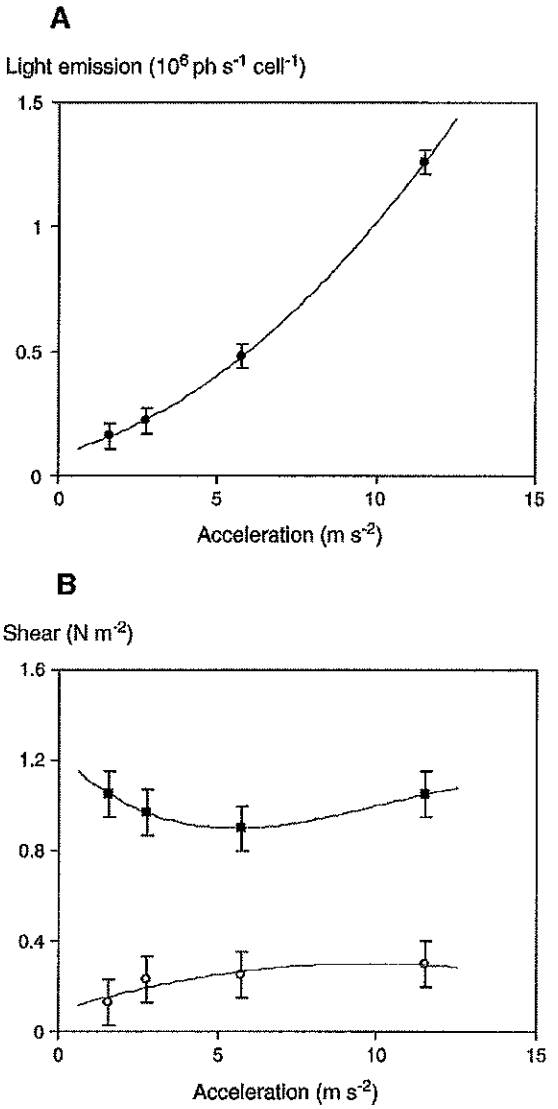

Fig. 5. (A) Maximum of bioluminescence response as a function of acceleration. For weak acceleration, the intensity of emitted light vanishes but is an increasing function of the acceleration. (B) Evolution of the threshold (solid circles) and of the stiffness (open circles) of the bioluminescence response as a function of the acceleration. No clear evolution is visible. Error bars are estimated from the experimental data scattering and solid lines are plotted to guide the eye.

and Le Gal (2004). The critical shear value and the stiffness of the curves show only slight evolutions with acceleration (Fig. 5B). Therefore, although it exists for most of the cells, a critical shear equal to 1 N m^{-2} in the present case (note that this critical shear is equal to 0.7 N m^{-2} for *P. noctiluca*, Cussatlegras and Le Gal, 2005), the bioluminescent emission of the *P. lunula* population is controlled by the temporal change of shear, that is acceleration in the flow. For a weak acceleration, the quantity of emitted light vanishes as was the case in the stationary Couette flow experiments in Cussatlegras and Le Gal (2004). This effect is also described by Von Dassow et al. (2005) and these authors show that it is linked to a desensitization process.

3.3. Derivation of the response curve

When subject to a mechanical stimulation, a certain proportion of the cell population emits light. It may be concluded that the concerned organisms possess a bioluminescent threshold which is lower than the applied one. Let us point out that this threshold corresponds to the bioluminescent threshold of a given sub-group of cells among the whole population and not to the bioluminescent threshold of the whole population as usually defined. Therefore, the bioluminescence response curve can be interpreted as a cumulated probability to trigger bioluminescence. The error function that fits the experimental bioluminescence response is the integral of the Gaussian function. Hence Fig. 6 shows the derivative of both the experimental data points and the fitting curve of Fig. 3. These Gaussian curves represent the probability density function and the experimental normalized histogram to trigger bioluminescence among a population of the dinoflagellates *P. lunula* when subject to a given shear. As previously observed, the mean bioluminescent critical shear (averaged on the whole population), corresponding to the maximum of the distribution and its standard

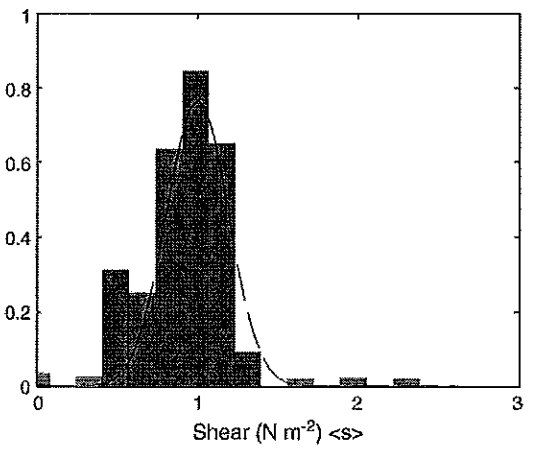

Fig. 6. Normalized probability density function of bioluminescence thresholds in *Pyrocystis lunula* as a function of shear and under a given acceleration (here 11.5 m s^{-2}). The mean threshold is 1 N m^{-2}, and the standard deviation of the probability is 0.3 N m^{-2}. The histogram is calculated from the experimental measurements and the dotted line is obtained from the derivation of the response fit. It illustrates the diversity and variability of the dinoflagellate bioluminescence response to mechanical constraints.

deviation, which controls the stiffness of the response, are only slightly dependent on the acceleration. The very left part of the probability distribution, that comes from the most sensitive cells, represents therefore the classical "bioluminescent threshold" (as defined in Von Dassow et al. (2005) for instance). As a direct consequence of Fig. 5B, this threshold does not depend neither on the acceleration (or on the rate of change of shear) as it was shown by Von Dassow et al. (2005) in their Fig. 6. However, due to the exponential shape of the probability distribution, we emphasize here the general difficulty to define and measure a clear "bioluminescent threshold" which is hidden by spontaneous bioluminescence or by the limited accuracy of very weak light measurements. Moreover, this "bioluminescent threshold" would be by definition, sensitive to the total number of cells: as observed by Von Dassow et al. (2005), only several flashes (or even a single flash) can be recorded at low shear and consequently the small number of these sensitive cells may then interfere in the measurement. For all these reasons, we prefer to consider a distribution of thresholds with a typical and well-defined averaged critical shear. Despite these difficulties, it can be seen from Fig. 4, that the classical "bioluminescent threshold" (the very left part of the histogram) of our own experiments can be estimated around a value less than 0.2 or 0.3 N m^{-2} in agreement with admitted values (Latz et al., 1994) and smaller by a factor of 5 than the population averaged critical value.

4. Conclusion and discussion

Our first set of experiments have demonstrated that the shear is indeed one of the control parameters of bioluminescence stimulation in *P. lunula*. The measurements of light emission from three sets of experiments which were performed with different mechanical stimuli collapse on a single response curve when plotted as a function of the average shear in the flow. This response curve has been fitted with an error function. In a second set of experiments, we have quantified the role of the flow acceleration or temporal change of shear, which is found to determine the quantity of emitted light. This result is in complete agreement with the recent experiments of Blaser et al. (2002) where the acceleration of an oscillating plate also controlled the intensity of light emitted by the dinoflagellate *Pyrocystis fusiformis*. It is also consistent with the recent findings that acceleration alone does not stimulate bioluminescence (Latz et al., 2004; Cussatlegras and Le Gal, 2005; Von Dassow et al., 2005). More generally, these results are also in agreement with recent observations of the critical role of the stretching velocity in mechanical stimulation of muscle cells (Burkholder, 2003) or endothelial tissues (Mabasheri et al., 2001; Bao et al., 1999).

Concerning fluid mechanical studies, the use of phytoplankton bioluminescence as a flow diagnostic was for the first time evoked by Rohr et al., 1997. In particular, these authors showed that there was a relation between the intensity of the emitted light and the wall shear stress produced by laminar and turbulent pipe flows. Thus, it could be possible to get quantitative measurements of flow fields if the bioluminescent response was clearly scaled versus the flow parameters. Visualization of the flow patterns around dolphins, in the wakes of spheres (Rohr et al., 1998) or in breaking waves (Stokes et al., 2004) are today the more advanced tentatives in this direction. However, as shown in this study, it appears that the double dependence on acceleration and shear that we exhibit in this study, will complicate a lot the interpretation of the quantity of emitted light. A weak shear reached with a high acceleration can give the same luminescent response as a high shear achieved through a weak acceleration.

The derivative of the response curve leads to the distribution of bioluminescence threshold in the dinoflagellates population. We think that the lack of a unique bioluminescent threshold (above which all the organisms would emit the maximum of their potential light, and where the response curve would be a step function) but the existence of a quite large distribution of thresholds comes from the natural diversity of the cell population. This diversity can originate from the age or the shape of the cells and/or from each organism variability at the molecular level: differences in the stretch activated channels number or channels with different sensitivities. Finally, we show that the probability distribution of the bioluminescent response (mean and standard deviation) is a characteristic of the diversity of the dinoflagellates and consequently is species dependant as shown in Cussatlegras and Le Gal (2005) for *P. noctiluca*.

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