

## OXYGEN CONSUMPTION AND LUMINESCENCE OF *MAUROLICUS* PHOTOPHORES STIMULATED BY POTASSIUM CYANIDE

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### SUMMARY

1. Isolated photophores of *Maurolicus muelleri*, maintained in saline at 20°C, consume oxygen at a mean rate of  $1.25 \pm 0.07 \text{ nmol O}_2 \text{ min}^{-1}$  ( $N = 50$ ).
2. In the presence of  $5 \times 10^{-6} \text{ mol l}^{-1}$  KCN, seven preparations did not luminesce while two showed only dim luminescence. In all nine preparations, the resting oxygen consumption decreased by about 50%.
3. In the presence of  $5 \times 10^{-5} \text{ mol l}^{-1}$  KCN, all of the photophores produced a slow, low luminescence and their oxygen consumption decreased by about 75%.
4. In the presence of  $5 \times 10^{-4} \text{ mol l}^{-1}$  KCN, all of the photophores produced a slow, high luminescence and their oxygen consumption decreased rapidly by about 92%.
5. It is suggested that the oxygen needs for light production by the isolated photophores of the mesopelagic fish, *Maurolicus*, differ from those of the epipelagic fish, *Porichthys*.

### INTRODUCTION

The metabolic inhibitor potassium cyanide (KCN) triggers glowing and enhances the oxygen consumption of isolated photophores of the epipelagic luminescent fish *Porichthys* (Mallefet & Baguet, 1984). To explain this paradoxical effect of KCN, it has been suggested that the increase in oxygen consumption during the light response corresponds to that involved in the luciferin–luciferase light reaction (Cormier, Crane & Nakano, 1967). The aim of the present work was to investigate, in isolated photophores of a mesopelagic fish, whether KCN can also evoke a light emission associated with a simultaneous increase in oxygen consumption. The results show that KCN evokes a light response but blocks the oxygen consumption of the light organ. It is suggested that oxygen has different roles in the control of light production in *Maurolicus* and *Porichthys* photophores.

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## MATERIALS AND METHODS

*Dissection and mounting the photophores*

Fresh specimens of *Maurolicus muelleri* were collected, as previously described (Baguet & Christophe, 1983), during 8 days between 05.00 and 06.00 h in the Strait of Messina and brought to the Istituto Talassografico where they were transferred to glass vessels in cooled sea water and stored at 7°C.

The two rows of abdominal photophores are partly fused, forming a common narrow central trunk (Fig. 1A). Each experiment was performed on a portion containing 12 photophores (Fig. 1B). Portions were carefully excised under a binocular microscope using fine scissors and forceps (Dumont no. 5). The ends of the preparation were tied to a thin stainless-steel needle which was fixed to the lower part of the oxygen electrode by an O-ring (Fig. 1C), the preparation being about 3 mm from the plane of the cathode. The oxygen electrode and the photophores were immersed in a glass tube (50 mm length; 14 mm internal diameter) in a temperature-controlled chamber (20°C); the tube was filled with 4 ml saline and sealed to the electrode at the top with paraffin.

*Measurement of luminescence*

The light-emitting area of the preparation was 10 mm from the photomultiplier (PM 270C, International Light) the photosensitive area of which was placed on the transparent wall of the thermostatic chamber. The signal was displayed on a two-channel strip-chart recorder. The apparatus was calibrated using a tritium-irradiated phosphor (Betalign, Saunders Roe, Nuclear Enterprises, Ltd) emitting on an area of 2 mm<sup>2</sup>, in the same location as the light organ.

*Measurement of oxygen consumption*

The oxygen consumption of the preparation was estimated from the oxygen content of the surrounding saline during a given time period, using a polarographic oxygen electrode as previously described (Mallefet & Baguet, 1984). To measure the oxygen consumption of the preparation, the electric current of the electrode was reduced by 90% with a back-off system and the remaining signal amplified.

*Experimental procedure*

In each experiment, the oxygen electrode was calibrated in oxygen-free saline (prepared by adding sodium dithionite, Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>, to reduce dissolved oxygen) and in fully aerated saline (20°C); the oxygen consumption of the electrode, without the light organ, was then measured for 10 min.

The preparation, attached to the electrode, was immersed in fully aerated saline to measure the oxygen consumption and the luminescence level for 10 min. After this, 0.4 ml KCN was injected into the saline, through a capillary fixed on the side of the electrode, to achieve dilutions of  $5 \times 10^{-4}$ ,  $5 \times 10^{-5}$  and  $5 \times 10^{-6}$  mol l<sup>-1</sup>. Oxygen

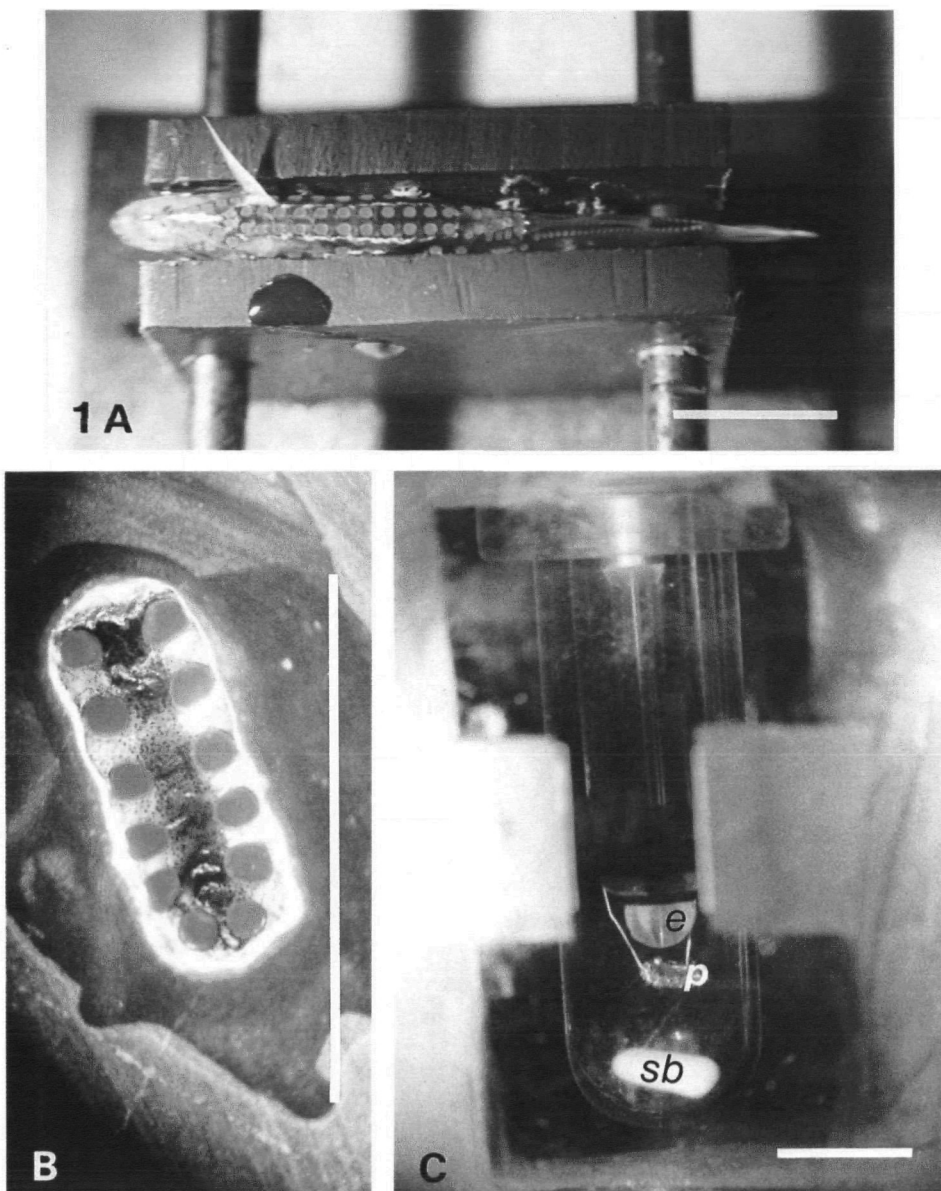


Fig. 1. (A) Ventral view of *Maurolicus* photophores. (B) Isolated row of abdominal *Maurolicus* photophores. (C) Oxygen electrode (*e*) and photophores (*p*) in saline with stirring bar (*sb*). Scale bars, 1 cm.

consumption and luminescence were followed for 40 min. Afterwards, the preparation was blotted with a filter paper and weighed with an electrobalance (Metler H10, accuracy 10  $\mu$ g).

From the measurements in aerated saline and the known volume of saline containing the preparation, the rate of oxygen consumption was calculated in  $\text{nmol min}^{-1}$ . The results are expressed as mean values with standard errors (mean  $\pm$  S.E.M.) and the number of preparations ( $N$ ), together with the statistical significance difference between different mean values.

## RESULTS

### *Oxygen consumption of resting photophores*

The resting respiration rate was measured on 50 preparations, the experiments being carried out from 2 to 13 h after fishing. The duration of storage of the specimens did not appear to affect the oxygen consumption of the isolated preparations. The mean oxygen consumption rate of preparations excised 2–5 h after capture of the fish ( $1.38 \pm 0.08 \text{ nmol min}^{-1}$ ) did not differ significantly from the mean rates of preparations excised 5–9 h ( $1.24 \pm 0.11 \text{ nmol min}^{-1}$ ) and 9–13 h ( $0.91 \pm 0.23 \text{ nmol min}^{-1}$ ) after capture (Table 1).

The mean value for 50 preparations was  $1.25 \pm 0.07 \text{ nmol O}_2 \text{ min}^{-1}$  for an average mass of  $10.24 \pm 0.38 \text{ mg}$ . Regression analysis of the resting oxygen consumption, plotted as a function of the mass of the preparations, shows a significant correlation of the two parameters ( $r = 0.69$ ;  $N = 50$ ) which is described by the equation  $\text{O}_2 = 0.126 \pm 0.019P - 0.037$ , where  $\text{O}_2$  is the oxygen consumption in  $\text{nmol min}^{-1}$  and  $P$  the fresh mass in mg (Fig. 2).

### *Effects of potassium cyanide (KCN) on luminescence and oxygen consumption*

In the presence of  $5 \times 10^{-6} \text{ mol l}^{-1}$  KCN, two preparations produced a dim luminescence, commencing  $303 \pm 33 \text{ s}$  after the application of cyanide and reaching a

Table 1. *Mean values ( $\pm$  S.E.M.) of the respiration rate of photophores isolated from fish at different times after capture*

	Time after capture (h)		
	2–5	5–9	9–13
Fish length (mm)	$40.5 \pm 1.2$	$42.9 \pm 1.1$	$41.3 \pm 1.6$
Fish weight (mg)	$997.8 \pm 73.0$	$1206.9 \pm 88.9$	$1059.5 \pm 97.1$
Photophore mass (mg)	$10.23 \pm 0.49$	$10.63 \pm 0.65$	$9.10 \pm 1.18$
Photophore $\text{O}_2$ consumption ( $\text{nmol min}^{-1}$ )	$1.381 \pm 0.08$	$1.241 \pm 0.11$	$0.91 \pm 0.23$
$N$	22	21	7

$N$  is the number of experiments carried out on 2–5, 5–9 and 9–13 h after fishing.

The lengths and masses of the fish, as well as the masses of the corresponding isolated preparation, were similar in the three groups; there is no significant difference in the oxygen consumption rates measured 2 or 13 h after capture.

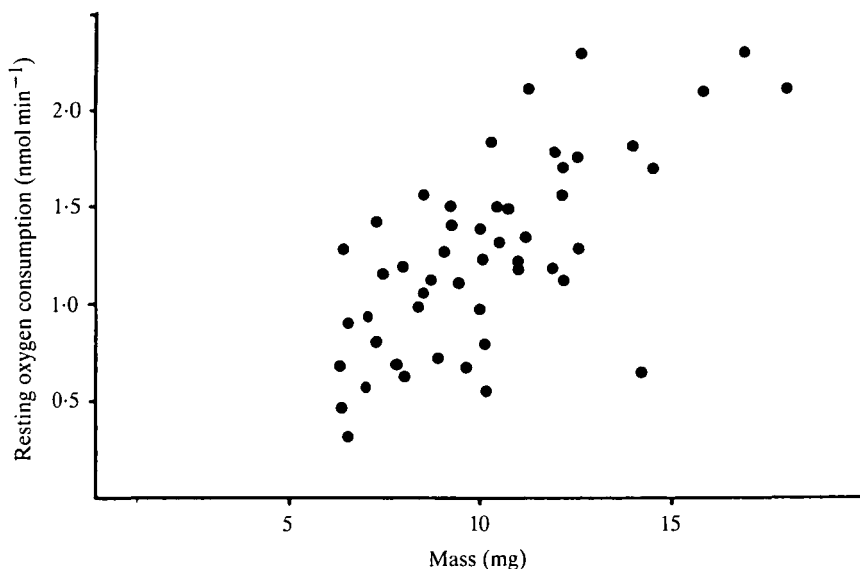


Fig. 2. Relationship between the resting oxygen consumption and the fresh mass of abdominal photophores excised from *Maurolicus muelleri*.

constant value of  $44.87 \pm 3.97$  Mquanta  $s^{-1}$  after  $1554 \pm 150$  s. The two glowing and seven non-glowing preparations showed similar time courses of oxygen consumption (Fig. 3A). The oxygen uptake decreased immediately after the application of cyanide. After 3 min the mean respiration rate was  $0.85 \pm 0.28$  nmol  $min^{-1}$ , which corresponds to about 70% of the previous resting level. Subsequently the oxygen uptake declined, at a slower rate, to reach a constant level after 27 min corresponding to about 50% of the previous resting level.

In  $5 \times 10^{-5}$  mol  $l^{-1}$  KCN, all the preparations ( $N = 9$ ) produced a slow, low light emission, beginning  $341.4 \pm 52.6$  s after the application of cyanide and reaching a plateau ( $49.73 \pm 21.18$  Mquanta  $s^{-1}$ ) after  $1228 \pm 188.8$  s. The mean oxygen consumption rate seemed to decrease in two steps (Fig. 3B): a rapid fall of 40% after 3 min, followed by a slower decrease for the next 30 min, when the oxygen uptake reached a steady level corresponding to about 23% of the initial resting level.

The time course of the mean oxygen consumption and the mean production of light in  $5 \times 10^{-4}$  mol  $l^{-1}$  KCN are shown in Fig. 3C. All 24 preparations responded with a long-lasting luminescence, commencing  $102.6 \pm 13.9$  s after addition of KCN and reaching a maximal value ( $826.8 \pm 174.9$  Mquanta  $s^{-1}$ ) after  $1466.1 \pm 95.2$  s. 16 min later, at the end of the experimental period, the light emission level dropped to 42% of the peak value. The oxygen uptake dropped dramatically on addition of KCN, before the preparation had begun to luminesce, and reached a mean value of  $0.45 \pm 0.08$  nmol  $min^{-1}$  after 3 min, which corresponded to 35% of the initial resting level. Subsequently, there was a further, slower decrease: after 15 min oxygen consumption had decreased by 92% and it remained at this steady level for the next 25 min. It is noteworthy that most of the light was produced during this period, i.e. after the oxygen consumption had been virtually blocked (Fig. 3C). Although these

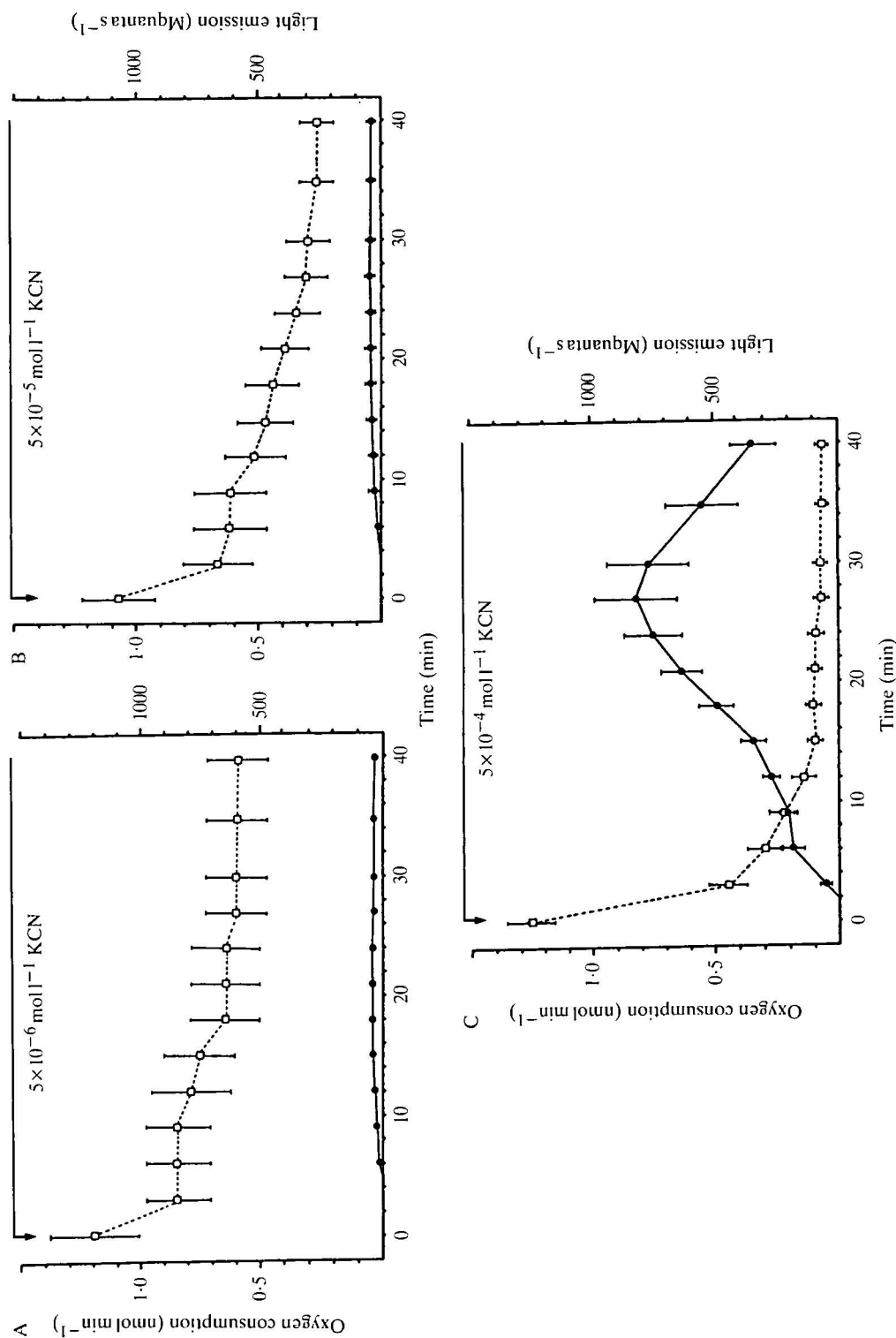


Fig. 3. Oxygen consumption ( $\square$ , in  $\text{nmol min}^{-1}$ ) and light emission ( $\bullet$ , in  $\text{Mquantas}^{-1}$ ) of abdominal photophores of *Maurolicus muelleri* in response to (A)  $5 \times 10^{-6} \text{ mol l}^{-1} \text{ KCN}$ , (B)  $5 \times 10^{-5} \text{ mol l}^{-1} \text{ KCN}$  and (C)  $5 \times 10^{-4} \text{ mol l}^{-1} \text{ KCN}$ . The oxygen consumption at zero time is the resting oxygen consumption of the preparation.

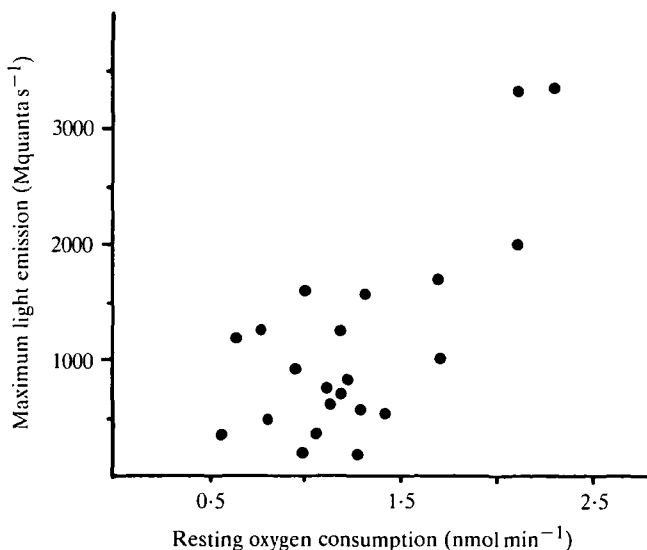


Fig. 4. Relationship between the maximal light emission in response to  $5 \times 10^{-4}$  mol l<sup>-1</sup> KCN and the resting oxygen consumption of abdominal photophores excised from *Maurolicus muelleri*. This significant relationship can be described by the equation ( $r = 0.73$ ;  $N = 22$ )  $y = 1350 (\pm 282)x - 580 (\pm 1460)$ , where  $y$  is the maximum light (in Mquanta s<sup>-1</sup>) and  $x$  is the oxygen consumption (in nmol min<sup>-1</sup>). The regression line crosses the y-axis at  $580 \pm 1460$  Mquanta s<sup>-1</sup>.

results indicate that light production is independent of oxygen uptake, it seems that the ability of an isolated preparation to emit light is dependent on the intensity of its resting oxygen consumption (Fig. 4). The data in Fig. 4 show that light production is not significantly different from zero when there is no oxygen consumption; the slope,  $b$ , of the calculated regression line indicates that for 1 nmol oxygen consumption per minute at rest, a preparation produces  $1350 \text{ Mquanta s}^{-1}$  in response to  $5 \times 10^{-4} \text{ mol l}^{-1}$  KCN.

#### *Effects of adrenergic inhibitors on the light response to KCN*

The sensitivity of isolated photophores of *Maurolicus* to adrenaline and nor-adrenaline, and the significant decrease of the light response in the presence of  $\alpha$ - and  $\beta$ -adrenergic antagonists (Baguet & Christophe, 1983), suggest that they could be innervated by catecholaminergic neurones. It could be that KCN causes release of a neural transmitter, thus inducing luminescence of the light organ. We tested this hypothesis by comparing the light emission evoked by KCN in preparations which had been pretreated with phentolamine ( $\alpha$ -adrenergic antagonist) and propranolol ( $\beta$ -adrenergic antagonist).

To do this, two preparations were isolated from each fish and immersed either in normal saline or in saline containing phentolamine or propranolol ( $5 \times 10^{-4} \text{ mol l}^{-1}$ ) for 10 min; KCN was then added, to achieve a concentration of  $5 \times 10^{-4} \text{ mol l}^{-1}$ . The mean differences from controls of the light emission responses were  $49.7 \pm$

$41.1 \text{ Mquanta s}^{-1}$  ( $N=11$ ) in phentolamine-saline and  $31.3 \pm 34.8 \text{ Mquanta s}^{-1}$  ( $N=11$ ) in propranolol-saline.

Thus neither phentolamine nor propranolol significantly affected the intensity of the light emitted by *Maurolicus* photophores in the presence of  $5 \times 10^{-4} \text{ mol l}^{-1}$  KCN. Similarly, no change occurred in the time course of the oxygen consumption in the presence of either adrenergic antagonist.

## DISCUSSION

### *Resting respiration*

Previous research (Baguet & Christophe, 1983) has shown that isolated photophores from freshly collected specimens remain chemically excitable and luminesce in the presence of adrenaline and noradrenaline. The present work shows that the abdominal photophores exhibit a stable resting respiratory rate even when stored for 13 h and are therefore in a good physiological condition. The resting respiratory rate of 1 g of isolated photophores of *Maurolicus* is  $126.5 \pm 19.1 \text{ nmol O}_2 \text{ min}^{-1}$  ( $20^\circ\text{C}$ ;  $N=50$ ). This value is not significantly different from those of  $87.0 \pm 15.6 \text{ nmol O}_2 \text{ g}^{-1} \text{ min}^{-1}$  ( $N=18$ ) for the isolated ventral light organ of the mesopelagic fish *Argyrops leucostictus* (Mallefet & Baguet, 1985) and  $102.6 \pm 21.4 \text{ nmol O}_2 \text{ g}^{-1} \text{ min}^{-1}$  ( $N=12$ ) for the isolated photophores of the mesopelagic fish *Myctophum punctatum*. However, the resting respiration rate ( $335 \pm 24.4 \text{ nmol O}_2 \text{ g}^{-1} \text{ min}^{-1}$ ) of the isolated photophores of the epipelagic fish *Porichthys* is much higher (J. Mallefet & F. Baguet, unpublished observation).

According to Torres, Belman & Childress (1979) the oxygen consumption of mesopelagic fish is about two orders of magnitude less than that of epipelagic species; most of the difference appears to be due to lower aerobic metabolism in the muscle tissues (Siebenaller & Yancey, 1984). We therefore suggest that the amount of metabolically active tissue is higher in *Porichthys* than in *Maurolicus* photophores. Differences exist, but they are opposite to what could be expected: the protein content is greater in *Maurolicus* ( $312 \pm 84 \text{ mg g}^{-1}$ ) than in *Porichthys* ( $112 \pm 33 \text{ mg g}^{-1}$ ) photophores (D. Sempoux-Thinès & F. Baguet, unpublished observations) and the photocyte mass represents about 35% of the overall mass of the photophore in *Maurolicus* as compared with only 2.4–4.2% in *Porichthys* (LaRivière & Ancil, 1984). The present results confirm our suggestion (Mallefet & Baguet, 1985) that the lower rate of aerobic metabolism of photophores of a mesopelagic fish might be an inherent characteristic of these deep-living luminescent species.

### *Effects of KCN*

Our results suggest that KCN has a dual effect on isolated photophores of *Maurolicus*: (i) it induces occasional light production at  $5 \times 10^{-6} \text{ mol l}^{-1}$  and always induces luminescence at  $5 \times 10^{-5}$  and  $5 \times 10^{-4} \text{ mol l}^{-1}$ , and (ii) it decreases the oxygen consumption of the photophores whether they produce light or not, the inhibitory effect increasing from  $5 \times 10^{-6}$  to  $5 \times 10^{-4} \text{ mol l}^{-1}$ . When KCN induces light production in *Maurolicus* photophores it decreases the oxygen consumption prior to the

production of light. As there is good evidence that cyanide blocks the mitochondrial respiration of fishes in the same way as in mammals (Gumbmann & Tappel, 1962) it is likely that the inhibitory effect on the *Maurolicus* photophores can be explained by an inhibition of the resting respiration rate of the tissues. As  $5 \times 10^{-4} \text{ mol l}^{-1}$  KCN evokes a large and long-lasting luminescence, our results emphasize that mitochondrial respiration is not essential for the process of light generation. Moreover, the decrease in oxygen consumption suggests the inhibition of a mechanism that must prevent spontaneous luminescence of the photophore. By analogy with the photophores of the epipelagic luminescent fish *Porichthys*, which also luminesce in the presence of  $10^{-5}$ – $10^{-3} \text{ mol l}^{-1}$  KCN (Mallefet & Baguet, 1984), it might be that, by inhibiting cellular respiration, the mechanism which sequesters intracellular calcium would be inhibited by KCN, so that the increase in free  $\text{Ca}^{2+}$  could stimulate the luminescent system.

Since histological and anatomical studies on the photophores of *Maurolicus* have demonstrated the presence of nerves (Oshima, 1911), it might be argued that KCN does not act directly on the photocytes but indirectly on the neural processes of the photophore. The absence of any significant effect of adrenergic inhibitors on the luminescence induced by KCN rules out the possibility that this metabolic inhibitor triggers release of an adrenergic neuromediator.

The biochemical mechanism for the luminescence in *Porichthys* photophores consists of a luciferase-catalysed oxidation of luciferin by oxygen (Tsuji *et al.* 1975). It has been suggested that the increase in oxygen consumption associated with the light emission evoked by KCN originates from the oxidation of luciferin by molecular oxygen (Mallefet & Baguet, 1984).

Since the production of light by *Maurolicus* photophores is not associated with increased oxygen consumption, it is likely that oxygen plays different roles in the control of light generation in *Maurolicus* and *Porichthys* photophores. All known bioluminescent systems require oxygen to produce light *in vitro* (Hastings, 1983); a bioluminescent system such as coelenterazine (coelenterate luciferin) can be induced to produce light *in vitro* simply by the addition of calcium, oxygen apparently not being required (Shimomura, Johnson & Saiga, 1962). However, Hastings & Morin (1969) have shown that coelenterazine is precharged with oxygen to form a stable peroxide. In this case, it would be expected that no oxygen would be consumed during light emission, since it is stored as a preoxygenated intermediate. Although we have not examined the biochemical nature of the bioluminescent system in *Maurolicus* photophores, it seems likely that some preoxygenated substance could also be involved in luminescence.

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