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UCL **The luminescence of the deep-sea shark *Etmopterus spinax* is not bacterial.**

Mallefet Jérôme^{1,2*}, Duchateau Laurent¹, Henry Pablo², Olivier Henriet², and Jacques Mahillon²
¹ Laboratory of Marine Biology, ELIB, Université Catholique de Louvain, Louvain-la-Neuve, Belgium
² Applied microbiology laboratory, ELIM, Université catholique de Louvain, Louvain-la-Neuve, Belgium
 *Corresponding author. E-mail: Jerome.mallefet@uclouvain.be

INTRODUCTION

Five years ago, a first attempt to characterize the chemistry of the luminous system in the velvet belly lantern shark, *Etmopterus spinax*, was not conclusive [1]. Despite that three potential sources of luciferin were found in the diet of the shark, none of the classical cross-reaction using tetrapyrrole luciferin, coelenterazine and cyprinid luciferin were able to highlight a luciferin/luciferase reaction within the photophores extract of the shark. In this work, the possibility of the presence of extrinsic luminescence, i.e. light production from bacterial origin within the photophore was tested using transmission electron microscopy, in situ hybridization and PCR methods.

MATERIALS & METHODS

Fish collection

Transmission electron microscopy (TEM)

Transmission in situ hybridization (FISH)

PCR

RESULTS & DISCUSSION

TEM

FISH

qPCR

CONCLUSIONS

- Nor bacteria neither bacterial tubules observed in the photocyte/photophore.
- No trace of bacteria in the photophore detected by FISH (except in positive control).
- Very low number of bacteria measured by qPCR in the ventral skin of *E. spinax*.

References

[1] Mallefet J, et al. (2011) The luminescence of the deep-sea shark *Etmopterus spinax* is not bacterial. *PLoS One* 6(12): e28111. doi:10.1371/journal.pone.0281111

PHOTODIODE CALIBRATION METHOD FOR REPORTING BIOLUMINESCENCE MEASUREMENTS IN STANDARDIZED UNITS

DA BARTHOLOMEW, DI ANDRAGÉ
 Dept of Bioluminescence, University of Utah, Salt Lake City, Utah 84132-5020, USA
 Email: dbartholomew@uconn.edu

INTRODUCTION

Bioluminescence measurements are reported in relative light units (RLU), which are dependent on the specific detector used and the operating conditions. To make comparisons between measurements from different detectors and assays, or to determine the quantum efficiency of a particular assay, photometers need to be calibrated such that measurements are reported in absolute photons (photon⁻¹). This allows intensity measurements to be reported in units normalized to the collection angle and independent of the device. We explore a calibration method using a collimated light emitting diode (LED) as a light source. The emission light source is characterized in terms of its radiance and the collection angle. The intensity of the collected light source is then used to calibrate a photomultiplier tube (PMT) based bioluminescence and a charge-coupled device (CCD) in terms of photons. The method allows for generalization of detector response and light source.

METHODS

In order to calibrate a detector, the intensity of a source $I_s(r, \theta)$ (in radiance units of W/m²sr) must be known as a function of time, position in the detection plane, r (in μ m), and wavelength λ (nm). An LED operating at a steady state temperature with a constant current source and a diffuser plate is used in order to define the intensity as a function of r , θ , and λ . In this case, I_s is a constant value for all r and θ due to the constant temperature and source. Since the Lambertian diffuse plane average only varies as $1/r^2$ due to inverse temperature on the surface of the LED, I_s can be integrated over the emission spectrum independent of r . $I_s(r, \theta, \lambda)$ can be further simplified by normalizing r and θ according to their respective peak intensities in position and wavelength. Thus, $I_s(r, \theta, \lambda)$ for the LED source described becomes:

$$I_s(r, \theta, \lambda) = I_0 \exp(-\alpha r) \exp(-\beta \theta) \exp(-\gamma \lambda)$$

Equation 1.
 The optical power of the source at the detection plane, P_d (to collect the units of watts), is the integration of the intensity, I , normal to the detector over the area, A , of the detector aperture. P_d is the collected photon density (equal from incident source power, P_s , on the detection plane as a function of the detector

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