Comp. Biochem. Physiol., 1976, Vol. 53C, pp. 75 to 82. Pergamon Press. Printed in Great Britain

BIOLUMINESCENCE OF BATHYPELAGIC FISH FROM THE STRAIT OF MESSINA

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(Received 7 July 1975)

Abstract-1. Photophores isolated from bathypelagic fish emit bright flashes (6×10^7 to 6×10^9 quanta/sec) lasting a few msec when they are electrically stimulated. In *Argyropelecus, Diaphus* and *Ichthyococcus.* the flashes are short. fatigable and have short latency. In *Chauliodus* and *Stomias* the flashes are multimodal, and have a long latency.

2. Adrenaline, nor-adrenaline, serotonin and acetylcholine are without effect on the isolated photophores, and do not influence the electrical stimulation.

3. Photophores of *Argyropelecus* have a low amount of ATP, less than 02 itmole/g, and larger amount of ADP (about 1 innole/g) and AMP (0-5 innole/g). No inosine monophosphate was detected, nor inosine nor adenosine.

4. Oral and caudal extremities of the gut harbour photomicrobes, which appears to be normal hosts of bathypelagic fish.

INTRODUCTION

LUMINESCENT bathypelagic fish seldom reach the surface alive: they are usually scaled, skinned or otherwise damaged (Robinson, 1973). The useful working time with netted animals during cruises rarely exceed 2 hr, owing to their rapid deterioration (Barnes & Case, 1974); moreover, attempts to study the activity of excised photophores have been so far unsuccessful (Anctil, 1972; Barnes & Case, 1974).

The physiology and the biochemistry of the photophores of bathypelagic fish is therefore quite difficult to study (for a review see Nicol, 1969; Baguet, 1975a).

It so happens, however, that bathypelagic fish may be obtained alive in the strait of Messina: *Argyropelecus hemigymnus* and *Chauliodus sloanei* survive up to 16 hr in appropriate conditions. These fish provide an unique opportunity to carry out a study of isolated photophores.t It turned out that the photophores were fairly well excitable by electrical stimulation and showed responses very distinct from those of the isolated photophores of *Porichthys,* an epipelagic fish (Baguet & Case, 1971). On the other hand, pharmacological agents like adrenaline, nor-adrenaline or 5-hydroxytryptamine (5-HT) never trigger light emission when applied on excised photophores.

We tried to correlate the nucleotide content and some other biochemical parameters with the responses of the light organs: it was found, surprisingly, that their ATP concentration is very low.

METHODS

1. *Collection of fish*

Twice a month from November to March, a maximum tide occurs in the strait of Messina (Italy). The currents in the strait become fast and powerful. The deep water currents are deflected upward by the shallow at Messina, dragging with them fish and many animals which live in the depths of the Ionian sea. Living luminescent fish may be captured in two different ways: (i) If the tide and wind conditions are favourable, fish may be stranded: this phenomenon, "the spiaggiamento", has been thoroughly studied by Genovese *et al.* (1971). Some of the stranded fish collected early in the morning still show movements when touched: their colours are bright and their muscles are soft, indicating that these fish are still living, albeit shocked. (ii) They can easily be captured in a handnet from the shallow waters of the strait. These fish show splendid iridescent colours and swim vigorously.

The species collected during January included: *Argyropelecus hemigymnus* (95), *Ceratoscopelus mederensis (1), Chauliodus sloanei (5), Diaphus holi* (5), *Gonostoma denudaturn* (2), *Ichthyococcus ovatus* (4), *Maurolicus muelleri (5), Myctophum punctatum* (3), *Cyclothone braueri* (25), *Stomias boa (1), Vincinguerria poweriae* (8).

The fish were brought to the station of Ganzirri, near Messina, where they were transferred to glass vessels filled with cooled sea water (7°C) from the strait and stored in a refrigerator at 7°C. In these conditions, many fish survived for 12-18 hr (Baguet, 1975b).

2. *Dissection of the photophores*

(a) Stranded fish. The fish were laid on a filter paper wetted with sea water and some photophores were excised with as little surrounding tissue as possible. No contractions of the body occurred, except when photophores of *Argyropelecus* are cut out of its tail: this causes a vigorous movement of the whole body.

(b) Netted fish. The following procedure was used successfully without requiring any anaesthetization: the fish was quickly transferred from cooled sea water (7°C) to sea water at 20-25°C and exposed for 40-60 sec to the light of an electric-light bulb. Afterwards, it was laid on a filter paper wetted with sea water and dissected. This short exposure to tepid water and to light prevented movements during dissection. All the fish withstood this handling and recovered after a few minutes in cooled sea water.

3. *Measurements of the light emission*

The photophores of *Argyropelecus* are tube-like structures about 1.5 mm length; those of the other fish investigated *(Chauliodus sloanei, Diaphus holi, Ichthyococcus*

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t A preliminary report of these studies has been published (Baguet & Marechal, 1974).

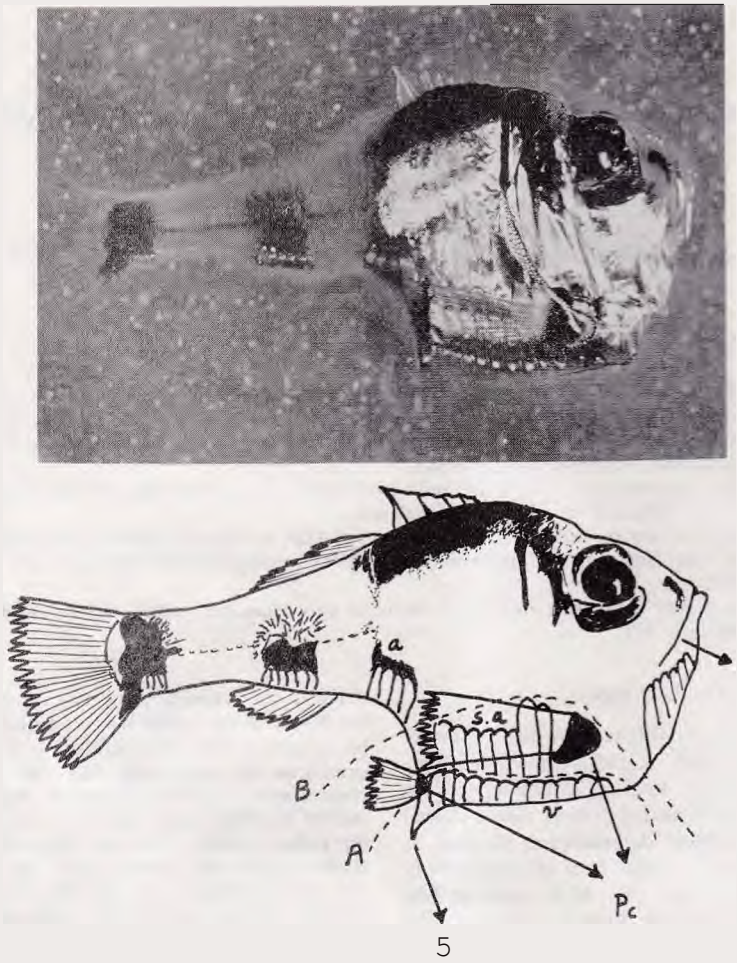


Fig. 1. *Argyropelecus hemigymnus Cocco,* photographed (uppermost) and schematically sketched to show the localization of the rows of photophores dissected for the biochemical analysises. a, anal photophores; m, mouth; s.a, Supra-abdominal photophores; v, ventral photophores; Pc, Pectoral fins; S, Spur; A, separation line of the "ventral photophores" preparation; B, separation line of the "ventro-supra-abdominal photophores" preparation. The animals length is 3.5 cm.

ovatus, Stomias boa) look like small beads about 1 mm dia. After dissection, they were placed at room temperature (12-14°C) in a small vessel filled with air-saturated saline (Baguet & Case, 1971). After 5-10 min, one photophore was placed in a chamber (Baguet, 1975a), with its dermal surface in contact with two platinum electrodes (dia 100 gm) which were connected to a home-made stimulator delivering square pulses. The luminescent response obtained from the specimen was sensed by a photomultiplier 1P 21 operating at 935 V, at a distance of 14-17 mm. The signals from the photomultiplier anode and the stimulus markers were displayed on a Tektronix 5100 storage oscilloscope and recorded with a Polardid camera.

The drugs, adrenaline hydrochloride (Fluka), noradrenaline hydrochloride (Fluka), acetylcholine (Roche), 5-hydroxytryptamine creatinine sulfate (Sigma) were dissolved in distilled water or in saline just before use.

The correct identification of the fish was checked after each experiment.

4. *The dissection for the biochemical analysis*

Only *Argyropelecus hemigymnus* were used for biochemical studies. These fish were the most numerous in the catches, and, furthermore, their photophores were conveniently located. Indeed the lower ventral portion of this fish contained only photophores, with the pectoral fins and associated muscles. There were no visceral organs. Photophores were dissected quickly by either of the following two methods.

In the first method. the pectoral fins (cf. Pc Fig. 1) were cut near their insertion: the ventro-caudal spur (s) was cut near the rows of photophores. Then with one scissor stroke, the lower ventral row of photophores was separated from the body along the line "A". The strip thus obtained contained the right and the left lower ventral rows of photophores, still attached to each other. There were 24 photophores. The strip weighed about 10 mg, .for a fish of 0.75 g average weight. This preparation will be referred to as "ventral photophores" (V).

In the second method, pectoral fins and spur were cut out as previously. Then a piece of the fish was cut out as indicated by the line B in Fig. 1, leaving out the pectoral bones and muscles. This preparation contained the ventral photophores and also the supra-abdominal (s.a) but not the anal photophores (a) nor the ones which are under the mouth (m). This preparation is refered to as the "ventro-supra-abdominal photophores".

5. *Extraction procedure*

"Ventral photophores" and "Ventro-supra-abdominal photophores" were quickly frozen after dissection by immersion into acetone cooled with dry ice. The frozen

organs were rapidly weighed, and, without allowing them to thaw, they were dropped into a tube which contained 200 pl of 0.5 N $HC1O_4$ at dry-ice temperature. They were ground with a glass rod; after a few minutes. the mixture was slightly warmed with the hand, without interruption of the grinding, until it thawed. The tubes were cooled again to — I 5°C for 2 hr. They were then allowed to thaw, and they were centrifuged at 4000 rev/min for 5 min. After centrifugation, three layers were observed: a white precipitate P, at the bottom; on top of it, a thin layer of black material (presumably melanin), and then a clear fluid. The fluid was transfered into a tube containing 20 µl of 5 N NaOH. There occurred a precipitate white and fibrinous, **P2.** The centrifuged precipitate P, was washed once with 200 pl of distilled water; the wash was added to the supernatant. The supernatant was centrifuged 5 min at 4000 rev/min. A white floconous precipitate, **P3** (seemingly the same as P_2), of unknown origin lay on top of the $KC10_4$ precipitate. The clear fluid which was above the precipitates was used for the enzymic measurements and the chromatographic analysis of the nucleotides.

6. *Analysis of the nucleotides*

(a) Enzymatic. A slightly modified version of the method of Jaworek *et al.* (1970) was used for ATP measurements. The test tube contained: 1.0 ml of distilled water: 0.4 ml of a solution made by mixing 10 ml of a solution containing 55.8 mg of glycerate-3-phosphate, 49.2 mg of $MgSO_4$, 3.31 ml of triethanolamine (pH 7.6) made up to 50 ml, and 0.25 ml of NADH (1 mg/ml); 0.01 mg of an enzyme mixture consisting of 0.7 ml of glyceraldehyde-3-phosphate kinase, 0.1 ml of 3-phosphoglycerate kinase, 0.2 ml of glycerinphosphate—deshydrogenase and 0.05 ml of triose—phosphate—isomerase.

The fluorescence (light greater than 400 nm) of the solution was excitated by a 360 nm light, and it was measured by a Perkin—Elmer spectrofluorometer MPF-3 or a Locarte spectrofluorometer. Thirty microliters of the sample was added to the test tube. The decreases in fluorescence were calibrated with an ATP solution of known concentration. This reaction was completed in 5 min. As very little ATP was found in the extracts we have controlled that the reaction is not inhibited by the photophores extracts, by checking its sensitivity with internal standards. It appeared that no inhibitory substances was present in the extracts of photophores.

ADP and AMP were measured according to the method of Jaworek *et al.* (1970) slightly modified to adapt it to fluorescence measurements. The test tube contained: 0-6 ml distilled water; 0.2 ml of a buffer solution made by dissolving 8 g of triethanolamine hydrochloride and 7.5 g of K_2CO_3 in 100 ml of water (pH 7.5); 0.05 ml of NADH (0.18 mg/ml); 0.02 ml of a neutral solution containing 10 mg of Na-phosphoenolpyruvate, 185 mg of $MgSO_4 \cdot 7 H_2O$, 200 mg of KCl and 1.5 ml of water; 0.01 ml of lactate deshydrogenase (1 mg/ml) and 30 /21 of a photophore extract. The NADH contained in the tube is excited with a u.v. light of 360 nm wavelength, and the resulting fluorescence is measured (light with a wavelength longer than 400 nm).

Ten microliters of pyruvate kinase (1 mg/ml) were added to the tube, and the decrease in fluorescence, read 10 min later was a measure of the ADP content. Ten microliters of myokinase (2 mg/ml) were then added; the decrease in fluorescence was measured 30 min later and it was a measure of the AMP content. The fluorescence changes were calibrated with external standards.

All the enzymes were purchased from Boehringer (Germany).

(b) Chromatographic analysis of the nucleotides were done on photophore extracts according to the thin layer procedure described by Canfield & Marechal (1973). The analysis confirmed the results obtained with the enzymatic methods and demonstrated the absence of inosine monophosphate, of adenosine and of inosine.

RESULTS

1. *Electrical stimulation*

Photophores excised from living or stranded fish never luminesce spontaneously when lying in the chamber designed for the light recording. However, they respond to single as well as to multiple electrical stimuli with a brief light emission, either a few minutes after dissection or after 3-4 hr bathing in saline at 12°C (Fig. 2).

(a) *Response to a single electrical stimulus.* Isolated photophores respond to single electrical shocks in different ways depending on the genus of the fish from which they were excised. Photophores from *Argyropelecus, Diaphus* and *Ichthyococcus,* always respond to a single electrical stimulus of 4 msec duration with one brief flash and to a stimulus of 8-16 msec by two or three flashes of increasing amplitude. For longer duration of the stimulus, no further flashes are elicited.

Figure 2a shows the time course of the flash evoked by a 4 msec stimulus applied to a photophore of the ventral region of *Argyropelecus.* The light emission begins 3 msec after the onset of the stimulus and reaches a maximum in 0.5 msec. Extinction is completed in about 7 msec. The flashes recorded from

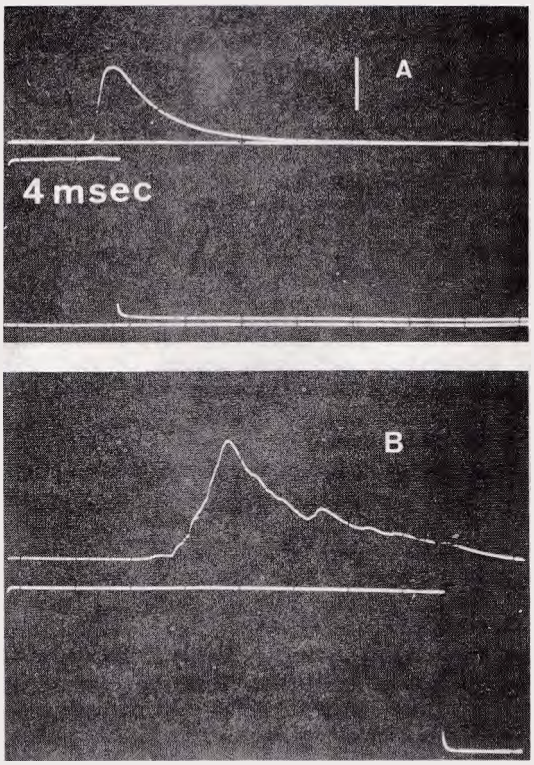


Fig. 2. Luminescence of a photophore isolated from *Argyropelecus hemigymnus.* **A, response to a single stimulus (square wave, 4 msec duration, 25 V) 5 min after dissection;** B, response to a single **stimulus (square** wave, 16 msec, duration, 25 V) after **4 hr bathing in aerated saline. The upper trace corresponds** to the **light** emission, the lower trace to the electrical stimulus. Vertical bar deflection: 2.4 x 10^8 quanta/sec.; **time** markers: 2 msec.

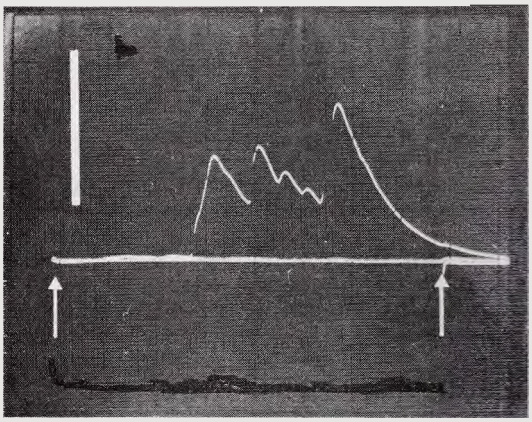


Fig. 3. Time course of the flash evoked by one stimulus (20 V; 16 msec) applied between the two arrows on a photophore from the anterior ventral row of *Chauliodus sloanei.* Vertical bar deflection 5 x 10^8 quanta/sec.

photophores of *Diaphus* and *Ichthyococcus* are similar in intensity and time course. The main variability is in the emission latency time: the shortest value was measured on *Diaphus* (14 msec) and the longest on *Ichthyococcus* (6.1 msec).

On the other hand, the photophores from *Chauliodus sloanei* and *Stomias boa* never respond to a single short electrical stimulus (4-16 msec). Stimuli of longer duration, at least 20-40 msec, are required to trigger a light response from an excised photophore.

Figure 3 shows a typical response of a ventral photophore of *Chauliodus sloanei* stimulated by a 15 msec-duration stimulus (20 V): the light emission which occurs about 6 msec after the onset of the stimulus, is much more complex than that described in the case of *Argyropelecus, Diaphus* or *Ichthyococcus.* It is not a single flash but a series of unfused flashes produced at irregular intervals lasting about 6 msec. The time to reach the peak value is 1 msec for the first flash; similar values were measured in six other experiments. This rate of flashing is thus half as slow as that measured for the photophores of *Argyropelecus* and related genera. On the other hand, the rate of extinction is rigorously similar, i.e. 7-10 msec.

When the stimulus duration is prolonged, the number of flashes increases; though they are produced as long as the stimulus is maintained, their amplitude decreases progressively.

(b) *Response to repetitive electrical stimulation.* Repetitive stimuli trigger flashes that never fuse together.

In the case of *Argyropelecus,* the photophores respond to a series of stimuli (4-16 msec) as long as the repetition rate does not exceed some threshold, otherwise the light emission stops. For example, a photophore does not respond to a series of 16 msec stimuli at a frequency of 50/sec, but it will respond to the same stimuli applied at 12/sec. In this case, individual flashes of maximum amplitude are produced during 1 sec, then their amplitude decreases and the response stops 2 sec later. For such stimuli at 2/sec, the response lasts 6 sec, and at 1/sec it lasts 16 sec. Two successive series of stimuli induce similar responses only if the interval between them is 4 min or longer: no response occurs to the second stimulation if it occurs sooner.

In the case of *Ichthyoccocus.* the response to a series of stimuli is quite different. Repetitive 2 or 4 msec pulses trigger flashes even when their rate is high (25-50/sec). At 50 sec. stimuli evoke a series of flashes of variable intensity at irregular intervals (Fig. 4). Successive 8 or 16 msec stimuli at a low rate, i.e. 1 or 2,/sec, produce flashes the amplitude of which periodically increases and decreases (Fig. 5).

In the case of *Chauliodus* and *Stomias,* a series of stimuli either at low or at high frequency (1/sec to 50/sec) triggers only sporadic flashes: Fig. 6 shows a typical response of a ventral photophore of *Chauliodus sloanei* stimulated t;).y a series of 16 msec-duration stimuli (20 V) at a frequency of I2/sec. After 20 stimuli, three flashes are produced at stimuli number 21, 22 and 23 and afterwards there is not any more response.

(c) *Response of aged photophores to electrical stimulation.* A photophore aged for 3 or 4 hr in aerated saline responds to an electrical shock in a somewhat different way than that of a fresh photophore. Comparison of Fig. 2a.b emphasizes two striking differences between the flash emission of two photophores of the ventral region of *Argyropelecus,* stimulated 5 min after dissection or after a 3 hr bath in aerated saline: (i) the shape of the light response in Fig. 2b is not as regular as that shown in Fig. 2a: the flash shows a series of discrete and irregular indentations. (ii) the rate of the light emission is lowered; the peak of the response is reached only about 3 msec after the onset of the stimulus.

A few isolated photophores glow spontaneously if they are left 5 hr or more in a saline solution. When this occurs, they do not respond any more to a single short stimulus. nor to a series of stimuli. Figure 7 shows a typical response of a spontaneously glowing photophore of *Argyropelecus,* to a cathodal stimulus of 5 sec duration. The luminescence increases slowly during the stimulation and reaches a peak about 2 sec after the end of stimulation. It took about 35 sec for the light emission to return to the baseline. In this case, an electrical stimulus of long duration can still modulate the light level of the photophore, but this occurs at an extremely slow pace. The slowness of

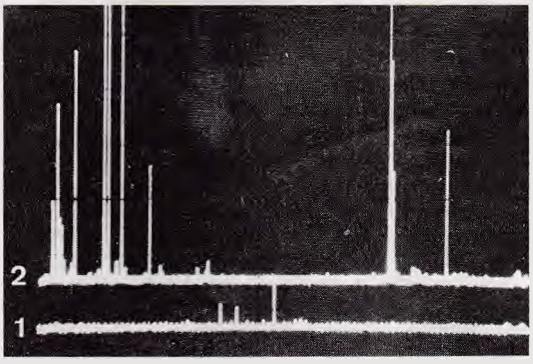


Fig. 4. Light responses of a photophore of *Ichthyococcus* to two series of electrical shocks (16 msec, 20 V, lower traces) applied at a frequency of 12/sec (trace number 1) and 50/sec (trace number 2).

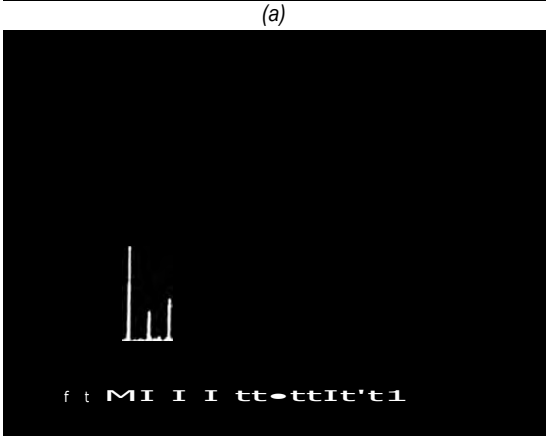
the response suggests that the mechanism controlling the rate of luminescent reactions is perhaps damaged.

2. *Chemical stimulation*

In order to study a possible involvement of neural transmitters in photophore activation, a preliminary neuropharmacological survey was conducted to determine the effects of adrenaline, nor-adrenaline, 5-hydroxytryptamine (5-HT) and acetylcholine on excised photophores luminescence. None of these pharmacological agents (10^{-6} to 10^{-3} M), applied either separately on different photophores, or successively on the same photophore, ever induced any detectable light emission from freshly isolated organs of fish studied in previous section.

On the other hand, two photophores excised from *Stomias* and one from *Chauliodus* 3 hr after death, emitted a weak luminescence about 10 sec after addition of adrenaline at a final concentration of 10^{-5} M. This low production of light lasted about 80 sec. However these photophores were already glowing faintly before the application of the pharmacological agents. Similarly, some photophores of the ventral region of some *Argyropelecus* were glowing 6-8 hr after death; when isolated from the fish, the glow increased slightly and temporarily by application of adrenaline 10^{-3} M. In more recent experiments on photophores isolated from freshly captured and non luminous *Chauliodus,* no luminescence was observed after application of these pharmacological agents on the photophores.

(a)



(b)

Fig. 5. Effect of the stimulus duration on the flashes emitted by a photophore of *Ichthyococcus.* A. Repetitive stimuli (dots, lower trace) at a frequency of I sec do not evoke any light response for duration of 2 msec (trace 1) or 4 msec (trace 2); flashes of varying amplitude are emitted when the duration of the stimulus is 8 msec (trace 3). B. Stimuli of 16 msec duration (dots, lower trace) evoke flashes of higher amplitude; flashes of high and low amplitude alternate periodically. Vertical bar deflection: 5 x 10^8 quanta/sec.

Fig. 6. Three sporadic flashes evoked by the stimulation (dots of lower trace: 20 V; 16 msec; 12/sec) of a photophore isolated from *Chauliodus sloanei.* Vertical bar deflection: 5 x 10^8 quanta/sec.

3. Light emission of the fishes

Several dozen of bathypelagic fish have been observed by visual inspection for light emission.

None of the fish freshly picked up from the strand did luminesce. The species observed included: *Argyropelecus hemigymnus (5), Myctophum punctatum* (3), *Maurolicus muelleri* (5), *Vincinguerria poweriae* (8), *Gonostoma denudatum* (2), *Ichthyococcus ovatus* (3), *Chauliodus sloanei* (5), *Stomias boa (1), ceratoscopelus mederensis* (1), and *Chlorophtalmus aganizi (* I). Coastal epipelagic fish were rarely stranded; some of them quite small and young were also examined in that respect, but only three could be identified as *Sardinia;* none of them did luminesce.

However, most abyssal fish became luminescent 12-24 hr after they had been collected.

Figure 8 shows a typical result. This *Argyropelecus* collected one day earlier was conspicuously glowing in the dark. The upper half of the figure, shows a photograph of the fish illuminated with artificial light. The lower half shows the fish at the same magnification photographed in total darkness (exposure time: 25 min, f. 8, ASA: 3000). Many parts of the animal glow. Ventral, ventrolateral, lateral and mouth photophores are visible. Photophores of the middle part of the tail glow as well, but the photophores near the caudal fins are not visible. What is however conspicuous is the fact that parts of the body devoid of any photophores do also glow (e.g. abdomen). Dissection of some argyropelecus showed which internal viscers glowed: the lingual glands, the stomacal gland or the perianal portion of the gut (cf. Brauer, 1908).

4. Nucleotides contents of the photophores

ATP, ADP and AMP content are reported in Table 1 for three samples of "ventral photophores" (1, 2 and 3) and five samples of "ventro-supra-abdominal photophores" (4-8).

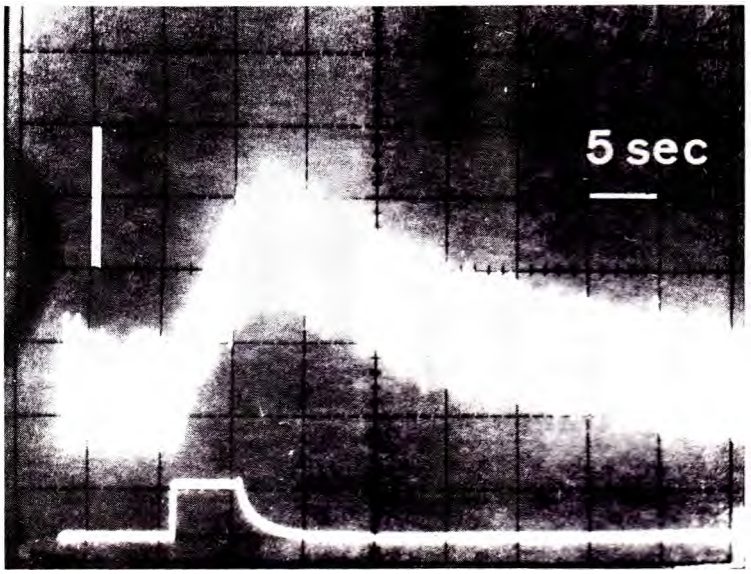


Fig. 7. Response of a spontaneous glowing photophore of *Argyropelecus* (upper trace) to a 5 sec duration stimulus (lower trace). Vertical bar deflection: 5 x 10^8 quantum/sec.

Very little ATP was found, less than 0-2 fanole/g, but sizeable contents in ADP, about 1 fanole/g, and of AMP, about 0.5 fanole/g were found. There does not seem to be any differences between ventral and ventro—supra—abdominal samples.

The organs were dissected a few hours after the collection of the fish. It was not checked whether they were luminous, but in parallel electrophysiological experiments, no spontaneous glow of the photophores were noticed, although they were excitable. Furthermore, we never observed spontaneous glow so early after the collection. Presumably, these organs would have glowed if they had been examined a few hours later.

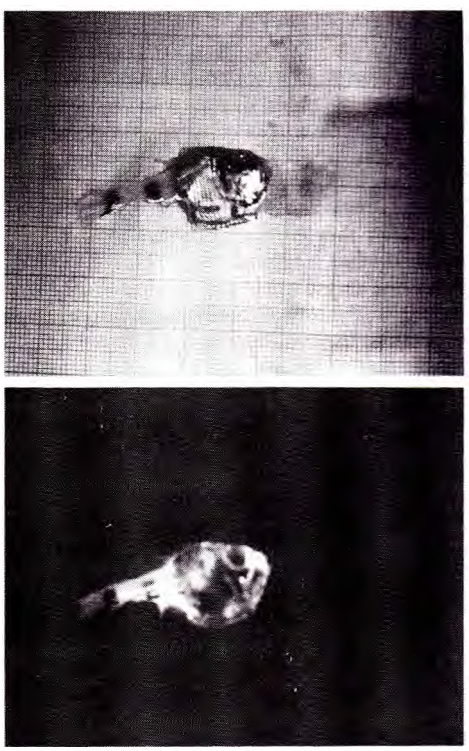


Fig. 8. *Argyropelecus hemigymnus Cocco,* photographed by lamp-light (uppermost) and in dark-room by its own light (see the text).

Table 1. Nucleotides in photophores of *Argyropelecus* (µmole/g)

N'	Weight mg	ATP	ADP -	AMP
1	10.6	0.10	–	–
2	20.0		–	
3	12.0	0.03	1.02	0.77
4	20.0	0	1.12	0.40
5		0.03	1.29	0.48
6		0.03	0.85	0.71
7	31.4	0.12	1.44	0.47
8	33.6	0.07	0.86	0.41

The fact that the ATP concentration is so low is surprising. It might suggest that the organs were already in an advanced state of autolysis as is shown, for instance by beef muscle (Valin & Charpentier, 1969). However, if it was true, one should find high amounts of adenosine, inosine or inosine monophosphate. Thin layer chromatography on polyethylenimine did show some ATP, ADP and AMP, but no inosine monophosphate, inosine or adenosine was detected. Therefore, these facts suggest that the low concentration of ATP might be physiological; this may indicate that ATP is not required for the light production mechanism, as we know that such photophores are electrically excitable.

DISCUSSION

1. The light response to electrical stimulation

The analysis of the light production by isolated photophores has now advanced so far that it is time

to propose a first scheme for the classification of photophores.

In a first class (type F photophores), the light production is a brief pulse, lasting a few milliseconds, but of high intensity (6×10^7 to 6×10^9 quanta/sec). Such photophores respond to repetitive stimuli by repetitive flashes which may fatigue rapidly when triggered at a high rate, but which never fuse in a sustained light response. This property which recalls that of action potentials lead us to call this type of response a "lumispike". Photophores of all bathypelagic fish studied up to now belong to this type. Sub-classes may however be described.

In the type Fa (photophores from *Argyropelecus, Diaphus* and *Ichthyococcus),* a short (4-16 msec) or a long stimulus (1 sec) triggers one or three flashes during the first milliseconds of the stimulation. In the type Fb (photophores from *Chauliodus* and *Stomias) a* stimulus of at least 40 msec is required **to** evoke a flash composed of several subunits of varying amplitude; flashing continues during 1 to 2 sec if the stimulus is maintained. Repetitive stimuli evoke occasionally sporadic flashes.

In the second class (type S photophores) the light produced by a stimulus is slow and low, but the light responses fuse easily together (Baguet & Case, 1971). By analogy with the contractions of a striated muscle, we propose to call such a response a "lumitwitch" if unfused and a "luminus" if fused. Luminuses are typical of photophores of *Porichthys.*

2. Light response to pharmacological agents

In the case of the batrachciid teleostean **fish *Porichthys,* adrenaline triggers a luminescent response** of isolated **photophores, whilst 5-HT inhibits or reduces the luminescence (Baguet, 1975b). Although these drugs are ineffective on isolated photophores, they trigger powerful responses if they are injected into the fish. This was first shown by Bertelson & Grontved (1949) for *Argyropelecus olfersi.* This fish does not luminesce** spontaneously, but **an injection** of **adrenaline triggers** a luminous response **from all** the **photophores of the** trunk. A similar effect is **obtained if *Argyropelecus hemigymnus*** is injected **with adrenaline at a concentration as** low as 10^{-7}M (Baguet, **19756). We have** found these effects **many** times in ***Argyropelecus hemigymnus*** and in ***Chauliodius*** *sloanei,* and, **furthermore, we** have observed that an injection of **5-HT 10^{-7}M in** *Argyropelecus* **induces** a luminescence **of those** photophores located on the tail. These observations indicate that these neurotransmitters **may play a part** in the control of the luminescence in ***Argyropelecus*** and *Chauliodus;* their action is however **indirect, on** some tissues which are not isolated with the photophores or perhaps which are damaged by dissection.

*3. The origin **of the light** emitted after death*

The facts described in section 3 of the results, suggest strongly that the spontaneous light emission which occurs some hours after the death of luminescent bathypelagic fish originates from bacteria or some other microorganisms. The early light production starts in some visceral organs, then it invades the light organs and some part of the skin; later on, it diffuses to the surrounding sea water. It can be transferred to a salty agar-peptonized medium, where light productive bacteria-like organisms have been maintained several weeks. The problem is to decide the significance of these observations. Three possibilities should be discussed. First, they are contaminating photobacteria which have nothing to do with bathypelagic fish. Second, these photobacteria live in the deep sea and are ingested by the bathypelagic fish. Third, the photobacteria are somehow associated with the physiological operation of the photocells, being perhaps the source of the light production of photophores.

The first hypothesis is the most probable. It is well known that *Photobacterium phosphoreum, Ph. fischeri, Vibrio luminosus* and other photomicrobes grow quickly on dead fish (Spencer, 1955). These species thrive well in the cold (under 25°C) and require a salty medium (at least 3% salt). These requirements are the same as those of the photomicrobes isolated from the bathypelagic fish. However we are reluctant to admit this hypothesis for two reasons. First, a few non bathypelagic fish were collected and handled in the same way as the bathypelagic species: none of them was ever seen to glow. Secondly, it occurred several times that a fish would not glow but that a careful dissection of the melanine sheath which is under the skin or around visceral organs would uncover luminescent perianal or perioral glands.

The latter observation supports the second hypothesis. The glow of visceral organs is limited to some parts of the gastrointestinal tube. This phenomenon has not yet been thoroughly studied, but it suggests that luminescent microbes may normally thrive in the gut of bathypelagic fish.

The third hypothesis seems unlikely. A first reason is that we observed that a species without any light organs *(Chlorophthalmus)* had a luminescent anal organ. A second reason rests on the histology of the light organs of the bathypelagic fish (Bassot, 1966) which suggests that the origin of the light is intracellular. It is certainly possible to imagine within the photocells some kind of symbionts which would prove to be the true source of the light. We have tried to isolate photobacteria from excised photophores but no trial has been successful. It must however be pointed out that although we have observed that light organs may begin to glow some time after the collection, this glow did not seem to arise from microbial invasion from the visceral organs, as it still arises in isolated "ventral photophores" or "ventrodorsal" photophores of *Argyropelecus.* Furthermore, this glow is strictly localized to the photophores. This latter fact is also true of whole glowing animals, as can be seen from inspection of Fig. 8. These two arguments suggest that light organs may switch on by themselves.

In conclusion, it is very likely that bathypelagic fish normally harbour bathypelagic luminescent microbes in their alimentary tract.

Acknowledgements—This study was made possible by a grant of the *"Fonds National de la Recherche Scientifigue",* of Belgium, and of the *"Centre beige d'Oceanographie".*

We wish to acknowledge the help of Prof. S. Genovese, director of the Istituto di Idrobiologia of the University of Messina (Italy) who provided us with the necessary information and bench space. We are also grateful to Dr. Chiricosta, who helped us to make the fluorescence measurements with the equipment kindly lent by Prof. d'Amore, of the Istituto di Merceologia of the University of Messina. Prof. Gangemi, Dr. Guglielmo, Mr. Coshetta and Mr. Donato provided us with several specimens. We thank them very much for their gifts.

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