

Technical Discussion I

Underwater light measurement and light absorption by algae*

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SUMMARY: In this paper concepts and nomenclature of light measurements are discussed. The particular problems of underwater light measurements and the calibration of the equipment were presented. The pros and cons with spectroradiometers and broad band sensors were discussed. The use of specific ultraviolet-B sources for algal photobiology is recommended.

Key words: Irradiance, light measurements, nomenclature, photon fluence rate, spectroradiometer, technical discussion, UV sources.

CONCEPTS AND NOMENCLATURE

From a geometrical standpoint there are two main ways of quantifying light that are important for the biologist, but there is no general agreement on the terminology for describing the corresponding quantities. One way is to describe the incident light on a flat surface of unit area, the other is to describe

that incident on a sphere with unit cross-section. In addition one has a choice of expressing light in terms of energy, or in terms of number of photons or moles of photons. Part of the discussion concerned the terms to be preferred, and whether some terms should be weeded out completely. In Table 1 a listing of the synonyms is indicated. Irradiance and vectorial irradiance are synonyms used to designate the energy per unit time and unit area incident on a flat surface, often confused with fluence rate and scalar irradiance, properly used to designate the

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TABLE 1. – Concepts and units for the quantification of light

Sensor geometry	Energy System	Photon system
Flat		
time integrated	exposure, energy applied, time-integrated (energy) irradiance* unit: J m^{-2}	time integrated photon irradiance unit: mol m^{-2}
per unit time and unit area	(energy) irradiance, vector (energy) irradiance unit: W m^{-2}	photon irradiance (photon flux density) unit: $\text{mol m}^{-2} \text{s}^{-1}$ **
Spherical		
time integrated	(energy) fluence unit: J m^{-2}	photon fluence unit: mol m^{-2}
per unit time and unit area	(energy) fluence rate, scalar (energy) irradiance unit: W m^{-2}	photon fluence rate unit: $\text{mol m}^{-2} \text{s}^{-1}$

* This is one point where no accepted term exists. There are different proposals.

** The unit in the International system of units is $\text{mol m}^{-2} \text{s}^{-1}$. Einstein has also been also used for 1 mol of photons. However this term should no longer be used.

energy per incident on a sphere of unit cross-section per unit time. The insertion of the qualifier “energy” as opposed to “photon” is usually not necessary since it is evident from the unit, e.g. W m^{-2} , what is meant (Table 1).

REMARKS

1. Because irradiance (vectorial irradiance) is expressed in the same units as fluence rate (scalar irradiance), it is necessary to specify whether irradiance or fluence rate are meant. Thus, is not sufficient to write “The plants were exposed to 15 W m^{-2} of white light”.

2. The terms photon flux density (PFD) and photosynthetic photon flux density (PPFD) are often used by the researchers on photosynthesis. This is most unfortunate because (a) different people do not mean the same quantity; PFD may be either irradiance or fluence rate, (b) in other areas “flux” implies something expressed per unit area, and thus flux density is a tautology.

Although the discussion group did not arrive at any single terminology to recommend for future use, there was unanimous condemnation of use of the term photon flux density. Most people felt uncomfortable with the term exposure for time integrated irradiance. Often the term dose is used. Björn remarked that dose is used by people working with ionizing radiation only for absorbed radiation, not incident radiation, but it seems impossible to reach uniformity throughout the scientific community on this point.

There was some discussion about whether fluence rate should be considered as light impinging on a sphere or arriving at a point. One argument was that since a point has no area, light at a certain point cannot be expressed per unit area. However, there is no real difficulty. The proper way of handling this theoretically is to think of a sphere that is getting smaller and smaller until the required spatial resolution is reached. On the other hand, for practical measurements, only sensors of finite size can be used, and the values obtained must be regarded as spatial averages.

As for spectral measurements, in photobiology they are usually taken on a “per unit wavelength” basis, and the qualifier “spectral” has to be added to the terms in the Table 1, for instance in the energy system spectral fluence rate expressed in the unit $\text{W m}^{-2} \text{nm}^{-1}$ and in the photon system, the photon fluence rate expressed in the unit $\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$. It should be noted that physicists often express spectra on a “per unit frequency” basis, and that such spectra have a different shape. It should also be noted that spectra have different shapes whether expressed as energy fluence rate or photon fluence rate and giving a value for, e.g. the maximum wavelength of a light spectrum has no meaning unless one also specifies whether the light is expressed on an energy or a photon basis.

The term “light intensity” for the the quantification of light should be avoided because of its ambiguity, and should never be used when fluence rate or irradiance are appropriate terms. In several texts the term light quantity is used as the energy

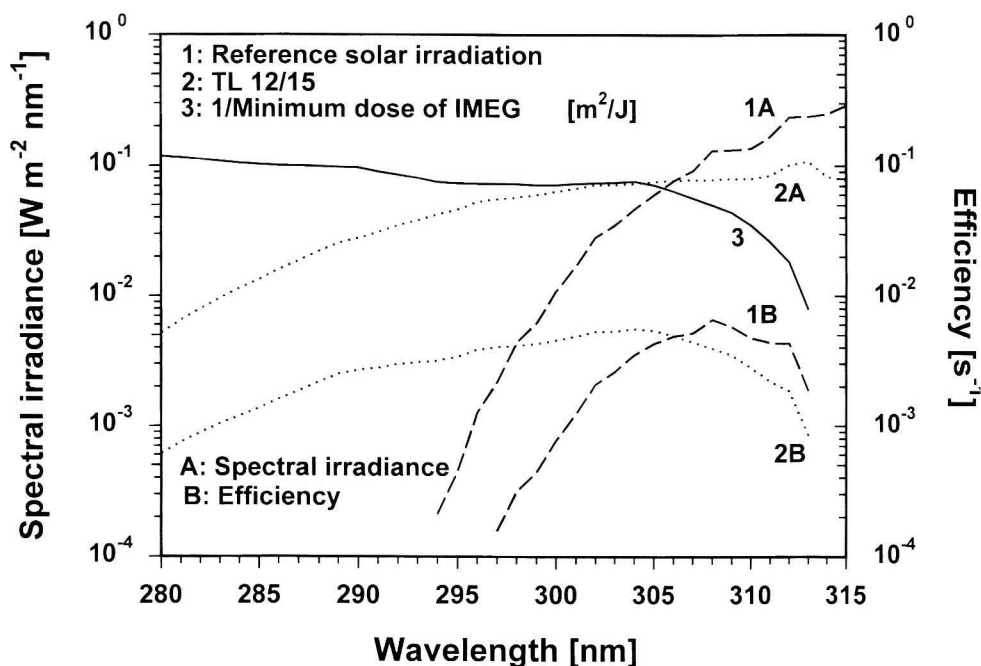


FIG. 1. – Emission spectrum of the sun (curve 1A) and of a UV light source namely Philips TL 12/15 (curve 2A), action spectrum of the motility in the flagellate *Euglena gracilis* (curve 3) and spectra of the responses produced by multiplication of the emission spectra and action spectra (curve 1B and 2B).

integration over a determined wavelength interval. On the other hand light quality is used to indicate the color of the light or the light per unit wavelength (spectral basis).

Describing the irradiance from a light source may not be sufficient to characterize the effect of radiation on an organism, since because of the spectral weighting of different wavelengths by an organism (action spectra), light sources with different emission spectra may have different effects. Figure 1 compares the emission spectrum of the sun (curve 1A, sea level, elevation 90°, 320 Dobson Units) with that of a UV light source (curve 2A, Philips TL12/15). Both sources yield the same irradiance of 1.81 W m⁻² in the UV-B range (280-315 nm). In order to compare the photobiological efficiency of these two lights on an organism an action spectrum is used. Example is the inhibition of motility in the flagellate *Euglena gracilis* (curve 3). When the action spectra are convoluted with the two emission spectra the integrated irradiances differ considerably (curves 1B and 2B): the artificial UV source is more effective by a factor 2. As a consequence, a photobiologist should indicate the effective irradiance for the described response. The effective UV irradiance, E, or dose rate (exposure), is given by

$$E = \int F(\lambda) W(\lambda) d\lambda$$

where $W(\lambda)$ is the weighting function, or action spectrum, for a biological or chemical effect and $F(\lambda)$ is the spectral irradiance, either computed or measured for a given time and location. Hourly, daily, and yearly weighted doses may then computed by time-integration of the dose rates (Madronich *et al.* 1995).

However, in the most cases the action spectrum of the responses is not known; in these cases one must indicate the light source used (emission spectrum of the lamp plus filter combination) and also describe how the irradiance was measured.

Spectroradiometers versus Broad Band Sensors

The pros and cons with spectroradiometers and broad band sensors were discussed. Most biologists still have only broad band sensors such as PAR meters (sensors for photosynthetically available radiation or photosynthetically active radiation, by convention defined as light from 400 to 700 nm wavelength) or broad band UV-B and UV-A sensors available. For many purposes the broad band sensors give relevant information. The PAR sensor can be constructed either to have equal response for all photons between 400 and 700 nm or to have the same energy sensitivity for all light in that range. The commercial UV-B sensors are usually construc-

ted to have a spectral response similar to the skin (erythral spectrum or cancer induction spectra) which differs considerably from the generalized plant spectrum defined by Cadwell (1971) and also for the inhibition spectra determined specifically for algae, such as that of Cullen *et al.* (1992). With a spectroradiometer one is more flexible, and can afterwards weight and sum the wavelength components in any desired way. On the other hand, apart from the high cost, spectroradiometers are less portable, more vulnerable to damage, and because they usually employ photomultipliers as light sensors, require frequent recalibration.

For spectral measurements of ultraviolet-B radiation only spectroradiometers with double monochromators have sufficiently low stray light.

The intercomparison and the intercalibration among different equipment for light measurements, including spectroradiometers and broad light sensors, is very important. The comparison of the data of UV-B radiation measured in different parts of the world with different sensors is crucial to know the real increase of UV-B as a consequence of ozone depletion (Gardiner *et al.* 1993). Seckmeyer *et al.* (1994) carried out an intercomparison of spectral-UV-radiation measurement systems (spectroradiometers). The measured irradiances at noon differed by factors up to 100. The large differences demonstrated the great difficulties with this type of measurement. Some instrument systems, however, agreed within $\pm 10\%$. The indiscriminate use of simple linear factors as the radiation amplification factor (RAF), used to convey the relative increase in harmful UV radiation with the decrease in ozone concentration, is leading to serious underestimates of UV radiation when large changes in ozone concentration are involved (Booth and Madronich, 1994). The errors in reporting solar radiation using moderate bandwidth radiometers have been reviewed by Booth *et al.* (1994). Kirk *et al.* (1994) carried out a comparison of irradiance in air and irradiance and attenuation coefficients at several water depths at UV-B wavelengths determined with some commercial instruments used by photobiologists e.g. Biospherical Instruments, International Light, LI-COR and Optronic Laboratories. This analysis revealed differences in spectral resolution, accuracy, sensitivity to low light energy and convenience in generating depth-specific profiles. Broadband instruments trade off resolution for simplicity and in some cases greater sensitivity, but are subject to

errors when the solar spectrum is modified by sun angle, atmospheric column ozone, and the inherent optical properties of the water. As a conclusion none of the instruments tested matched the ideal, in having at the same time narrow bandwidth, sensitive and temperature-stable light detector and wavelength filter/selector with high rejection of stray light, well-characterized cosine response and immersion coefficients (Kirk *et al.* 1994).

Particular problems for underwater measurements

Because water has a much higher refractive index than air, the reflection and refraction at the surface of the sensor is quite different depending on whether this is in air or immersed in water. Therefore sensors for underwater use should be calibrated below water, but it is not easy, since available standard lamps and calibrator systems are intended for use in air. The only way to come around this is to compute the effect of water immersion using Fresnel's reflection law and Snell's refraction law. In several commercial spectroradiometers used for air and underwater light measurements, data from the immersion effect are available from the companies to correct the calibration determined in air.

Häder discussed the types of natural waters with different optical properties. The classical work is that by Jerlov (1976) but recently other papers have been published on the subject (Smith and Baker, 1978, 1979; Baker and Smith, 1982; Smith *et al.* 1989). A recent book covering both theoretical aspects and the practical problems of measuring underwater light is that by Kirk (1994).

For delving deeper into the problems of air and underwater light measurements we recommend reading the paper Radiation measurements (Holmes, 1984) published in "Techniques in Photomorphogenesis", edited by Smith and Holmes (1984) and several chapters of the books: "General Photobiology" by Häder and Tevini (1987), "Seaweeds: Their Environment, Biogeography and Ecophysiology" by Lüning (1990), "Light and Plant Growth" by Hart (1988) and "Photomorphogenesis in Plants" edited by Kendrick and Kronenberg (1994).

Ultraviolet-B sources for algal photobiology

Lüning pointed out the great disadvantage in using cellulose acetate foil for filtering out the UV-C contaminating radiation from UV-B fluorescent

lamps. The only alternative filters available are Schott glass filters, but they are too expensive for experiments where large filter surfaces are required and also too difficult to handle in the field and under water experiments. Lüning recommended the use of lamps which have a suitable spectrum without filtering and mentioned Philips TL1 330. The lamps from Q-Panel Company, UVA 351 and UVA 340 are recommended. The UVB-313 from Q-Panel and the TL12 from Philips contain appreciable amounts of UV-C radiation. Ultraphan and Folex filters are recommended to cut-off UV-A (filter 320), UV-A and UV-B (filter 395) and UV-C (filter 295).

REFERENCES

- Baker, K.S. and R.C. Smith. – 1982. Bio-optical classification and model of natural waters II. *Limnol. Oceanogr.* 27:500-509.
- Booth, C.R. and S. Madronich. – 1994. Radiation amplification factors: improved formulation accounts for large increases in ultraviolet radiation associated with antarctic ozone depletion. *Antarctica Res. Series* 62: 39-42.
- Booth, C.R., T. Mestechkina and J.H. Morrow. – 1994. Errors in the reporting of solar radiation using moderate bandwidth radiometers: an experimental investigation. *Ocean Optics* XII 2258: 654-663.
- Caldwell, M.M. – 1971. Solar UV radiation and the growth and development of higher plants. In: A. C. Giese [Ed.] *Photophysiology* vol 6, pp.131-177. Academic Press, New York.
- Cullen, J.J., P.J. Neale and M.P. Lesser. – 1992. Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. *Science* 258: 646-650.
- Gardiner, B.G., A.R. Webb, A.F. Bais, M. Blumthaler, I. Dimhirn, P. Forster, D. Gillotai, K. Henriksen, P.J.M. Huberkirsch, P. C. Simon, T. Svenoe, P. Weihs and C. S. Zerefos. – 1993. European intercomparison of ultraviolet spectroradiometers. *Environ. Tech.* 14: 25-43.
- Häder, D.-P. and M. Tevini. – 1987. *General Photobiology*. Pergamon Press, Oxford, 323 p.
- Hart, J.W. – 1988. Light and plant growth. M. Black and J. Chapman [Eds.], *Topics in Plant Physiology*, 204 pp. Unwin Hyman Ltd, London.
- Holmes, M.G. – 1984. Radiation measurement. In: H. Smith and M.G. Holmes [Eds.] *Techniques in photomorphogenesis* pp. 81-97. Academic Press, London.
- Jerlov, N.G. – 1976. *Marine optics*. Elsevier Amsterdam, 231 pp.
- Kendrick, R.E. and G.H.M. Kronenberg. – 1993. *Photomorphogenesis in plants* 2nd edition. Kluwer Academic Publishers, 828 pp.
- Kirk, J.T.O. – 1994. *Light and photosynthesis in aquatic ecosystems*, 2nd ed.. Cambridge University Press, 509 pp.
- Kirk, J.T.O., B.R. Hargreaves, D.P. Morris, R.B. Coffin, B. David, D. Frederickson, D. Karentz, D.R.S. Lean, M.P. Lesser, S. Madronich, J.H. Morrow, N.B. Nelson, and N.M. Scully. – 1994. Measurements of UV-B radiation in two freshwater lakes: an instrument intercomparison. *Arch. Hydrobiol. Beih* 43: 71-99.
- Lüning, K. – 1990. *Seaweeds. Their environment, biogeography and ecophysiology*. John Wiley & Sons, Inc, New York, 527 p.
- Madronich, S., R.L. McKenzie, M.M. Caldwell and L.O. Björn. – 1995. Changes in ultraviolet radiation reaching the earth's surface. *Ambio* 24: 143-152.
- Seckmeyer, G., S. Thiel, M. Blumthaler, P. Fabian, S. Gerber, A. Gugg-Helminger, D.-P. Häder, M. Hüber, C. Kettner, U. Köhler, P. Köpke, H. Maier, J. Schäfer, P. Suppan, E. Tamm and E. Thomalla. – 1994. Intercomparison of spectral-UV-radiation measurement systems. *Applied Optics* 33: 7805-7812.
- Smith, R.C. and K.S. Baker. – 1978. Optical classification of natural waters. *Limnol. Oceanogr.* 23: 260-267.
- Smith, R.C. and K.S. Baker. – 1979. Penetration of UV-B and biological dose rates effective in natural waters. *Photochem. Photobiol.* 29: 311-323.
- Smith, R.C., B.B. Prezelin, R.R. Bidigare and K.S. Baker. – 1979. Bio-optical modeling of photosynthetic production in coastal waters. *Limnol. Oceanogr.* 34: 1524-1544.