Couette Flow−induced bioluminescence in the dinoflagellate *Pyrocystis noctiluca*.

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ABSTRACT

Plankton organisms living in the sea undergo turbulence at a great range of different scales. This turbulence affects phytoplankton in many ways such as nutrient assimilation, spatial distribution, cell division and growth rate. Mechanical agitation is also responsible of the light emission in bioluminescent dinoflagellate species (Biggley 1969). These organisms are responsible of luminous trails observed around moving ships, dolphins and breaking waves (Rohr et al. 1998, 2002). Dinoflagellates respond to mechanical stimulation in less than 20 ms (Widder and Case 1981) with a light flash of approximately 100 ms in duration. The total mechanically stimulated bioluminescence has been measured in different species and varies from 10^8 photons $cell^{-1}$ in *Gonyaulax sp*. to $0.5 - 6.10^{10}$ photons $cell^{-1}$ in *Pyrocystis sp*. The light emission is in the blue−green wavelengths, with the maximum centered around 473 − 478 nm (Hastings and Morin 1991). It has been shown that bioluminescent dinoflagellates exhibit a circadian rhythm with light emission that can be 100 times brighter at night than during the day.

Turbulence has always been used to stimulate bioluminescence in all kind of devices devoted to measure bioluminescence . This turbulence can be generated by different manners such as a stirrer (Aiken and Kelly 1984), or a grid (Widder et al. 1993). The exact cellular mechanism by which the light emission is triggered is not completely understood. The physical deformation of the cell membrane is believed to trigger an action potential propagating along the internal vacuolar membrane (Widder and Case 1981). This action potential generates the migration of small vesicles and the liberation of their content : luciferin and luciferase.

In order to show that a constant shear can induce bioluminescence, Latz et al. (1994) used a Couette flow produced in the gap between two concentric cylinders. They found that stationary laminar flow stimulates bioluminescence with a response threshold of 0.1 N m^{-2} to 0.3 N m^{-2}. Similar values were obtained with cultured or freshly collected plankton flowing through a fully developed pipe flow (Latz and Rohr 1999, 2000). However, they also report that a dramatic increase of bioluminescence is observed when transition occurs from laminar to turbulent flow (Rohr et al. 1990).

This paper is devoted to the study of bioluminescence in the dinoflagellate *Pyrocystis noctiluca.* Revisiting the Couette experiments of Latz et al. (1994), we studied the response of the bioluminescent reaction to controlled mechanical constraints. Our Couette device (Fig. 1) was designed after the one used by Latz et al. (1994) given the fact that our primary goal was to study dinoflagellates bioluminescence following Latz et al. results (1994). The length of the two cylinders is 190 mm. The inner diameter of the outer cylinder is 52.3 mm and the diameter of the inner cylinder is 46.3 mm, conferring a 3 mm gap between the two concentric cylinders. The cylinders are positioned vertically. The inner cylinder is made of black polished acetal plastic and the outer one is in transparent glass so the light can be measured by the camera and the photomultiplier tube. This

external cylinder is driven by a d.c. servo− motor. Rotational speed is measured by an optical encoder with an accuracy better than 1 %.

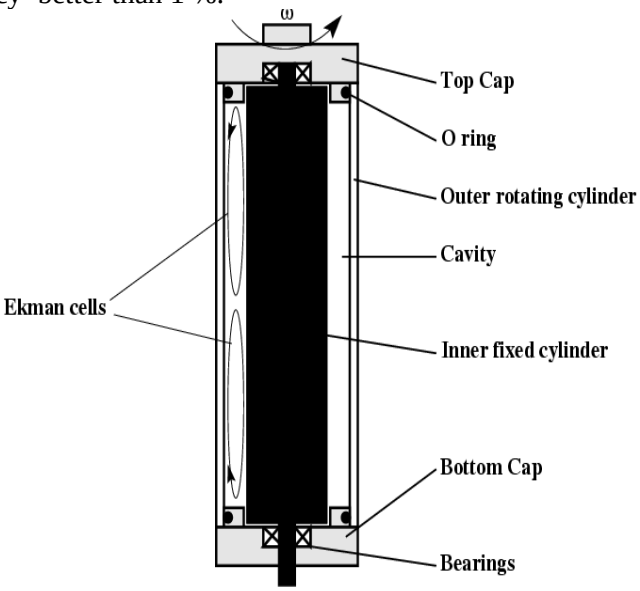

Figure 1: *Experimental set−up with the indication of the Ekman cells in the flow.*

Laboratory cultures of dinoflagellates *Pyrocystis noctiluca* Murray et Haeckel (strain number 732 obtained from the CCMP, Bigelow) were grown in enriched f/2 media and maintained in a culture chamber at 20°C +/− 2°C on a 12:12 L:D cycle. Subjective day always started at 12 am and subjective night at 12 pm. Experiments were always conducted between 3 pm to 6 pm so the cells were in the middle of the dark phase and the level of emitted light was maximum, according to the bioluminescence rhythmic cycle in dinoflagellates.

As the gap width is small compared to the cylinder radius (ratio equal to 0.06), the rotating flow can be approximated at first order by a linear function. Thus, the shear stress is homogeneous in the whole flow and is proportional to the rotation frequency of the outer cylinder: In fact, this description of the flow field is only valid for infinitely long cylinders. Because of the finite size effects, a three dimensional flow takes place between the cylinders. This flow, superimposed on the Couette primary flow, generates complex three dimensional shear constraints at both cylinder ends and also in the equatorial plane of the flow. This phenomenon due to the rotating extremity caps is well known in fluid mechanics and is called the Ekman pumping. It creates two contrary recirculating eddies called the Ekman cells that meet each other in the equatorial plane of the set−up (Fig. 1). Thus, the exact shape of the stream lines is more like an helix with a very small pitch than a circle. An intensified video camera (ULL509, Lhéritier) was used to record images of the whole cylinder. A photomultiplier tube (Hamamatsu H6180−01) was used to measure the light flux emitted by the dinoflagellates. An interferential filter centered on 475 nm is mounted on the head of the photomultiplier. The photon number was integrated over a chosen period of 10 ms. The photomultiplier was placed at 40 cm of the test cylinder so a correction factor was applied to obtain absolute values of the total light emitted in each experiment. The absolute values of photons fluxes were then normalized by the total number of cells contained in the solution between the two cylinders (75 ml). So for each experiment, the light emission is given in number of photons per second and per cell (Photons s^{-1} $cell^{-1}$). In addition, we have checked that the total photons number is directly proportional to the cell concentration (for concentrations between 10 and 800 cell ml^{-1}).Two sets of experiments were conducted by increasing progressively the rotation speed of the cylinder from 0 to 26 Hz in a total duration of 2 mn.

In the first experiments, we realized that turbulence could appear at different (non unique) rotation rates. Indeed, the transition to turbulence in the cylindrical Couette flow is not given by a continuous instability process as it is, for instance, the case for the Taylor−Couette flow. On the contrary, in the case of interest here, turbulence appears through sudden bursts of finite amplitude (Daviaud et al. 1992). Therefore, the transition is hysteretic and care must be taken in order to get reproducible results. In our case, we choose to trigger turbulence (run labeled A in Fig. 2) by placing a small rubber band on the top of the fixed inner cylinder. In this way, transition to turbulence is under control, reproducible, and appears around 13 Hz This value is the lowest rotation rate for which turbulence was obtained. Without the rubber tape, the transition appears naturally at a

higher value of the rotation speed if this latter is increased very slowly and if no air bubble is stuck between the two cylinders (run labeled B).

Contrary to the findings of Latz et al. (1994) we show that the stationary homogeneous laminar shear flow does not excite the bioluminescent reaction in *Pyrocystis noctiluca*. Transition to turbulence is absolutely required to stimulate massive bioluminescence in this species. Our photometric measurements and video image analysis quantify these findings and confirm the conclusions of Anderson et al. (1988). Further experiments considering transient flows give indications on the nature of the plankton response to changes in mechanical stimulation. However, because of recirculating Ekman cells induced by unavoidable finite size effects, a smaller response of the dinoflagellates to the non homogeneous three dimensional flow is observed. This light emission is visualized at the end caps and in the equatorial plane of the Couette flow. These findings allow a new interpretation of the conclusions made by Latz et al. (1994).

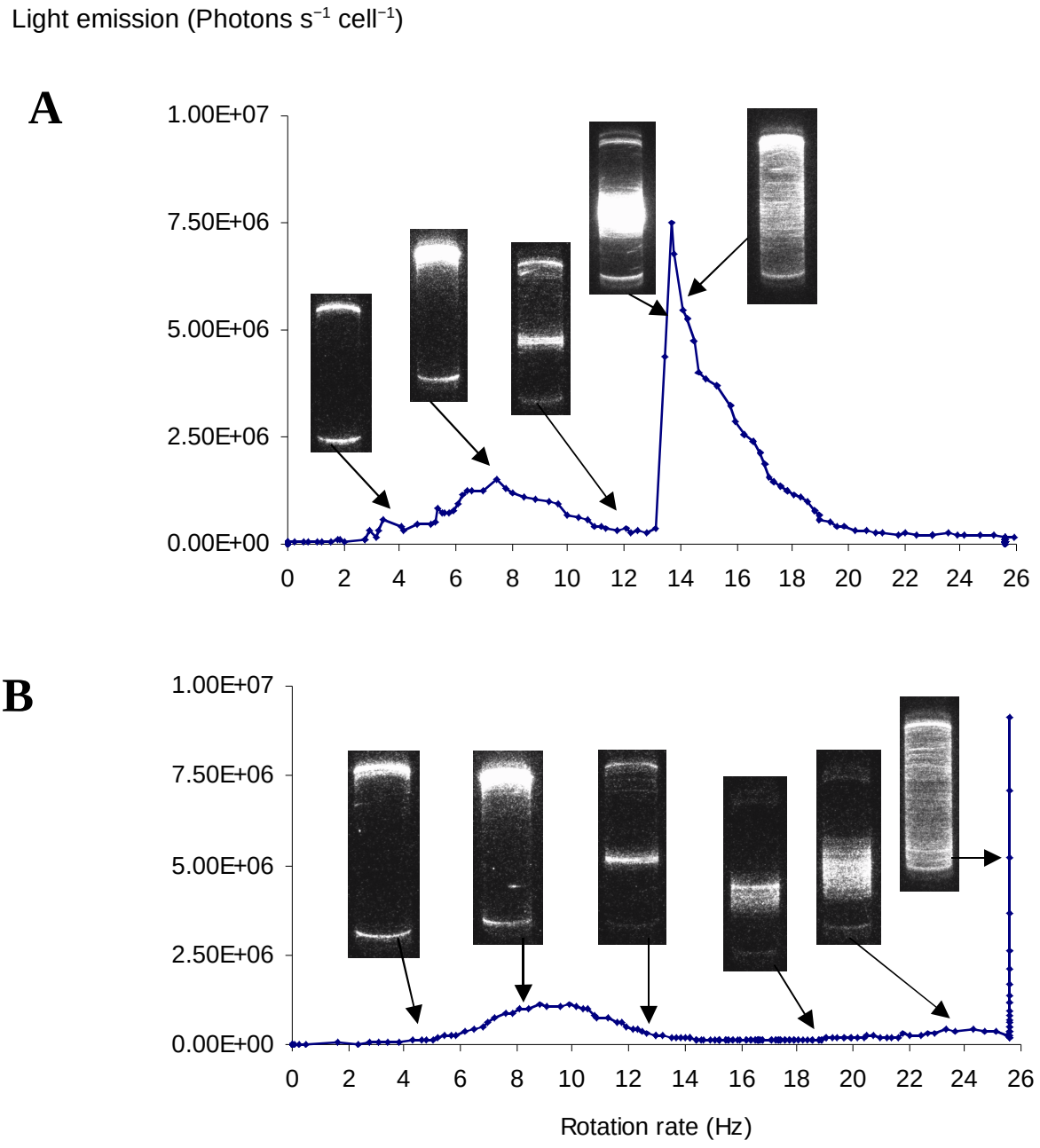

Figure 2. *Light emission with increasing rotation speed. The cylinder extremities are visible. The first bell−shape part of the curve corresponds to the light emission produced at both extremities of the cylinder, where high mechanical constraints occur. Bioluminescence is triggered when turbulence occurs at 13.6 Hz (A) and 25.6 Hz (B). Cells concentration is around 325 cells ml^{-1}*

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