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Bioluminescence Based Imaging Technique for Pressure Measurement in Water

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Abstract The dinoflagellate *Pyrocystis lunula* emits light in response to water motion. We developed a new imaging technique for measuring pressure using plankton that emit light in response to mechanical stimulation. The bioluminescence emitted by *P. lunula* was used to measure impact water pressure produced using weight drop tests.

The maximum mean luminescence intensity correlated with the maximum impact pressure that the cells receive when the circadian and diurnal biological rhythms are appropriately controlled. Thus, with appropriate calibration of experimentally determined parameters, the dynamic impact pressure can be estimated by measuring the cell-flash distribution. Statistical features of the evolution of flash intensity and the probability distribution during the impacting event, which are described by both biological and mechanical response parameters, are also discussed in this paper.

The practical applicability of this bioluminescence imaging technique is examined through a water drop test. The maximum dynamic pressure, occurring at the impact of a water jet against a wall, was estimated from the flash intensity of the dinoflagellate.

Keywords Bioluminescence · Impact pressure · Imaging measurement

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1 Introduction

When breaking waves collide with a vertical structure, impact wave pressure occurs on the vertical wall, which often damages coastal structures and facilities. In particular, very strong impacts are often observed for waves breaking just in front of a vertical structure during the so-called flip-through process in which the projecting wave crest and forward wave trough converge and are focused onto the wall [2,3], the pressure of which can be 10 to 100 times higher than normal wave pressure.

A number of investigations of wave impact have been performed in the laboratory setting on the basis of single point measurements using pressure transducers or load cells arrayed on a wall, which is currently the only available method to measure the impact pressures of waves (see the recent review by Cuomo et al. 2010[1]). A better understanding of the impacting process would be obtained if instantaneous spatial distributions of the pressure could be measured, because as-yet unknown hydrodynamic mechanisms are subjected to local concentrations of pressure gradients in breaking jets of water.

Some species of marine planktons emit lights as a response to mechanical stimulation [4]. Blaser et al. (2002) [5] studied the flash responses of the bioluminescent dinoflagellate *Pyrocystis fusiformis* in shearing pipe flow and oscillatory flow. Detailed analyses of the luminescent behaviors of dinoflagellate *Lingulodinium polyedrum* in turbulent shear flow, performed by Latz and Rohr (1999) [6] and Rhor et al. (1997)[7], revealed a unique correlation of the flash intensity with wall shear stress. These studies indicated the possibility of measuring the shear flow field using bioluminescent planktons. Stokes et al. (2004) [8] applied the bioluminescence imaging technique to ocean breaking waves, where

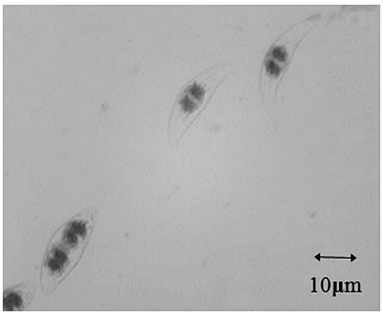

Fig. 1 Dinoflagellate plankton *Pyrocystis lunula*.

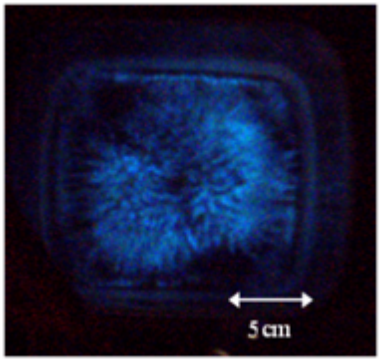

Fig. 2 Flashing *Pyrocystis lunula*.

strong turbulence and shears are organized and developed during wave propagation [9], suggesting a possible application in which a statistical calibration of the cell-flash could be used to measure the actual wave field. Bioluminescence imaging has potential advantages for determining planar or volumetric distribution of mechanical stress and its gradients within a fluid region.

In this study, the bioluminescent dinoflagellate *Pyrocystis lunula* was used as a biological pressure sensor for measuring impulsive dynamic water pressures aimed at determining the impact of a water jet on a wall as a model of a breaking wave. Weight-drop experiments in the laboratory were used to assess the cell-flash properties during water impact, which is similar to the impact model test reported by Bullock et al. (2001)[10]. We investigated the flash response and developed statistical calibration models to estimate the impact pressure based on the flash intensity. The model was examined through a physical test under a model of breaking waves.

This paper is organized as follows. The experimental set-up for the weight drop tests and pre-and post-processing of the images are explained in §2. The mechanical and biological parameters to determine the flash intensity, and statistical descriptions of the flash responses are discussed in §3. In §4, we present the applicability of the current calibration model to the water impact using a model jet. The results are summarized in §5.

2 Experiments

2.1 Dinoflagellate Bioluminescence

The dinoflagellate planktons *Pyrocystis lunula* (see Figure 1), provided by the National Institute for Environmental Studies (NIES-609), are 20 to 30 μm long, and, in response to external stimuli, emit blue light (see Figure 2) with a dominant wavelength in the range of 470 to 480 nm; the light is produced by a luciferin-luciferase reaction (for details of the chemical reaction, see Simomura 2006[11]). The dinoflagellates are observed worldwide on the sea surface and sometimes form colored patches called ‘ red tide ’ on the sea surface due to excessive proliferation.

The plankton were cultured in enriched f/2 media at 20 ^{o}C under cool-white fluorescent lighting with a 12 h light / 12 h dark cycle. The cell population increased 100 – 200 times after 4 weeks of the cultivation, and therefore sufficient numbers of the cells required in the experiment were obtained every week. Stokes et al. (2004) cultivated large numbers of dinoflagellate *P. fusiformis* to use as flow indicators in experimental waves in a laboratory wave flume of 33 m long. It may also be possible to cultivate a high volume of *P. lunula*, which increases with the identical growth rate to another dinoflagellate, required in a future wave experiment.

The biological behavior of dinoflagellates depends on a circadian biological rhythm; that is, when photosynthesis is active during the light phase, less luminescence activity is expected, and the luminescence is generally intensified during the dark phase (see Figure 3). The diurnal activity of the plankton may also affect bioluminescence. In this experiment, these biological factors were taken into account when characterizing the luminescent intensity for the dynamic impact pressure.

2.2 Experimental Setup

Seawater mixed with *Pyrocystis lunula* at a density of approximately 200 cm^{-3} was placed in a rectangular transparent acrylic container with an inner side length of 50 mm (See Figure 4). A steel weight of 1.3 kg was released from height h to strike the cylindrical lid of the container to generate a single pulse of impact pressure that was uniformly transmitted to all of the sea-

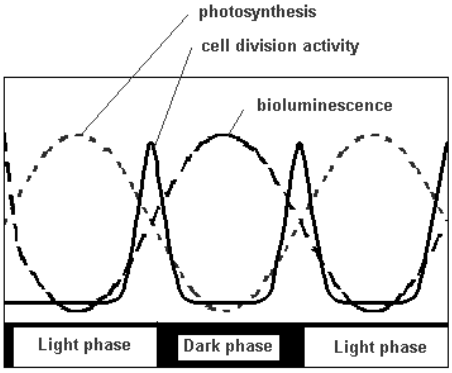

Fig. 3 Typical circadian biological rhythm of the dinoflagellate.

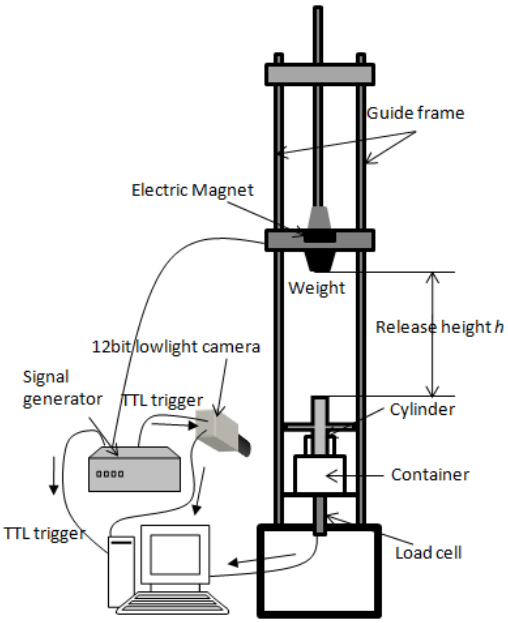

Fig. 4 Experimental apparatus and image acquisition system.

water in the container. Dinoflagellates do not respond to hydrostatic pressure, and a flash is produced only in response to a rapid change in pressure. A 12-bit high-sensitivity digital video camera (Ropper, QICAM) with an acquisition frequency of 40 Hz was placed to record the planar distribution of cells over the bottom surface of the container. While a camera with higher temporal resolution should be used for precisely capturing maximum luminescent intensity, a long exposure was required for detecting weak luminescence. The possible out-of-phase error associated with the unresolved intensity is discussed later.

The camera was connected to an image grabber installed in a personal computer where the image data were stored. The pressure was also measured using a load-cell (Kyowa, LTZ-100KA) fixed to the bottom of the container at a recording frequency of 25 kHz. When the weight was released, a TTL (Transistor-Transistor-Logic) trigger signal was sent to the computers to simultaneously start both the image acquisition and load-cell measurement to obtain the bioluminescence images synchronized with the pressure records. The image resolution was 1392 × 1040 pixels. The flash responses were investigated for the both the mechanical and biological experimental parameters; initial release height of the weight (h=10, 20, 30, 40, 50, 70, 100 mm), effects of the circadian clock, and media exchange. The water temperature in the test container was confirmed to be the same as that of the culture medium (20^{o}C). The mean population of the plankton in the container was 2.5×10^5 for all test cases.

When the water containing the plankton was placed in the container, most of the initially suspended cells gradually settled down onto the bottom of the container. The bioluminescence of the settled plankton was recorded because each the location of each cell was readily identified with the image processing system described in §2.4. Although it may be possible to identify the three-dimensional location of the suspended cells to obtain the volumetric flash distribution using a recently described stereoscopic imaging technique (*e.g.*, Watanabe et al., 2006), this study aimed to evaluate the fundamental statistics of the cell-flash under a uniform impact rather than the pressure distribution in the inner fluid region.

Sequential images of the cell-flash over the container bottom after the impact are shown in Figure 5. The luminescent intensity monotonically increases until the maximum cell-flash is achieved at phase (c), and it then gradually decays over time.

2.3 Calibration for light intensity

The acquired image intensity was calibrated with the optical intensity of the bioluminescence. A LED array panel emitting continuous blue light with the peak wavelength of 474 nm, which was identical to the dinoflagellate luminescence, was recorded with the camera. The photoconductivity power for the light intensities was measured using a photodiode for calibrating the image intensity of the corresponding light. In this calibration procedure, the cell-flash intensity was expressed as a measure of the photoconductivity power (Watt).

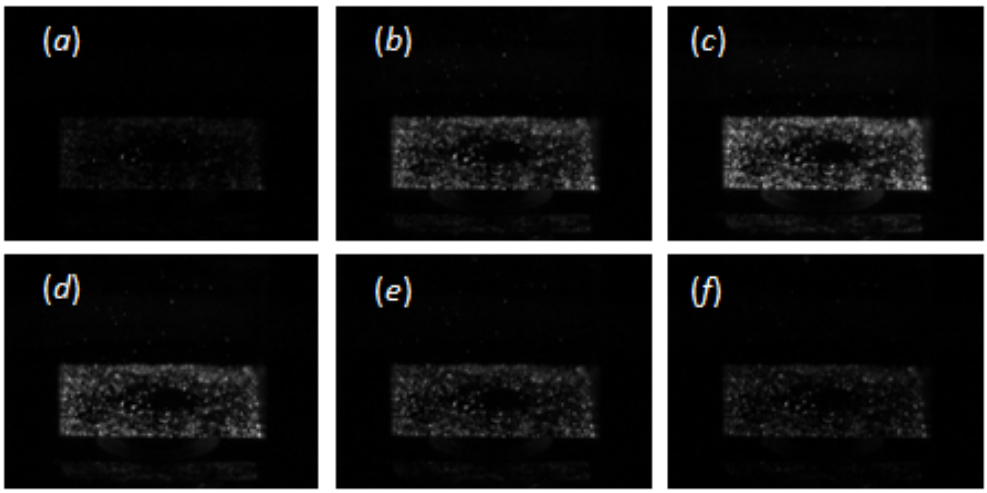

Fig. 5 Sequential images of the bioluminescence of the dinoflagellate *P. lunula* at the bottom of the container at impact. Time interval, 50 ms. Release height, h=40 mm.

2.4 Image processing

The image coordinates were transformed on the basis of a perspective projection to orthogonal real coordinates over the bottom of container. A median digital filter was used to reduce spike noise on the images.

Dinoflagellates were defined to be at the locations with the local maxima of the flash during the impact event (see Figure 6). The minimum absolute gradients of the image intensities within each flash region (an average size of 60 pixels) were calculated to determine the pixel with the local peak intensity. The time records of the luminescent intensities at all cell locations throughout the event were statistically analyzed in this study.

The ensemble mean of the luminescent intensity was taken over the detected cell population N:

$$\overline{I(t)} = \frac{1}{N}\sum_{n=1}^{N} I_n(t). \quad (1)$$

The standard deviation with respect to the population mean (1) was given by

$$\sigma(t) = \sqrt{\frac{1}{N}\sum_{n=1}^{N}(I_n(t) - \overline{I(t)})^2}. \quad (2)$$

The maximum mean intensity was defined by

$$I_{max} = \max(\overline{I(t)}). \quad (3)$$

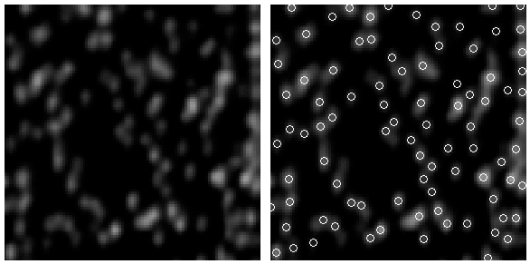

Fig. 6 A partial image of the cell flash (left) and detected cell locations (right).

3 Results

3.1 Fundamental features of the bioluminescence

The time records of the water pressure, simultaneous mean flash intensities $\overline{I(t)}$, and standard deviation $\sigma(t)$ are shown in Figure 7. The maximum pressure was rapidly established at 0.12 ms after initiation of the pressure increase. Both $\overline{I(t)}$ and $\sigma(t)$ also monotonically increased to a maximum after the impact at approximately 75 ms for any release height h, indicating a biological-based time delay in the flash response, which was independent of pressure strength. Typical exponential decays of $\overline{I(t)}$ and $\sigma(t)$ were observed after the maximum luminescence phase. These time-scales describing the evolution of the flash intensity are characterized in §3.3.

The maximum flash intensity produced by three drop heights appeared to correlate with the maximum pressure strength (Figure 7). This qualitative correlation suggests that maximum impact pressure can be measured utilizing the bioluminescence, which is quantitatively discussed in §3.5.

3.2 Biological conditions

As mentioned in §2.1, the flash behavior of the dinoflagellates varies depending on the circadian and diurnal bi-

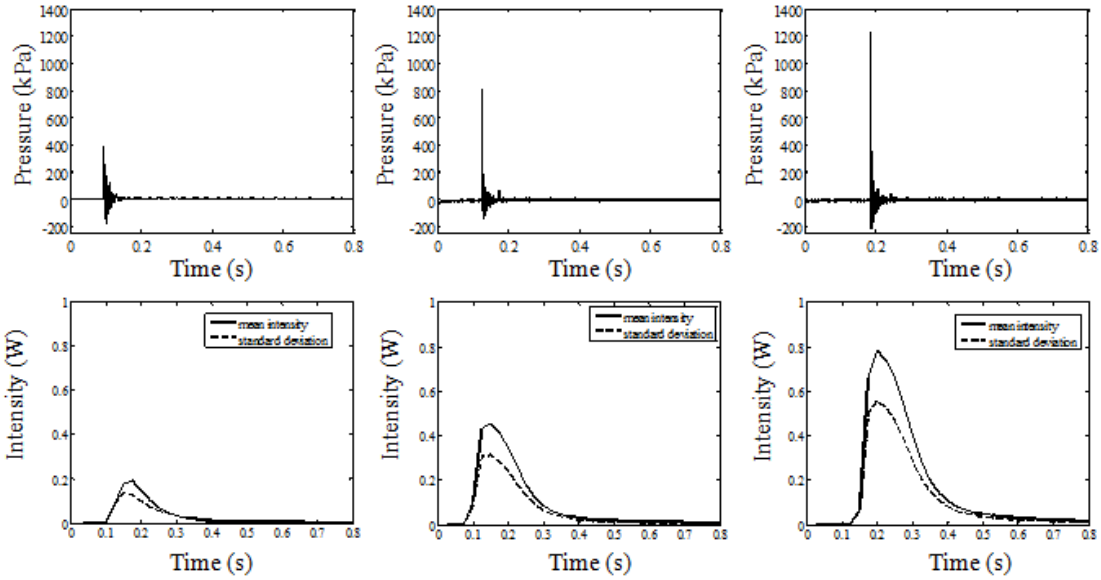

Fig. 7 Time records of the pressure measured by the load cell (top) and luminescence intensity (bottom) for h= 30 mm (left), 50 mm (middle), 100mm (right).

ological activities. To determine a unique relation of the flash intensity and the dynamic pressure, the variable features of the flash intensity for the biological activity are discussed below.

The maximum mean flash intensity, I_{max}, which was observed with a pressure of approximately 1250 kPa (h=100 mm) in experiments performed at different times throughout the dark phase, is shown in Figure 8. While relatively high intensities were maintained from 2 to 9 hours after beginning the dark phase, the luminescence activity significantly decreased after 10 hours in the dark as cell division may have begun, suggesting that unstable luminescence can be expected if measurements are made during the late portion of the dark phase. Therefore, all results discussed in this paper were obtained at 7 hours after beginning the dark phase, when the highest luminescence activity was expected.

Diurnal cell activity is also known to affect the luminescence response. Swift and Durbin (1971) found that the flash activity of the dinoflagellate *Pyrocystis fusiformis* varies at six different stages until cell division is established. Cell growth through a series of the activity stages depends on the following environmental conditions: water temperature, light levels, and culture medium nutrition. While the first two factors are controlled during cell cultivation, the influence of changes in the nutrient levels on flash intensity should be taken into consideration because the culture medium is replaced every 2 weeks to induce cell division.

Figure 9 shows the maximum mean intensity, I_{max}, of various heights for 6 days after medium replacement. The major feature of the diurnal luminescence activity for each height changed on day 4 after medium replacement. Mean I_{max} for h=100 mm on day 5 was twice that of the initial 3 days, while mean I_{max} decreased significantly at the other heights (h=50, 70 mm); that is, the correlation of the flash intensity with the release height disappears during days 4 and 5 after changing the medium. Therefore, cells cultivated within the first 3 days after the medium replacement should be used for the measurements. In the present experiments, we used cells from day 3 after the medium change.

In summary, there are two biological conditions that must be considered for pressure measurements based on the bioluminescence imaging: the duration of the dark phase (avoiding performing experiments late in the dark phase), and the diurnal activity pattern (day 2 or 3 after the medium replacement).

3.3 Evolution of luminescence intensity

The time-scales characterizing the time-varying luminescence responses to the impact pressure are discussed in this section.

As indicated in § 3.1, the mean flash intensity increases linearly after impact, and then shows a typical exponential decay after the peak intensity phase (see Figure 7). The simple form for these characteristic

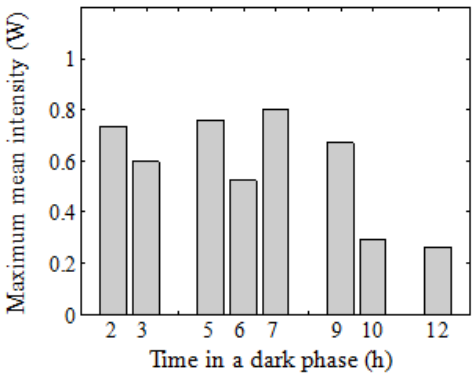

Fig. 8 Variations of the maximum mean intensity, I_{max}, at h=100 mm for the circadian clock.

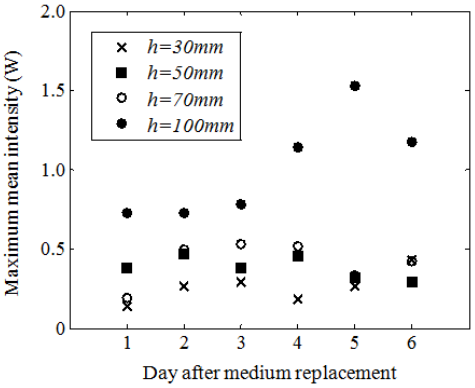

Fig. 9 Variations of the maximum mean intensity , I_{max}, for diurnal activity.

variations of the luminescence may be described by the following combination of linear and exponential terms:

$$\overline{I} = t' e^{-\beta t'}, \tag{4}$$

where the normalized time $t' = t/\alpha$, α is the rise time scaling parameter, and β represents the decay parameter. At the initial stage ($t \to 0$), the increase rate of the approximated intensity $\frac{d\overline{I}}{dt'} \approx 1$ because $e^{-\beta t'} \approx 1$. The luminescence increases at the rate $\frac{d\overline{I}}{dt'} = (1 - t'\beta)e^{-\beta t'}$ and then gradually decreases over time until the maximum intensity is achieved at $t' = 1/\beta$. Figure 10 presents the relations of the parameters α and β with the maximum pressure. Both parameters roughly decrease linearly with increasing maximum pressure p_{max}, which is approximately determined on the basis of the method of least squares; $\alpha = -4.32\times10^{-5}p_{max}+0.0822$ and $\beta = -9.63 \times 10^{-4}p_{max} + 1.6629$. The correlations of the observed and approximated parameters are 0.774 for α and 0.897 for β. Using these time-scale parameters, major features of the evolution of the mean intensities for various impact pressures are consistently determined as shown in Figure 11. In this way, the varying luminescent responses to a single pressure impact can be estimated by equation (4) with α and β, which are defined by the relation of I_{max} and p_{max} given in §3.5.

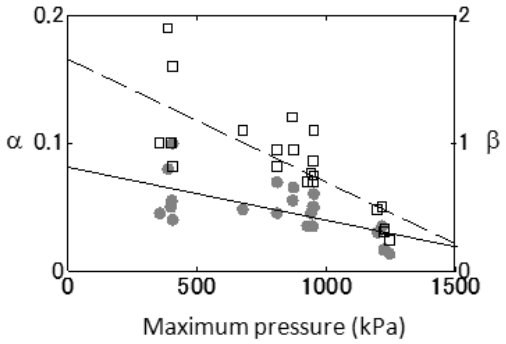

Fig. 10 The scaling parameters α (circle) and β (square) versus the maximum pressure p_{max}.

The current measurements at 40 Hz might not precisely resolve the maximum cell flash as already mentioned. The rate of this out-of-phase error to the maximum intensity I_{max} becomes relatively large in the cases of low I_{max} (see Figure 7), causing the scattered time scaling parameters at lower pressures in Figure 10, which may be reduced when a high-speed lowlight camera is used in measurements.

3.4 Probability distribution of cell-flash

The individual differences of a flash response, which can be influenced by both biological and mechanical factors, are discussed in this section.

Figure 12 shows time-varying histograms of the luminescence intensity under different impact pressures. With relatively weak pressure (h=30 mm), the cell-flash appeared to decay exponentially. With increased pressure (h=50 mm, h=100 mm), the flash intensity distribution changed from exponential to normal-like one. The intensity range became narrow and the histogram shapes returned to the exponential distribution after the maximum intensity phase ($t = t_m$).

The impact pressure-induced luminescent activity depends on the biological conditions, as discussed in §3.2. The probability of the cell-flash is conditionally described by two different probability densities of the biological factors and the stimulus response. The probability density of the biological factors should be defined as the probability of producing the maximum flash intensity that the cells are able to emit, which is determined only by the biological parameters such as water temperature, circadian clock, and diurnal cell activ-

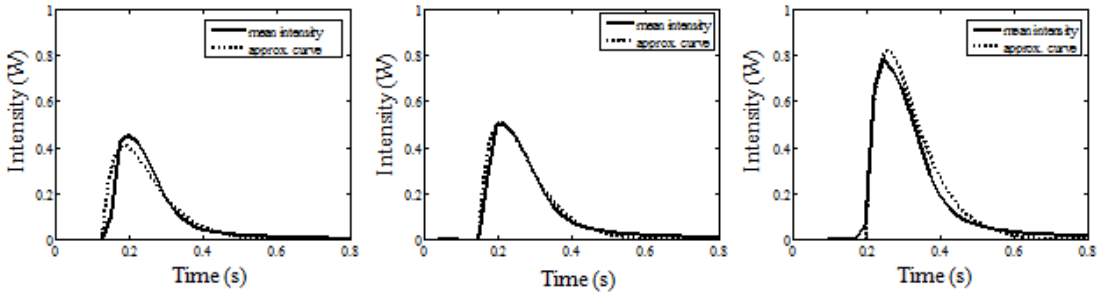

Fig. 11 Comparisons of the measured (solid) and calculated (dot) time series of the mean flash intensity.

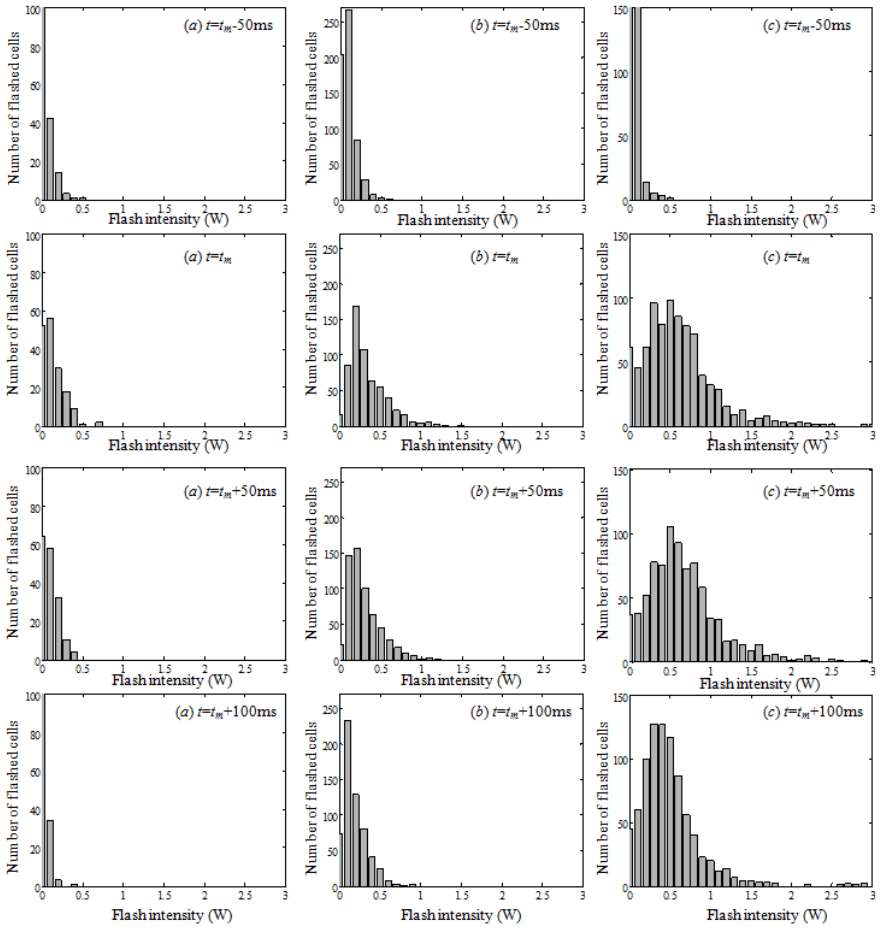

Fig. 12 Evolution of the histograms of the flash intensities for h=30 mm (a), 50 mm (b), and 100 mm (c). The maximum mean intensity occurs at $t = t_m$.

ity (independent of pressure strength). The exponential distribution is assumed to describe this probability, which approximates the common histogram shapes associated particularly with the weaker impact strength (see Figure 12), described by

$$f = \frac{1}{\mu} e^{-\mu i}, \tag{5}$$

where i is the flash intensity, and μ is a function of the biological parameters. Because the biological conditions are controlled in this study, μ should be constant for all cases. The normal probability distribution with a mean intensity $\lambda(p)$ and dispersion $\alpha^2(p)$ is assumed for the flash responding to the dynamic pressure strength p;

$$g = \frac{A(p)}{\sqrt{2\pi}\alpha} e^{-(i-\lambda)^2/2\alpha^2}, \tag{6}$$

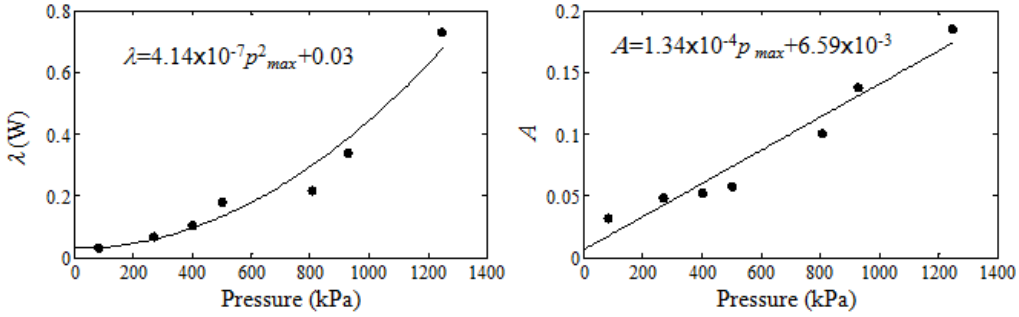

Fig. 13 Pressure response parameters λ and A with $\mu = 0.8$.

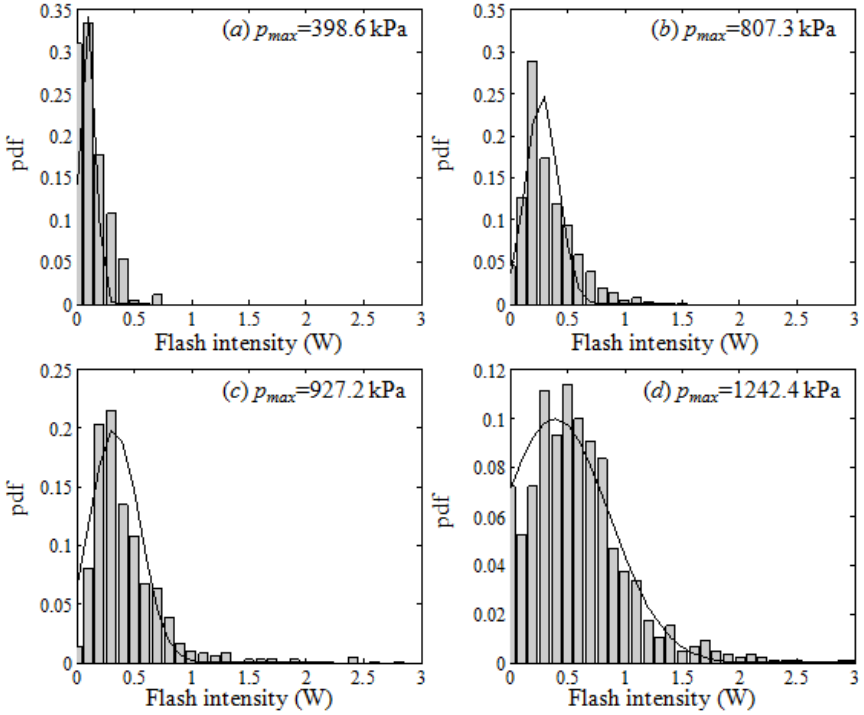

Fig. 14 Estimated probability density distributions (solid line) and normalized histograms at the maximum intensity phases for h=30 mm (a), 50 mm (b), 70 mm (c), and 100 mm (d).

where $A(p)$ is the response parameter.

Another form of the mean intensity (1) is therefore written in terms of the independent probability distributions f and g by

$$\overline{I} = \int_0^\infty fgdi = \int_0^\infty \frac{A}{\sqrt{2\pi}\alpha\mu} e^{-(\mu i + (i-\lambda)^2/2\alpha^2)} di. \quad (7)$$

The unknown parameters A, μ, α and λ should be found to determine the conditional probability density describing the flash responses to the pressure.

Assuming a proportional standard deviation α to λ, $\alpha = a\lambda$, the proportional rate a is estimated to be 0.65 by analogy with the maximum mean intensity, I_{max}, and standard deviation σ shown in Figure 7. The maximum probability density D_m appearing at the intensity i_m in the conditional distribution (7) is given by

$$D_m = \frac{A}{\sqrt{2\pi}\alpha\mu} e^{-(\mu i_m + (i_m-\lambda)^2/2\alpha^2)}, \quad (8)$$

where

$$i_m = \lambda - \alpha^2\mu = \lambda(1 - a^2\lambda\mu). \quad (9)$$

Substituting the observed i_m and D_m for all trials into the above equations (8) and (9), the constant biological parameter μ and response parameters A and λ can be approximately estimated to minimize the squared residuals. Figure 13 shows the estimated parameters λ and A with $\mu = 0.8$ at the maximum pressure phase.

Both parameters are uniquely determined by the maximum pressure; $\lambda = 4.14 \times 10^{-7} p_{max}^2 + 0.03$ and $A = 1.34 \times 10^{-4} p_{max} + 6.60 \times 10^{-3}$. The root-mean-square errors for the observed λ and A were 0.0465 and 0.0116, respectively. The probability density distributions configured by these parameters are consistent with the normalized histograms of the flash intensity under impact (see Figure 14), suggesting that the assumptions of the current conditional distribution for the biological and mechanical responses (7) are valid , which explains the major features of individual differences of the dinoflagellate luminescent activity under impact.

3.5 Relations of the luminescent intensities and maximum pressure

The maximum luminescent intensity (I_{max}) has a linear relation with both maximum pressure (p_{max}) and pressure impulse (p_{im}) (Figure 15). The strong correlations between I_{max} and p_{max} (0.83) and I_{max} and p_{im} (0.90) ensures that pressure can be estimated from bioluminescence imaging measurements. The approximate equations to describe these linear relations were determined using the method of least squares;

$$p_{max}(kPa) = C_1 I_{max} + C_2, \tag{10}$$
$$p_{im}(kPa \cdot s) = C_3 I_{max} + C_4, \tag{11}$$

where $C_1 = 2.12 \times 10^3$, $C_2 = 273.0$, $C_3 = 1.33$ and $C_4 = 0.59$.

The relative deviations of the observed pressure from equations (10) and (11) appears at relatively low flash intensities. As already indicated, the maximum pressure in this range might not be precisely resolved in the current measurement due to the out-of-phase error. However, in this study, assuming the error does not change overall features of the measured results, the above empirical equations is used to calibrate the luminescent intensity for estimating the pressure as a first step of application to a dynamic water pressure measurement, which is discussed in the next section.

4 Impact pressure from water jets

The impact pressure occurring when a water jet hits a wall, which is a physical model of a wave breaking against a vertical wall, estimated from the bioluminescence on the basis of the calibration equation (10) is examined in this section.

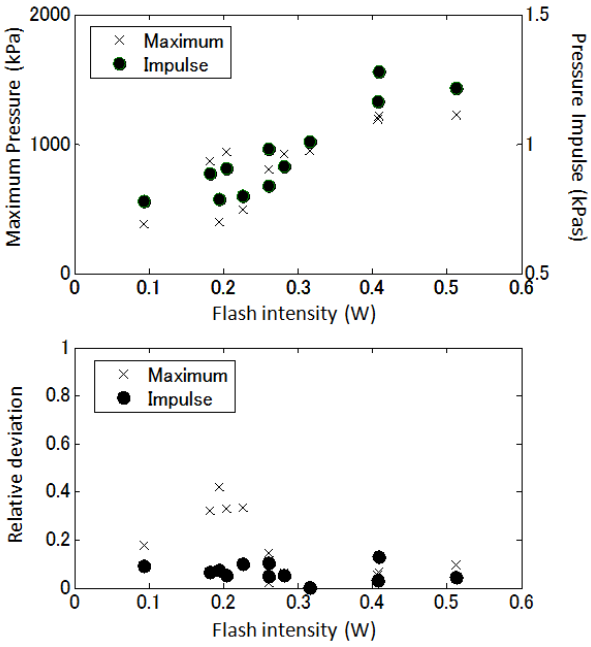

Fig. 15 Maximum mean intensity I_{max} versus maximum pressure p_{max} and pressure impulse p_{im} (top), and the relative deviations of the observed p_{max} and p_{im} from equations (10) and (11), respectively (bottom).

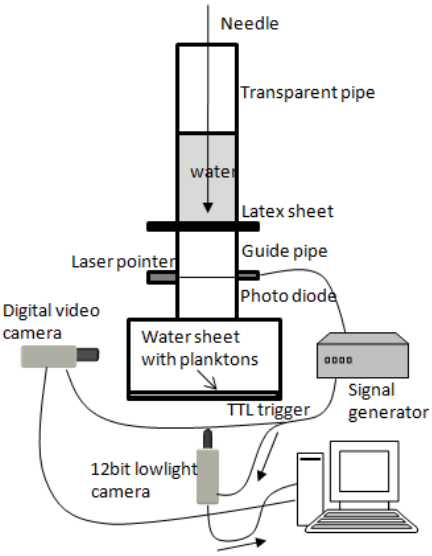

Fig. 16 Experimental set-up of the water jet impact.

4.1 Experimental set-up

The applicability of the current imaging technique was examined using the water drop test. An acrylic transparent pipe (inner diameter: 100 mm) with the bottom end covered by a stretched sheet of latex was filled with seawater (see Figure 16). A cylindrical water jet, formed by piercing the latex sheet with a needle posi-

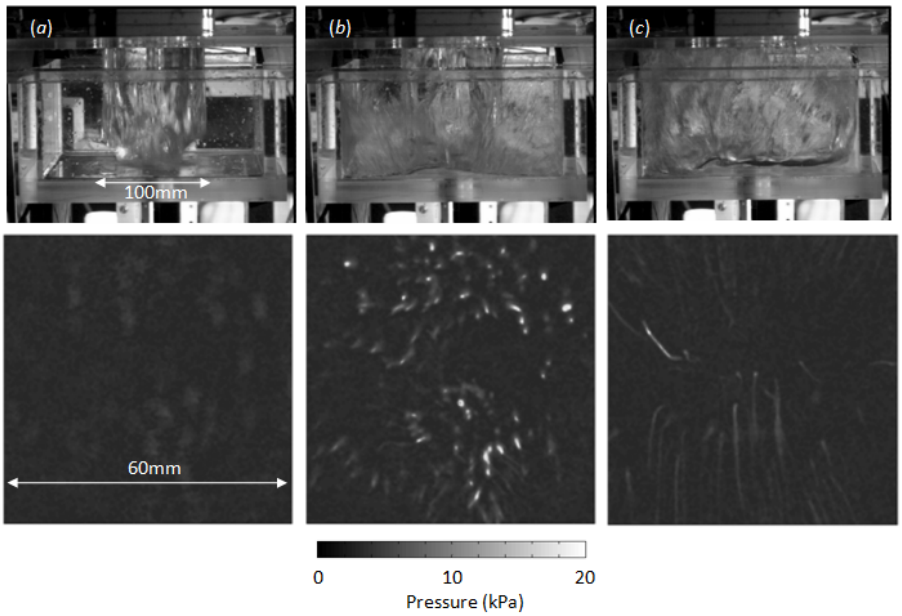

Fig. 17 Lateral view of the water jet (top) and simultaneous flash distributions near the center axis of the water jet on the horizontal measurement plane (bottom).

tioned at the center of the pipe (see also Figure 17), drops through a guide pipe and splashes on a thin water sheet (thickness: 5 mm) containing the dinoflagellates *P. lunura* in a transparent measurement box (200 × 200 × 100 mm). When the falling water jet intersects a laser beam, received by a photo diode fixed on the guide pipe, a photo-voltaic trigger signal was generated to start acquisition by the high-sensitivity camera mounted under the measurement box. The cell-flash distributions on the transparent bottom under the falling water jet were recorded at frequency of 40 Hz. The Field of View (FOV) was 60 × 60 mm, which covers the middle region of the water jet. The lateral surface shape of the water jet was simultaneously recorded at 250 Hz by a high-speed video camera (Photron, Rapid) for comparing with the luminescence images at the same phase. The water jet was released from 300 mm above the measurement plane, and the terminal velocity of the water was 2.50 m/s. The dynamic water pressure was also measured with a dynamic pressure transducer (PCB PIETRONICS, M106B) set at the center of the bottom wall of the measurement box recording at a frequency of 25 kHz under the same water jet conditions. The ensemble statistics of the population mean intensity (1) over 11 trials under the same jet conditions is discussed in this section.

4.2 Pressure under the water jets

When the vertical water jet touches down on the bottom wall, the cells compressed by the falling jet water emit light (see Figure 17 b). The axisymmetric flow formed over the wall then radically swept the cells away from the FOV (see Figure 17 c).

Figure 18 shows the comparison of the impact pressure measured by the pressure transducer with that estimated from the bioluminescence images using the calibration equation (10). While the rise time of the pressure measured by the transducer is approximately 2 ms, the current imaging estimation requires a much longer time of 0.025 s to achieve the peak pressure because of the biological time lag discussed in §3.1. On the other hand, identical maximum pressures were observed for both measurements, indicating that potential applicability of the current bioluminescence measurement to measure impact water pressure, although the result may contain the out-of-phase error associated with the low temporal resolution.

The flash emission lasts longer than the maximum intensity phase because shear stress occurring in the disturbed water flow in the measurement box may also induce additional cell-flash. It is difficult to obtain only the dynamic pressure in turbulent flows, since this technique cannot separate normal and shear stress in the measurement.

While any air bubble was not produced in an early stage of the impact, significant aeration was observed

in turbulent flow after 0.2 s from the impact. The result only in the initial stage of the impact reflect the pressure since additional flash emission might be induced in the aerated flow in the later phase. The effects of aeration should be identified in future research.

5 Conclusions

Bioluminescent responses of dinoflagellate *P. lunula* to impact pressures were studied using weight-drop tests. The luminescence activity was influenced by circadian and diurnal biological conditions. The maximum flash intensity significantly decreased during cell division 10 hours after starting the dark phase of the circadian clock. Unpredictable luminescence was also observed on days 4 and 5 after replacing the culture medium during the diurnal cell activity. When active cells are used under appropriate biological conditions, the maximum flash intensity highly correlated with the maximum impact pressure. The flash intensity obtained through imaging measurements of the luminescent dinoflagellate could be calibrated to estimate the maximum water pressure.

Fundamental features of evolution of the luminescence intensity at impact were identified. Time scaling parameters to characterize the initial increase and exponential decay of the mean flash intensity were described by the maximum pressure. A conditional probability distribution of the cell-flash at the maximum intensity phase was described in terms of biological and mechanical response parameters that were estimated from the observed results.

The bioluminescence measurement was confirmed to reasonably reflect the maximum impact pressure that occurs when a water jet hits a surface perpendicular to the water jet. However, further precise experiments using a high-speed camera need to be performed to examine practical reliability to the proposed measurement technique.

Flash responses to following practical parameters have yet been identified: dependency on cell-flash histories under multiple pressure spikes, effects of aeration, environmental conditions such as water temperature and salinity. Also applications of the current calibration model are limited to pressure measurements for no shear flow since this technique is unable to separate normal and shear stresses. However, to our knowledge, there are no other reports of a contactless measurement system to obtain the dynamic pressure in liquid. The current technique is potentially applicable for detecting and quantifying the pressure change in flow fields. This technique may be extended to obtain planer or volumetric pressure distributions within a fluid region, which is expected to extend our understanding of fluid mechanics.

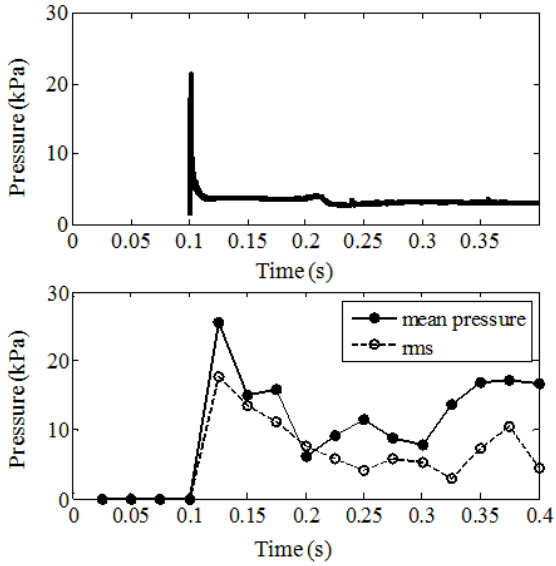

Fig. 18 Time records of the impact pressures measured by the pressure transducer (top) and estimated by the current bioluminescence imaging technique (bottom).

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