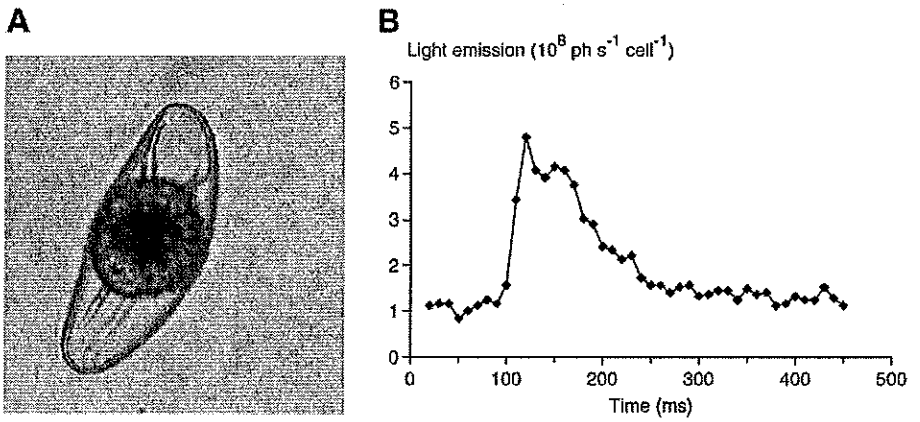

Fig. 1. (A) Optical microscope photography of a 200 μm length *Pyrocystis lunula* cell. (B) Temporal record of the light emitted during a spontaneous flash of *P. lunula*. The maximum value is of the order of 5 10^8 ph s^{-1} $cell^{-1}$. The flash duration is around 180 ms.

phase of exponential growth, cultures were kept in 800 mL culture flasks and diluted by a factor 1/10 approximately every 2 weeks. Cell abundances were determined by counting Lugol fixed samples in Nageotte slides under a dissecting microscope. Cell concentrations used for the experiments were 6240 cell ml^{-1} (+/−10%).

2.2. Experimental set-up

The experimental apparatus (Couette device, camera and photomultiplier tube) have been already used in our previous experiments on laminar and turbulent flows (Cussatlegras and Le Gal, 2004, 2005). The inner diameter of the outer glass cylinder is 52.3 mm and the diameter of the inner plastic (black acetal) cylinder is 46.3 mm. The length of the shearing chamber is 190 mm. The outer cylinder is set into rotation by a DC electric motor whose rotation rate is controlled by a feedback loop with an accuracy better than 0.2%. The rotation rate of this outer cylinder is measured by the mean of an optical coder which is mounted directly on its rotation axis. The totality of the experimental runs are recorded by an intensified video camera (ULL509, Lhétitier). The light emitted by the dinoflagellates is measured by a photomultiplier (Hamamatsu H6180-01) situated at a distance $L=40$ cm from the Couette chamber. The measure of the emitted light is given in number of photons per second and per cell (ph s^{-1} $cell^{-1}$) after integration over the whole sphere of radius L. As it will be shown in the following, the different measurements of the emitted photon numbers correspond to the classical levels of bioluminescence already published in the literature.

2.3. Measurement of individual flashes

Values for the flash intensity of *P. lunula* are not found in the literature and we have measured the intensity of single flashes in our cultures. Culture flasks were placed in the dark and spontaneous light emission has been measured by the photomultiplier. As spontaneous flashes are rare, individual flashes are clearly distinguishable and can be fully characterized. The maximum light emission is around 5.10^8 ph s^{-1} (Fig. 1B) and the duration of the flash is around 180 ms with a sharp increase (10–20 ms) and a long exponential decrease. The total integrated light emitted by a unique cell and for one flash is of the order 5.10^7 ph $cell^{-1}$. This value is fully consistent with values published in the literature if we consider that each cell can emit 20 flashes (Widder and Case, 1981. Indeed, as in autotrophic dinoflagellates, the total stimulable light is about 10^9 ph $cell^{-1}$ for *P. noctiluca* and *P. fusiformis* (Swift et al., 1985; Batchelder et al., 1992).

2.4. Experimental protocol

Before each experiment, fresh samples were transferred in a dark experimental room. The Couette chamber is filled with a culture sample by gently pouring it directly in the space between the cylinders. It was checked that less than 6% of the total emitted light was produced during the fill up operation.

In the first series of experiments, we have tested the effect of different shear flows under the same acceleration value. The Couette apparatus is started abruptly to produce three kinds of accelerated flows: 1) the two cylinders rotate in the opposite direction (called 'counter' for counter-rotation), 2) the two cylinders

rotate in the same direction (called 'corot' for co-rotation) and 3) only the outer cylinder rotates (called 'simple' for simple Couette flow). In a second series of experiments, simple transitory shear flow experiments have been performed but this time with different accelerations. Four values of acceleration have been obtained by placing four different-size aluminium inertial cylinders on the motor axis: 11.5 m s^{-2}, 5.75 m s^{-2}, 2.75 m s^{-2} and 1.6 m s^{-2}. Note that the centripetal acceleration due to the flow rotation has no effect as it is balanced by the radial pressure gradient.

3. Results

3.1. The bioluminescene response is determined by a critical shear

The magnitude of the bioluminescence response versus the mechanical stimuli applied on the dinoflagellate cells (shear and acceleration) is given by the value of the peak encountered in the temporal series recorded by the photomultiplier (Fig. 2). Most of the time, this peak is unique, but when the final rotational velocity is large, a subsequent transition to turbulence occurs in the flow (Cussatlegras and Le Gal, 2004). In this case, only the value of the first peak corresponding to the true response of the organisms to the applied controlled mechanical constraints is taken into consideration. Note that the subsequent peaks due to turbulence have the same order of magnitude of the first peak caused by the accelerated shear flow.

In the first series of experiments, we have tested the effect of different shear flows under the same acceleration value. The Couette apparatus is started abruptly and bioluminescence response is recorded by the photomultiplier tube (Fig. 2). Three kinds of accelerated flows are produced. In the first one, only the outer cylinder is set into rotation, thus the velocity $v_i(t)$ of the inner cylinder is kept equal to zero and the flow is the transient of the simple Couette shear flow ("simple" experiments). In the second series, both inner and outer cylinders are started together but rotate in counter-rotation: $v_o(t) = -v_i(t)$ ("counter" experiments). Finally, the third series concerns the transitory flow when both cylinders are rotated in the same direction i.e. in co-rotation: $v_o(t) = v_i(t)$ ("corot" experiments). In these three sets of experiments, the acceleration, when non-zero, is calculated at the inner surfaces of cylinders and is kept equal to 11.5 m s^{-2}. Fig. 2 displays the bioluminescent response corresponding to a final rotation frequency of 9 Hz and an acceleration of the outer cylinder of 11.5 m s^{-2}. The emitted light intensity is the highest when the two cylinders rotate in the opposite direction (- -'counter'), intermediate when the two cylinders rotate in the same direction (.... 'corot') and the weakest when only the outer cylinder rotates (— 'simple').

From a hydrodynamical point of view, it can be easily checked (Schichting, 1954) that more than 90% of the final speed is reached in about 2 s for the 'simple' experiments and in less than 0.5 s for the 'counter' experiments. Therefore, it is reasonable to think that the whole cell population has been stimulated by the accelerated shear flow. Thus the effective mechanical constraint applied on the dinoflagellate culture can be defined by the spatial average of the shear$\langle s(t) \rangle$. This is also in complete accordance with the time scales of the responses displayed in Fig. 2. Note that if only the cells nearby the walls were excited, the quantity of emitted light in the "corot" or "counter" experiments would be exactly twice that of the "simple" experiments. This is obviously not the case and thus confirms the fact that all the cells feel the non-stationary shear and allows the use of the effective shear$\langle s(t) \rangle$as a control parameter.

When considering the transitory regime that follows an abrupt start of one or both cylinders at a given constant acceleration, the fluid equation containing temporal derivatives of the velocity field must be considered. Supposing that the thickness of the fluid layer is small compared to its radius, the mean spatial shear intensity $\langle s(t) \rangle$inside the layer may be written as the integration of the norm of the velocity gradient

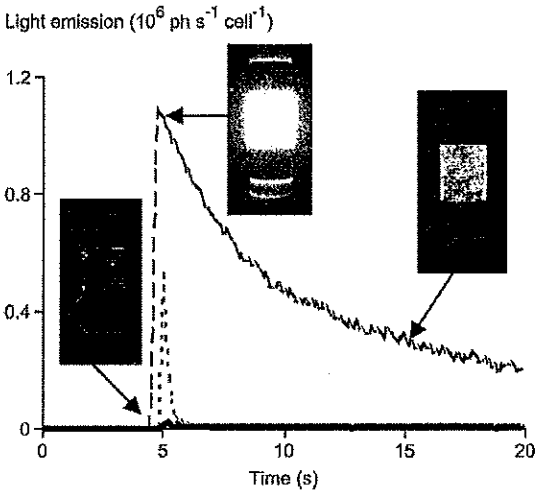

Fig. 2. Bioluminescence versus time stimulated by an abrupt start of the Couette shearing chamber. The final rotation frequency is 9 Hz and acceleration is 11.5 m s^{-2}. The light intensity is the highest when the two cylinders rotate in the opposite direction (- -'counter'), intermediate when the two cylinders rotate in the same direction (.... 'corot') and the weakest when only the outer cylinder rotates (— 'simple').

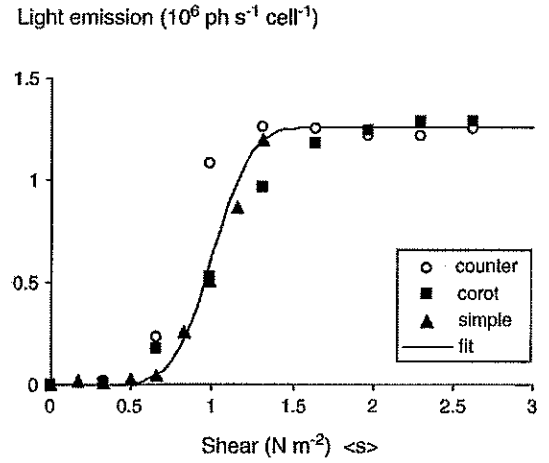

Fig. 3. Bioluminescence response in three kinds of accelerated Couette shear flows: 'counter' when the two cylinders rotate in the opposite direction, 'corot' when the two cylinders rotate in the same direction and 'simple' when only the outer cylinder rotate. The acceleration of these three runs is 11.5 m s^{-2} at outer cylinder. The three responses collapse on a single curve when plotted as a function of maximal shear. The solid line is an error function fit of the experimental data.

across the layer of thickness e. In each of the three cases, the mean intensity of the shear can be calculated as a function of the viscosity μ of water and $v_o(t)$, $v_i(t)$ and $v_{e/2}(t)$, the velocities of the outer and the inner cylinders respectively, and of the middle point inside the gap:

$$\text{—“simple” experiments : } \langle s(t)\rangle = \mu v_o(t)/e, \quad (1)$$

$$\text{—“counter” experiments : } \langle s(t)\rangle = \mu(v_o(t)-v_i(t)) = 2\ \mu v_o(t)/e, \quad (2)$$

$$\text{—“corot” experiments : } \langle s(t)\rangle = \mu(v_o(t) + v_i(t)-2v_m(t)) = 2\mu(v_o(t)-v_{e/2}(t))/e. \quad (3)$$

From these calculations, it appears that the mean shear produced during the experiments with counter-rotation is always and exactly twice the values it takes in the "simple" experiments. For the "corot" experiment, the shear is also exactly twice that produced in the "simple" experiments but only at the beginning of the transient when the velocity in the middle of the fluid layer $v_{e/2}(t)$ is inconsequential. It is then possible to plot together the three responses of the dinoflagellates population as functions of the maximum $\langle s\rangle$ of the mean shear $\langle s(t)\rangle$ encountered during the transient (Fig. 3). Results show that the three responses collapse on a single curve and therefore prove that the mean shear $\langle s(t)\rangle$ under a given acceleration is indeed one of the parameter that drives the bioluminescent response of *P. lunula*. The shape of the response to the applied mechanical stimuli is of sigmoid type and an error function can be fitted through the data points. The error function possesses 3 free parameters: its maximum, which is given by the value of the plateau reached at high shear, the position of the inflection point, which determinates the position of the critical shear, and finally the standard deviation, which gives the stiffness of the response.

3.2. Acceleration gives the response intensity

In a second series of experiments, only 'simple' transitory shear flow experiments have been performed but this time with different accelerations : 11.5 m s^{-2}, 5.75 m s^{-2}, 2.75 m s^{-2} and 1.6 m s^{-2}. The four corresponding bioluminescence responses are of sigmoid shape and can be fitted by an error function ('erf') (Fig. 4). Hence, The 4 fitted responses can be characterized by the maximum, the position of the inflection point and the standard deviation. The maximum value of the response is strongly dependent of the imposed acceleration (Fig. 5A). The maximum reached with the greatest acceleration is 1.2 10^6 ph s^{-1} $cell^{-1}$ and is in accordance with values published by Latz et al. (1994), Blaser et al. (2002) and Cussatlegras

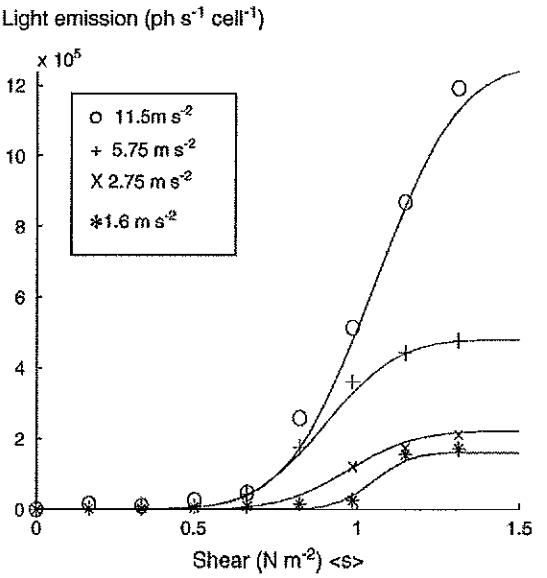

Fig. 4. Bioluminescence intensity versus mean final shear $\langle s\rangle$ for four different ramps corresponding to four accelerations of the outer tube of 11.5 m s^{-2}, 5.75 m s^{-2}, 2.75 m s^{-2} and 1.6 m s^{-2}. The four series of experimental data are fitted by four corresponding error functions. The maximum response intensity is clearly an increasing function of the acceleration.