BIOLUMINESCENCE OF SHARKS, A CASE STUDY: *ETMOPTERUS SPINAX*

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INTRODUCTION

Bioluminescence arose independently in a wide range of species, from bacteria to fishes, which are the only luminous vertebrates. Consequently, luminescent species demonstrate a great diversity in the structure, in the control, as well as in the function of their photogenic system.[1] Among luminous organisms, cartilaginous fishes are probably the least investigated group and incredibly few information is available concerning their bioluminescence.[2]

Even if it has been once suggested for some sharks of the genius *Somniosus* and *Megaschasma*.[3,4] symbiotic luminescence, common in teleosts, seems unlikely in chondrichtyes, This group contains however numerous self-luminous species, with at least one species of ray (*Benthobatis moresbyi*), and probably more than 50 different sharks (~13% of current shark species).[5,6] Luminescent sharks belong to 2 squalid subfamilies, the Etmopteridae (lantern sharks) and the Dalatiidae (dwarf mesopelagic sharks), which evolved separately 90 M.Y. ago, it is therefore possible that the bioluminescence arose 2 times independently in sharks.[6] Until now, only information regarding the photogenic structures of these sharks is available in the literature. Dalatiidae have photophores constituted of a single photocyte (=photogenic cell) placed in a pigmented cup and covered by a lens formed by a group of small cells, while photogenic organs of Etmopteridae are more elaborated, composed of a pigmented sheath containing several photocytes, one of several lens cells, and an iris-like structure which has been suggested to allow a control of light emission.[6,7] In both groups photocytes present granules supposed to contain the luminescent material.[6,8,9] Luminous sharks have also a specialized squamation allowing photophore accommodation in the skin.[2] The physiological control, the biochemistry, and the function of bioluminescence in these fishes remain totally unknown due to a lack of experimental data. Based on simple observation of the luminous pattern, authors have suggested that Dalatiidae would use their luminescence for counterillumination while Etmopteridae could in addition use it as a schooling aid. The aim of this work is to use morpho-physiological techniques to investigate the control and the function of bioluminescence in the velvet belly lantern shark *Etmopterus spinax*, a common etmopterid species.

MATERIALS AND METHODS

In February and December 2007, specimens of *E. spinax* (22.5-52.5 cm *TL*, total length) were collected in the Raunefjord, Norway. Light microscopy, fluorescence microscopy, and digital imaging analysis software were used to investigate bioluminescence of embryos and free-swimming specimens. We followed the elaboration of the luminous pattern and the development of photophores to determine when they become able to produce light. The density, the size of photophores, as well as the ventral surface occupied by photophores and luminous tissues were calculated for all the sharks.

Peroxide-induced luminescence was also recorded from luminous tissues of 30 different sharks, grouped by 10 cm categories, via a luminometer Berthold FB12. Light response was standardized using the maximal intensity of light in megaquanta per second per square centimetre for each luminous zone (L_{max} in $Mq.s^{-1}.cm^{-2}$).

A theoretical visual model was equally performed using these data as well as photophore density to estimate maximum visual range of luminous zones and the depth at which these zones match the downwelling light in adult sharks (> 30 cm).

A first screening of classical neurotransmitters and hormonal drugs was performed on adult sharks to investigate the control of luminescence in *E. spinax*.

RESULTS AND DISCUSSION

We established the sequential apparition of 9 different luminous zones during *E. spinax* embryogenesis (Fig.1). We followed the organogenesis of photophores which is a well controlled process whose the last observable event is the apparition of fluorescent vesicles inside the photocytes. These vesicles are also observed in photophores of adult *E. spinax* and *E. lucifer* (Fig. 2A). At this moment photophores can emit light after peroxide application. Spontaneous luminescence in embryos confirms that they are able to luminesce before birth (Fig. 2B). During embryogenesis the ventral surface covered by photophore and luminous zone increase, and attains 38% and 82%, respectively. During this period, the diameter of photophores increases while their density decreases. Although then number of tested embryos is limited, it seems that light capabilities induced by peroxide application attained its maximum just before birth (Fig. 3). All these results strongly suggest camouflage by countershading in juveniles, more subject to predation than adults.

The maximum theoretical visual ranges were obtained at 700 m when the shark is on its back, a behaviour frequently observed in aquarium. Even though these ranges were relatively weak (<1.5 m) they could be an aid for species recognition, for mating, and for schooling in *E. spinax*. All the zones would match the downwelling light around 600 m, a depth at which adults of this species are found in the Mediterranean Sea which would therefore be also able to counterilluminate.[10]

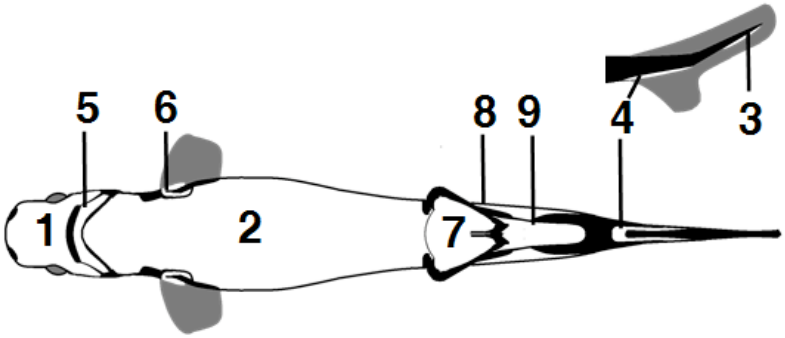

Figure 1. Luminous pattern of *Etmopterus spinax*. Each luminous zone has a number corresponding to its order of apparition: 1, rostral; 2, ventral; 3, caudal; 4, infra-caudal; 5, mandibular; 6, pectoral; 7, pelvic; 8, lateral; 9, infra-pelvic.

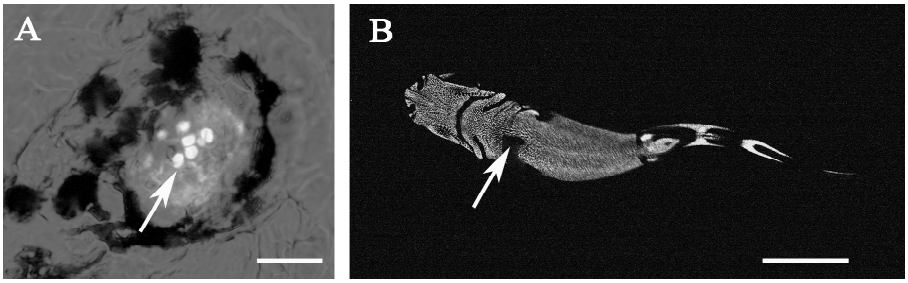

Figure 2. (A) Photocytes' fluorescent vesicles present in the centre of a photophore of *Etmopterus lucifer* (epifluorescent microcospy). Dashed line indicates the location of the pigmented sheath surrounding the photocytes. Scale bar = 50 µm. **(B)** Self glowing embryo (11 cm *TL*) of *Etmopterus spinax*. Arrow indicates the insertion of the yolk sac. Scale bar = 1 cm.

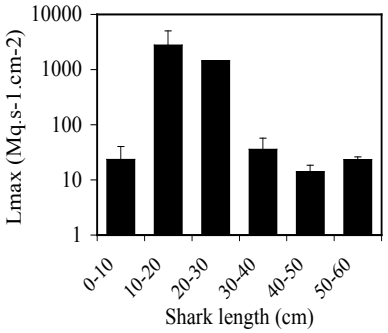

Figure 3. Maximum light emission of the ventral zone induced by hydrogen peroxide in relation to the size of the sharks. Dashed line separates embryos from free-swimming fish. Values are expressed as mean ± SEM.

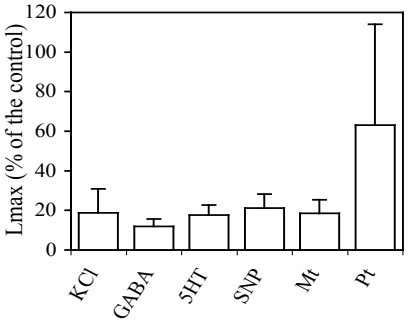

Figure 4. Drugs having triggered the light emission in *E. spinax*. SNP = Sodium nitroprusside (NO-donnor). Mt = Melatonin. Pt = prolactin. N = 7 (excepted for Pt where N = 3). Concentrations: KCl = 0.2 M, others 10^{-3} M. Control = H_2O_2 0.35 M.

Results of the pharmacological screening are shown in Fig. 4. Response to KCl as well as to GABA and 5HT strongly suggests a nervous control of luminescence in *E. spinax*. Moreover, high responses to melatonin and prolactin are in favour of an additional hormonal control of luminescence, which has never been highlighted in a fish before. NO-donnor (SNP), could have a modulator role in control of luminescence of *E. spinax* as in *Argyropelecus hemigymnus*, a luminous teleost.[11]

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