. Elution pattern of extract of *Parapriacanthus* light organs on Sephadex G-200 column.

far below that found in an equal number of *Apogon* organs. The low activity in *Parapriacanthus* seems to suggest low concentration in the luminous tissue, poor extraction, or deterioration of the enzyme during collection of the specimen and purification. Attempts to immunize rabbits with both crude extract and partially purified luciferase were not successful, probably due to low concentration of luciferase.

**Acknowledgement.** The authors wish to express their appreciation to Mr. David H. DONALD for his valuable technical assistance. Support is also gratefully acknowledged from the Japan Society for the Promotion of Science (to Y. H.) and from the Pacific Science Board, National Academy of Sciences—National Research Council (to F. I. T.).

## Summary

Unlike the luciferins, the luciferases of *Apogon* and *Cypridina* have been found to possess properties that are significantly different. *Apogon* luciferase is eluted from a Sephadex G-200 column as a main activity peak and a shoulder, whereas *Cypridina* luciferase is eluted as a single, homogenous peak. The position of the *Cypridina* peak corresponds almost exactly with the position of the shoulder of *Apogon*, but the identity of the two has not been conclusively established. The main *Apogon* peak, however, is eluted before the *Cypridina* peak and, hence, may be considered different. On standing in phosphate buffer, *Apogon* luciferase gradually increases in activity, whereas *Cypridina*

luciferase activity remains virtually unchanged. *Apogon* luciferase is relatively more sensitive to inhibition by its antibody than *Cypridina* luciferase, whereas *Cypridina* luciferase is more sensitive to its antibody than *Apogon* luciferase.

*Parapriacanthus* luciferase has been partially purified, but the yield has been considerably less than *Apogon* luciferase.

### References

HANEDA, Y. and F. H. JOHNSON. The luciferin-luciferase reaction in a fish, *Parapriacanthus beryciformes*, of newly discovered luminescence. Proc. Nat. Acad. Sci. U.S. **44**, 127 (1958).

HANEDA, Y. and F. H. JOHNSON. The photogenic organs of *Parapriacanthus beryciformes* Franz and other fish with the indirect type of luminescent system. J. Morph. **110**, 187 (1962).

HANEDA, Y., and F. H. JOHNSON, and O. SHIMOMURA. The origin of luciferin in the luminous ducts of *Parapriacanthus ransonneti, Pempheis klunzingeri,* and *Apogon ellioti.* Bioluminescence in Progress, F. H. Johnson and Y. Haneda, Editors, Princeton University Press, Princeton, New Jersey, 1966, p. 533.

HANEDA, Y., F. H. JOHNSON, and E. H.-C. SIE. Luciferin and luciferase extracts of a fish, *Apogon marginatus,* and their luminescent cross-reactions with those of a crustacean, *Cyperidina hilgendorfii.* Biol. Bulletin **115**, 336 (1958).

HANEDA. Y., F. H. JOHNSON, and E. H.-C. SIE. Luciferin and luciferase extracts of a fish, *Apogon ellioti* and their luminescent cross-reactions with those of a crustacean, *Cypridina hilgendorfii.* Science Report of the Yokosuka City Museum 4, 13 (1959).

JOHNSON, F. H., Y. HANEDA, and E. H.-C. SIE. An interphylum luciferin-luciferase reaction. Science **132**, 422 (1960).

JOHNSON, F. H., N. SUGIYAMA, O. SHIMOMURA, Y. SAIGA, and Y. HANEDA. Crystalline luciferin from a luminescent fish, *Parapriacanthus beryciformes.* Proc. Nat. Acad. Sci. U.S. **47**, 486 (1961).

SIE, E. H.-C., W. D. MCELROY, F. H. JOHNSON, and Y. HANEDA. Spectroscopy of the *Apogon* luminescent system and of its cross reaction with the Cypridina system. Archiv. Biochem. Biopys. **93**, 286 (1961).

TSUJI, F. I. and Y. HANEDA. Chemistry of the luciferases of *Cypridina hilgendorfii* and *Apogon ellioti.* Bioluminescence in Progress, F. H. Johnson and Y. Haneda, Editors, Princeton University Press, Princeton, New Jersey 1966, p. 137.

## 4. Synchonously Flashing Fireflies in the Malay Peninsula[1]

J-M. BASSOT and I. V. POLUNIN

Institut Océanographique, Paris, France and Dept. of Social Medicine and Public Health, University of Singapore

Numerous travellers have been impressed by the beautiful sight of large numbers of fireflies in trees flashing with a regular rhythm and in synchrony. Reports on this phenomenon have come from several tropical countries, stretching from India to New Guinea, and particularly from Thailand, Burma and Malaya.

Much speculation has arisen about the mechanisms underlying these displays, often by those who had never had the good fortune to witness them. Buck (1938) who has exhaustively reviewed the literature on synchronous displays, mentioned four main ways

[1] Presented at the Symposium on Bioluminescence in the Pacific Area, Eleventh Pacific Science Congress, August 21 to September 10, 1966, Tokyo, Japan.

of explaining synchrony. They are:

(1) The Accident Theory. Synchronism starts by accident, and is maintained by identical flashing frequencies of individuals.

(2) The Illusion Theory. Reports of synchronism are due to the tendency of the mind to read order into disorganised experiences, especially in an "emotional attiude bordering on the romantic".

This theory probably has no adherents among those who have witnessed synchronous firefly displays.

(3) The "Sense of Rhythm" Theory. Synchrony in fireflies is due to an ability analagous to that which allows human beings to beat time.

(4) The Leader or Pacemaker Theory.

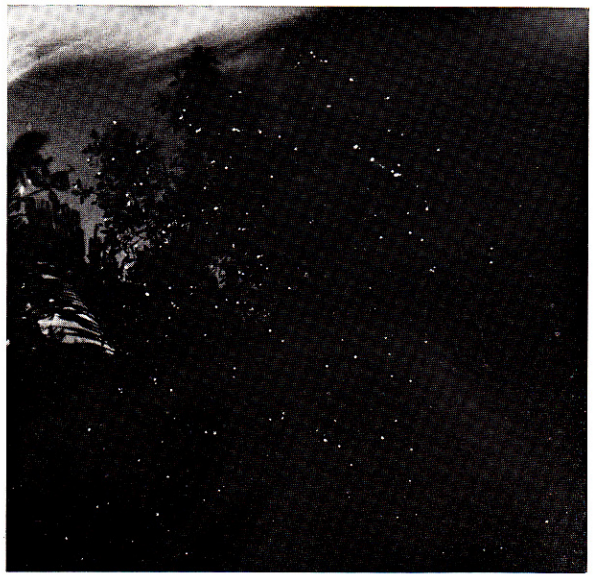


Fig. 1. Time exposure (about 5 minutes duration) of fireflies in a tree of *Sonneratia caseolaris*. The animals are either stationary or confine their movements to a very small area.

This paper describes some initial observations made in an attempt to explain the mechanism of synchronous flashing. The lowest three miles of the Benut River near the Southern end of the West Coast of the Malay Peninsula*, are lined with mangrove trees. The fireflies congregate in trees of *Sonneratia caseolaris* (*S. acida*), which are usually somewhat isolated (Fig. 1). This is the same species in which synchronous flash displays are seen in the lower reaches of the Menam Chao Phya in Central Thailand,** where they flash at double the frequency seen in Benut. Below the *Sonneratia*, fireflies are sometimes seen in a herbaceous *Acanthus*.

Synchronous flashing was noted in many trees, but from month to month fireflies

* Now officially called West Malaysia.

** Since this paper was written an extensive paper by Buck and Buck (1966) has appeared describing these displays.

have appeared to show a clear predilection for certain trees, while some trees have consistently given more precise displays of synchrony than others.

Though a majority of insects in each particular tree may be in synchrony, the populations of neighbouring trees are often out of synchrony with each other, and may be flashing at a slightly different frequency, for reasons that are not yet clear.

In one particular group of four small trees where most of the observations have been made, fireflies were found resting on the undersides of leaves in the afternoon. We have not been able to confirm the statement from Thailand that they flew in from outside as night fell.

Three main types of flash have been observed by fireflies on trees.

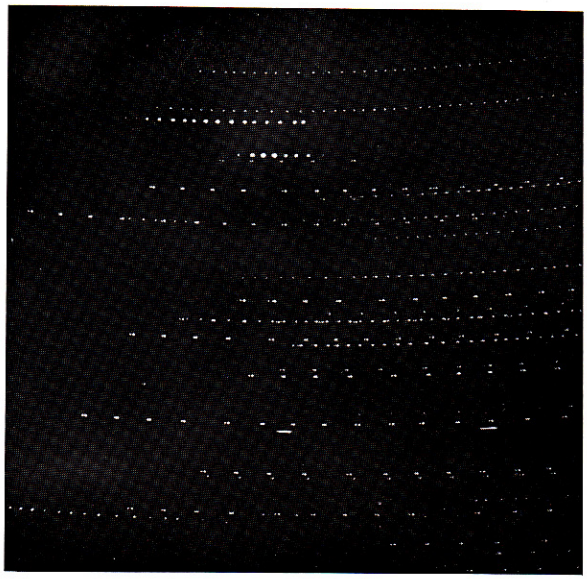


Fig. 2. Time exposure of a firefly tree made by slowly and regularly 'panoraming' the camera on a tripod. Some of the flashes are seen to be double. The distance between successive flashes depends both on flash frequency and the distance between the camera and the firefly.

(1) The rapid-rhythm flashes which appear fairly regular in rhythm. The frequency is over 120 beats per minute and can be up to 200 beats per minute. These flashes appear not to be synchronised, though what looks like synchrony is occasionally seen. Rapid flashing fireflies often walk round a leaf rather restlessly.

(2) Slow-rhythm flashes, with a frequency of 60-80 flashes per minute. These flashes are very bright, extremely regular and often show synchrony. The rest of this paper will describe observations on this type of flash.

(3) Rapid irregular flashes which can be elicited when insects giving slow or rapid regular flashes are considerably disturbed, as by handling.

Photographs of slow-rhythm flashes taken with a still camera by "Panoraming" the camera, (Fig. 2) or by moving film continually through the camera while the camera

shutter is open have shown that each visible flash consists of a double flash of light with an intervening period of darkness, or possibly of light emission which is too faint to be recorded on film. The first flash is fainter and shorter lasting than the second flash. The double flashes together last approximately 1/10th second, and the long dark period between, nearly 10 times as long.

The slow-rhythm flashes, whether produced by an independent individual or by a large population flashing in synchrony, show a remarkably regular rhythm. Thus one record of a single firefly made with a cinematograph camera running at constant speed showed that the insect could not have gained or lost more than 1/80th second in the 6½ seconds during which it was studied.

The frequency of flashes remains very constant over a short period. Determinations by stopwatch of the times taken to give 60 flashes by a large population flashing synchronously in one tree varied from 56.2 to 57.0 seconds in eight determinations made

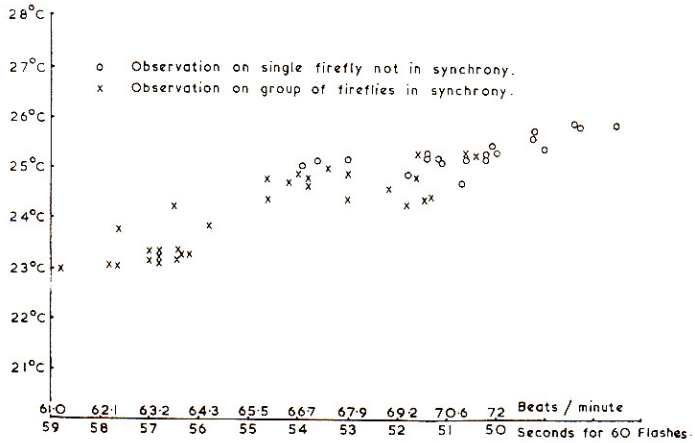



Fig. 3. Relationship between temperature and slow flash frequency.

over a period of 15 minutes. During this time the temperature had fallen by 0.2°C, which partly explained why the flashing was over 1% slower at the end.

Observations on slow-rhythm flashes showed that a given frequency of flash was only recorded within narrow temperature limits. Nearly all temperature readings fell within 0.5°C of the mean temperature for a particular flash frequency. This was true of readings made at different times on different nights and in different trees, and data of this type are presented in Fig. 3.

Individual fireflies flashing with their own individual rhythm, and small and large synchronously flashing groups showed no discernable tendency to flash at different rates. This shows that groups flashing in synchrony do so at a frequency which cannot very much from that of their intrinsic frequency.

These observations seem to us to eliminate the possibility that a pacemaker mechanism is operating, as then we would expect individual fireflies to flash at a slower rhythm than those which are being paced.

In spite of the incomplete view of the firefly population in a tree, and our admitted

tendency to pay attention to synchronous groups at the expense of others, we are convinced of the objective reality of synchronism.

Let us assume that the slow-rhythm flash occupies 1/10th of the cycle, and that a subjective judgement of 'synchrony' is made if the interval between the starts of the flashes by different animals is less than the duration of the individual flashes, we would only expect trios of 'synchronous' fireflies once in 100 possible trios, and quartets once in 1000 quartets, if all fireflies had identical flash-frequencies which were randomly distributed in time. In fact the flashes appear from film records to be more precisely synchronised than this. When for instance we count 12 fireflies in synchrony and one only out of synchrony, the probability of this being accidental is small indeed.

Objective records of synchrony are provided by comparing long exposure still photographs from small synchronous groups of stationary animals with photographs taken on film

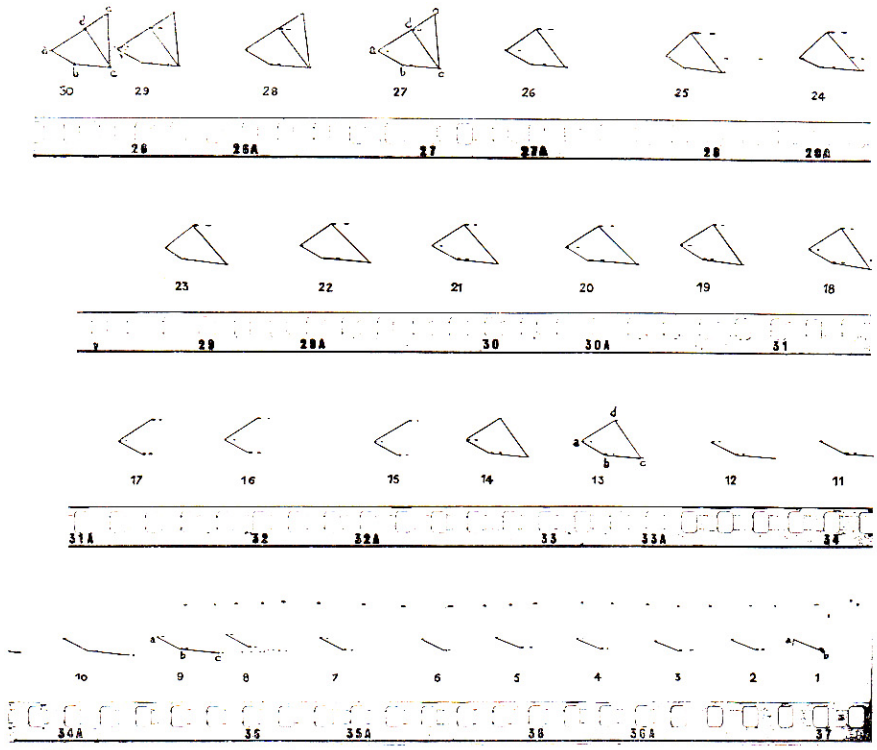



Fig. 4. Time exposure. Negative photograph of a small number of fireflies emitting slow rhythmic synchronous flashes. (Start reading from bottom right-hand corner. Lines have been drawn joining the first of the double flashes of which each slow rhythm flash is composed for ease of comparison of successive 'constellations'.)

The record begins at 1, with the film stationary in the camera for several seconds and shows 2 fireflies a and b. From 2 to 29 the film is wound continuously through the camera using the rewind handle. As this is turned by hand at an irregular speed the 'constellations' are unevenly spaced. At 30 the rewinding was stopped and the film exposed for several more seconds.

Note that the shape of constellations remains constant irrespective of whether film is stationary or moving, providing objective evidence of synchrony.

'New' fireflies appear at 9, 13 and 27, which appear to have 'switched on' in synchrony with the others.

running continuously through the same still camera, as in Fig. 4. The shape of photographs of a constellation of fireflies remains always the same, indicating synchrony. Ciné film records confirmed this and showed that members of a 'synchronous' group are not more than plus or minus 20 milliseconds out of synchrony, and may be much more accurate.

Fireflies flashing at similar frequency could develop synchrony if they tend to "switch on" in synchrony with a nearby individual or group. Alternatively a firefly might "lock" a variable frequency control when its timing coincides with that of the majority.

Fig. 4 shows what appears to be 3 fireflies switching on in synchrony with the majority. This slide is made with time exposures at beginning and end, with the film in between running continuously through the camera.

It shows synchrony between all slow-rhythm fireflies. We start with two fireflies in synchrony. A series of 8 brief flashes by a hitherto unlit firefly, is followed by it 'switching on' in synchrony, while at the 13th and 27th flashes, two more fireflies suddenly 'switch-on' in synchrony. It is impossible to generalise on the basis of the behaviour of three fireflies over half a minute and further studies will be needed to see whether synchronism commonly depends on such a 'switch-on' method. A further possibility is that fireflies apparently newly switched on may have appeared suddenly from behind a leaf.

It is also not yet clear whether fireflies can alter their frequencies to fit the timing of the majority, or whether these fireflies out of synchrony with the majority tend to switch off, or fly away. However, the impression was gained that a firefly out of synchrony with the majority was more liable to fly away or 'switch off' than were those in synchrony. When a trio or quartette in synchrony is watched, synchrony is often maintained for minutes. Such lack of individual variation would be surprising if the firefly had no means of altering the rate at which it gave slow rhythmic flashes.

### References

BUCK, J. B. (1938) Quart. Rev. Biol. 13, 301.
BUCK, J. and BUCK, E. (1966) Nature 211, 562.

## 5. Extraction and Characterization of Japanese Firefly Luciferin (Abstract)

KISHI, Y., GOTO, T., INOUE, S., SHIMOMURA, O., and Y. HIRATA
Chemical Institute, Faculty of Science, Nagoya University, Nagoya, Japan and
Department of Biology, Princeton University, Princeton, New Jersey, U.S.A.

Crystalline luciferine was obtained from abdomen, thorax, and head of *Luciola cruciata* (Japanese name Genji-Botaru) and characterized to be identical with that obtained from *Photinus pyralis* (American firefly) (IR, UU, ppc. Ms, etc.).

Extraction of luciferin from abdomen was done by means of a modified method of that used by MCELROY et al. (Arch, Biochem. and Biophys, 72, 358 (1957)), and the yield of luciferin was at least 2.5 times of that reported by McElroy in the case of *photinus pyralis* (5 mg of crystalline Luciferine from 3,000 firefly individuals).

Purification was done mainly by means of powdered cellulose partition chromatography and DEAE-cellulose ion-exchange column chromatography. The same luciferin was obtained from thorax and head by the similar treatments, yields of luciferin were ca. 15 mg and 0.5–0.6 mg from thorax and head, respectively, of 12.000 firefly individuals.

Other components having the benzothiazole skeleton will be also reported.

## 6. On the Mechanism of Chemiluminescence of Indole Derivatives* (Abstract)

N. SUGIYAMA, and M. AKUTAGAWA
Tokyo Kyoiku University, Tokyo, Japan

Recently it has been reported that several kinds of bioluminescent substances are indole derivatives[1] and TOTTER et al.[2] reported that some indole derivatives show chemiluminescent reaction. But they did not study the mechanism of this reaction in detail. So it seems interesting to study on this subject.

The chemiluminescence of about forty indole derivatives were studied in the following systems:

(a) EtOH—KOH—$H_2O_2$—$K_3Fe(CN)_6$ System

(b) DMS—KOH System

From the spectral studies the mechanism of chemiluminescence, especially, of 2, 3-dimethylindole and its hydroperoxide were studied in detail.

### References

1) HIRATA, Y. et al., Luminescence Conference at Hakone (1965)
CORMIER, M. J. et al., Biochem. Biophys. Acta, 64, 340 (1962); J. Biol. Chem., 238, 790 (1963).
2) TOTTER, J. R. et al., Photochem and Photobiol., 4, 869 (1965).

* This paper is published in full in the Bull. Chem. Soc., Japan (40), 240 and 347, 1967.

## 7. The similarity of Luminescent Mechanism of *Renilla reniformis* System and Indoxyl derivatives chemiluminescence (Abstract)

HORI, K., H. YAMADA, and M. J. CORMIER
Department of Chemistry, Tokyo Kyoiku University, Tokyo, Japan and
Department of Biochemistry, University of Georgia, Athens, Ga, U.S.A.

Previous studies have shown that *Renilla reniformis* luciferase catalyzes the luminescent oxidation of *Renilla* luciferin in the presence of $O_2$, $Ca^{++}$, and 3′, 5′-diphosphoadenosine (DPA). Luciferin can be quantitatively converted to a more reactive species non-enzymatically by heating at low pH. The product of this reaction reacts with luciferase and $O_2$ in the absence of DPA to produce light. Similar luminescent reaction was found in the syetem of indican and horseradish peroxidase. Horseradish peroxidae catalyzes the luminescent oxidation of indican in the precence of $H_2O_2$. Indican can be converted to

more reactive indoxyl by heating at low pH. Indoxl reacts with horseradish peroxidase and hydrogen peroxide to produce light.

Light is also emitted when indexyl or its derivatives are oxidized chemically with hydrogen peroxide and potassium ferricyanide at alkaline pH. To elucidate the chemiluminescent meckanism of indoxyl or its derivatives, the hydroperoxide of N-acetyl indoxyl was prepared by photochemical reaction. The hydroperoxide of N-acetyl indoxyl produces intensitive light by the addition of potassium hydroxide.

## 8. Luminous Fishes of Moreton Bay, Australia and Adjacent Waters[1] (Abstract)

Yata HANEDA
Yokosuka City Museum, Yokosuka, Japan

On the occasion of the research expedition to New Zealand under the U.S.-Japan Cooperative Science project from February to April 1965, I stayed in the laboratory of the department of harbours and marine, Brisbane, Australia for about two weeks through the courtesy of Mr. G. G. T. HARRISON, chief inspector of fisheries, and inspected some preserved specimens of luminous fishes from Moreton Bay and adjacent waters at the laboratory, and also had a chance to collect luminous fishes by crusing abroard a shrimp trawl boat.

The luninous fishes collected and examined were only shallow water forms, and belonged to the following families.

A. Monocentridae

*Cleidopus gloria maris*, the Australian pine cone fish. The paired luminous organs of this fish are situated on each side of the mandible. The surface of the luminous organ is reddish-orange in color. In a specimen 82 mm long, each luminous organ, elliptical in shape measured 4 mm in length and 2 mm in width. The luminous organ of this fish is larger than that of Japanese species *Monocentris japonicus*. I succeeded in cultivating luminous bacteria from the organ, (HANEDA, Y. 1966. On a luminous organ of the Australian pine-cone fish, *Cleidopus gloria maris*. Bioluminescence in Progress, ed. Johnson and Haneda, publ. Princeton University Press: 547–555).

B. Leiognathidae, Pony Fish.

1. *Equula equula* (FORSK.) Hump-Backed Pony fish
   Loc. Cleveland Bay, N. Queesland
2. *Equula decora* DE VIS, Hump nosed pony fish
   Loc. Plane River near Keppel Sands.

1) Acknowlegment is made of the partial financial support of this investigation through a grant from the Japan Society for the Promotion of Science as part of the Japan-U.S. Cooperative Science Program.
Presented at the Symposium on Bioluminescence in the Pacific Area, Eleventh Pacific Science Congress, August 21 to September 10, 1966 Tokyo, Japan.

3. *Gazza minuta* (BLOCK), Big-toothed pony fish
   Loc. Townsville N.Q.
4. *Secutor raconius* HAM, BUCK, Pig nosed pong fish
   Loc. Townsville Beach.
5. *Aurigequula longispinis* (C & V.)
   Long spined pony-fish
   Loc. Townsville N.Q.
6. *Eubleekeria splendens* (C & V.), Black tipped pong fish
   Loc. Townsville.
7. *Equulites virgatus* (FOWLER), Orang tip pony-fish
   Loc. Townsville.
8. *E. moretoniensis* OGL. Black banded pony fish
   Loc. Deception Bay, S.Q.
9. *E. hastatus* (OGILBY), Spear finned Pony Fish.
   Loc. Townsville.

The fish of the family Leiognathidae, commonly called "pony fish" are found only in shallow water, and abound in the waters of North and South Queensland. *Equulites moretoniensis* is common in Moreton Bay. Light is emitted from a swollen ring which encircles the esophagus and contains a gland with two openings leading into the aesophagus.

Symbiotic luminous bacteria live in the luminescent glands of all the above mentioned species.

C. Pempheridae

*Pempheris klunzingeri* MCCULL
Loc. Eclipse Island, N.Q.
*Parapriacanthus unwini* (OGILBY)
Loc. Keeper Reef, N.Q.
*Parapriacanthus elongatus*

Among the examined specimens of the genus *Pempheris P. compressus, P. macrolepis, P. lineatus* and *P. klunzingeri*, I found a V-shaped thoracic luminous duct and a Y-shaped anal luminous duct in *P. klunzingeri* similar to the ducts of *Parapriacanthus ransonneti*. *Parapriacauthus unwini* and *P. elongata* have luminous ducts similar to those of *P. ransonneti*.

Due to insufficient fresh mateial, it could not be proved that luciferin is stored in the pyloric caeca. However, since the structure of the thoracic and anal luminous organs is essentially the same as in *Parapriacauthus ransonneti*, it is highly probable that luciferin is stored in the pyloric caeca of these three species of fish.

(HANEDA, Y., F. H. JOHNSON and O. SHIMOMURA 1966. The origin of luciferin in the luminous ducts of *Parapriacauthus*, *Pempheris* and *Apogon*. Bioluminescence in Progress. Publ. Princeton Univ. Press. :533-545).

D. Apogonidae, Siphon Fish

*Apogon ellioti* var. *arafurae*
*Siphamia cunicups* WHITLEY, Siphon fish Loc. Moreton Bay
*Adenapogon roseigaster* RAMS of OGL.

Rose-Bellied Siphon Fish.

Loc. Moreton Bay, Brisbane River S.Q.

The light source of *Apogon ellioti* and *A. ellioti* var. *arafurae* is a chemical substance with a probable positive luciferin luciferase reaction, while that of the genera *Siphamia* and *Adenapogon* is symbiotic luminous bacteria. This contrast between closely related species is extremely interesting biologically.

The geographic distribution of crustacean ostracod, *Cypridina hilgendorfii* does not coincide with that of fishes of the waters of Australia. However *Cypridina noctiluca*, a tropical species, appears in the waters of Australia. So, if the luciferin of *Parapriacauthus ransonneti* and *Apogon ellioti* comes from *Cypridina hilgendorfii*, the luciferin of *Pempheris klunzingeri*, *Parapriacanthus unwini*, *P. elongata* and *Apogon ellioti* var. *arafurae* also might come from *Cypridina noctiluca*.

The shallow water luminous fishes of these regions are almost the same or closely related to the species of tropical Asia.

## 9. The Siera Luminous Millipeds, *Luminodesmus sequoiae* LOOMIS and DAVENPORT (Abstract)

Demorest DAVENPORT
Department of Zoology, University of California, Santa Barbara, California, U.S.A.

The natural history, ecology and distribution of the large luminous millipede of the Southern Sierra Nevada, *Luminodesmus sequoiae* are described. The possible source and biochemical nature of the luminescence is discussed, as well as the release of hydrocyanic acid by the organism. Photographs of the organisms taken in their own light and photographs of the fluorescence of the organisms when illuminated with ultra-violet will be presented.

## 10. Bioluminescence and the Irritability (Abstract)

Joseph Jin CHANG
Department of Biology, Sogang College, Seoul, Republic of Korea

Although it has been observed and known for many decades that most bioluminescent forms produce light only upon external stimulation, systematic investigations of bioluminescence as a stimulus-response system began only recently. According to the studies made since early 1950's bioluminescent intracellular granules, cells, tissues and organs represent an excellent material for work toward the further understanding of the irritability phenomena. As effector tissues the response characteristics of bioluminescent tissue show remarkable similarity to those of muscle. Light intensity increase as intensity or duration of stimuls increase, and repeated stimuli elicit luminescent responses similar to summation of twitches, stair-case phenomenon (facilitation), incomplete and complete tetanus of muscle. Relatively rapid fatigue of the light response is a characteristics of luminescent tissues. When neural connections are intact bioluminescent organ is subject to neural control and affers a good chance to study nerve-effector relationships. Most recently recordings of luminescent respones to stimulation from single intracellular light-producing granules have been made. Those subcellular luminescent granules will probably help us solve the mechanism of intracellular conduction and subcellular excitation.