Manual for Marine Monitoring in the

COMBINE

Programme of HELCOM

Part C

Programme for monitoring of eutrophication and its effects

Annex C-5

Phytoplankton primary production

Annex 3

Irradiance Differentiation and Control in the ICES incubator

ANNEX C-5 PHYTOPLANKTON PRIMARY PRODUCTION

ANNEX 3 IRRADIANCE DIFFERENTIATION AND CONTROL IN THE ICES INCUBATOR

Needed in order to be able to accomplish reliable P-I measurements with respect to phytoplankton primary production studies.

Performed by ZEMOKO, specialized in Radiometry and Marine Optics. By order of RIKZ, March 1994.

INTRODUCTION

A full discription of this incubator is given by Colijn et al. (1993). In essence the incubator consists of a TL-illumination set with ten TL33 fluorescent tubes inside, in front of which twelve rectangular shaped incubation flasks, with the flaskneck fixed on a turning wheel, are rotated. The whole flask assembly (see photo 1) is immersed in a ectangular clear perspex water tank. In principle a single-side illumination is foreseen, using a TL-illumination set at one side of the tank (see photo 2) and for higher efficiency a diffuse reflecting polystyrene foamlayer at the other side. [A higher irradiance can be achieved by a dual-side illumination, using one TL-illumination set at each side of the tank]. In both cases, even with all fluorescent tubes switched on, the irradiance distribution in the plane of the flasks is not uniform. That means that during revolution a time\position depending irradiance exists in the flasks and a time\position averaged value should be determined.

Photo 1. Flask assembly. Twelve rectangular shaped incubation flasks with the flaskneck fixed on a turning wheel to be immersed in the rectangular clear perspex water tank of the incubator. In the basic concept of the ICES incubator the possibility of irradiance differentiation is not provided. Only a maximum level can be chosen by the number of fluorescent tubes switched on. For measuring P-I relations an irradiance gradient

or a proper sequence of different irradiance values is needed. Changing the basic concept of the incubator as less as possible, the easiest way to achieve this irradiance differentiation is making a sequence by coating the incubation flasks, each in a different transmission density (see section 2). The light attenuation using such a density filtercoating should be preferably neutral, otherwise one needs a proper quantum irradiance measurement including a photosynthetic action response.
When neutral attenuation is achieved, the irradiance measurements can be done with an irradiance sensor having in fact any spectral response within (or partly in) the TL33-spectrum. For measuring total (scalar) irra- diance, a sensor with spatially uniform sensitivity which is small enough to pass through the neck of an incubation flask, is needed (see photo 3). Absolute irradiance measurement is attained by a proper calibration of the immersed irradiance sensor (see section 3). Photo 2. The complete incubator. In principle a single-side illumination is foreseen using a TL-illumination set at one side of the tank and for higher efficiency a diffuse reflecting polystyrene foam-layer (not shown) at the other side.

In the above indicated way one has a proper irradiance control, although some possible sources of error must be kept in mind:
The position of the sensor within the flask is rather critical. To find a proper average irradiance value a well defined central position in the flask should be achieved. The ater in the incubator and flasks should be kept free of airbubbles which cause lightscattering in an unpredictable way.
Influence of ambient light should be controlled, especially in daylight situations. With a polystyrene foam-layer around the incubator an ambient light shield can be realized but each shield may influence the light situation as well, due to reflections. Long term stability of the TL33-light output is still unknown. Besides a reduction in the absolute
irradiance value also variation in the spatial irradiance distribution of the tubes has to be expected. The irradiance value and uniformity in the flasks' plane will change after some time. The fluorescent tubes should therefore be frequently replaced, preferably before the tube-ends are burned in, to maintain the hereafter described situation (section 5). Last but not least there are always some optical imperfections and tolerances in sensor calibration as well (see section 4).

Photo 3. The mini spherical irradiance sensor. A provision is made to position the sensor in a more or less fixed central position in the incubation flask.

NEUTRAL DENSITY FILTERCOATING

NEUTRAL density water-resistant filtercoatings or filtermaterials are rather rare. After some research a good result is achieved with epoxy-resin in which dark pigments are mixed in different ratios. Compared with commercially available filtermaterials spectral neutrality is in most cases better with the advantage that every density value needed can be made.
A proper transmission density sequence for P-I measurements is made in epoxy filterlayers only 0.8 mm thick. The layers with the different transmission densities are integrated on the front- and backside of each flask. The other sides are made totally opaque. The spectral transmittance of the epoxy filterlayers is measured using a spectroradiometersystem and a tungsten halogen radiant source with daylight filter. The specified transmittance is actually the quotient of two spectral measurements, one with the filterlayer and another without the layer.

Fig.1 gives the relative spectral transmittance measured for the highest density filterlayer with a transmissionfactor of about 2%. This spectral transmittance can be considered as neutral as necessary for irradiance measurements with an arbitrary spectral response.
Additionally the measured relative spectral transmittance of a filterlayer with a 30% transmissionfactor is given.
The lowest line in Fig.1 is the relative spectral transmittance measured on a complete flask with filterlayers on each side with a transmissionfactor of 50% per layer. These layers are slightly polluted by the primer used for the opaque sides of the flask, resulting in a small increasing red transmittance.

Fig.1. >>

Experts to add figures

The thickness used for the epoxy-layers is rather small for a good control of the higher density values of these filterlayers; in fact the thickness forms a critical factor for these filters. Because the flasks have rims on both sides, it was most practical to use these rims to define the thickness of the epoxy-layers.
After only a small correction both rims where brought to an equal height of about 0.8 mm. A thickness of for instance 3 mm would be far less critical. These filters should then be made separately in the proper dimensions to fit on the flasks afterwards. Four identical filter/flask series are made to be used in four identical incubators. The 4 times 12 flasks are numbered 1-0 to 4-11, with the first number for the series and the second number indicating the transmission-step. Increasing numbers correspond with increasing transmittance- and irradiance values. So -0 corresponds with a totally opaque flask, -11 with a clear flask (front and backside only). The weight of these two extreme flasks, -0 and -11, is about 4 g lower as the weight of the other coated flasks because of the lack of filterlayers.
To balance the turning wheel these flasks shoud be mounted in opposite position. Fig.2 is a plot of the aimed transmission sequence together with transmissionfactors measured inside the flasks of the four separate filter/flask series in the incubator. The mutual differences are mainly caused by the above mentioned effect concerning the critical thickness of the filterlayers. The transmittance holds for the diffuse lightcondition in the incubator. Due to scattering in the somewhat diffuse filterlayers the transmittance is somewhat lower than expected. Nevertheless, the aimed trend is fairly good achieved. Table 1 gives an overview of the attained transmissionfactors for the four filter/flask series.

Fig.2. >>

Warning:
With these filterlayers transmission density is achieved by absorption. Exposing them to high irradiance values may result in an increasing temperature and distortion of the material. However, in a (cooled) water tank this effect does not appear.

In general the mechanical resistance of these filterlayers will be no problem at all, even at low temperatures (storage at -6 degrees Celsius for weeks of a coated flask did no harm).

Fig.3 shows the relative spectral reflectance of the 18 mm thick polystyrene foam-layer, used for higher efficiency of the TL-illumination and measured to be sure that the spectral influence is negligible, which indeed is the fact.

Fig.3. >>

SENSOR CONSTRUCTION AND CALIBRATION

Three mini spherical irradiance sensors are made, consisting of a silicon photodetector in front of which a green filter is mounted and a spherical collecting element made of diffuse epoxy. A provision is made to position the sensor in a more or less fixed central position in the flask (see photo 3). Fig.4 gives the measured typical spectral sensitivity for this type of sensor, restricted within the TL33-spectrum and Fig.5 the typical spatial sensitivity measured in two planes in front of a TL-set: one radial plane around the sensor, the other axial through the top of the sensor. In the axial plane the influence of the obstruction, caused by the connecting cable, is obvious.

Fig.4. >>

Fig.5. The typical spatial sensitivity of the sensor, measured by rotating the sensor in two planes in front of a TL-set: one radial plane around the sensor, the other axial through the top of the sensor.

For the absolute calibration of the sensors in Watt*m-2 and uEinst.*sec-1*m-2, the spectroradiometersystem is used again, consisting of a spherical collecting element as well, an optical fiber, a Jarrell Ash gratingmonochromator and a silicon photodetector. Furthermore a standard tungsten striplamp as a wellknown spectral radiance source. This source is used for calibration of the radiometersystem first. Immersed in water the mini sensors are intercalibrated for the TL33-spectrum using the calibrated spectroradiometersystem and a TL-illuminationset at 10 cm distance.

Fig.6 is the absolute spectral irradiance distribution of the TL33-set together with the integrated irradiance value measured at the calibration distance of 10 cm. From this integrated irradiance value the calibrationfactor or "multiplier" for the mini sensor results, as indicated on the labels of these sensors. Table 2 specifies the same multipliers as indicated on the labels for the three different mini irradiance sensors delivered. These multipliers have to be set in the photocurrentmeter used. There is a difference in relative sensitivity especially for the first sensor compared with the other two. With the appropriate multiplier the absolute sensitivity of the three sensors agrees within 2%.

Fig.6. >>

The measured spectral distribution of the TL33-set presented in fig.6 corresponds with manufacturers data. The spectral spikes on the curve result from the mercury discharge inside the tube.

TOLERANCES AND IMPERFECTIONS

Only an estimated maximum overall sensor calibration inaccuracy of +/- 15% can be mentioned, which is not an extreme tolerance in radiometric calibration (the overall inaccuracy can be roughly differentiated in: a 5% inaccuracy for the emission of the standard radiance source, 5% for the calibration of the radiometersystem and 5% for the sensor intercalibration). On the other hand, based on comparison with other calibrated irradiance sensors and a different radiometric system, the experience is that mostly the agreement turned out to be better than presumed by the above mentioned inaccuracies. So, the general experience is that the reliability of the present irradiance measurement is fairly good.
Because of aging effects or, in general, long term sensor instability, it is advisable to recalibrate sensors after at least a period of some years, depending on the aimed accuracy and general state of the sensor. Calibration is carried out at a temperature of 18 degrees Celcius. These sensors have a small negative temperature coefficient of about 0.17%/degr. for the selected wavelength, caused by the silicon detector incorporated. Because of the rather small temperature range in the incubator this effect can be ignored in most cases.

To be sure of a good linear response, these sensors should be used in combination with a low input impedance photocurrentmeter such as for instance a LI-COR LI-1000 or LI-189.
From the sensor configuration and the measured spatial sensitivity, it is clear that this sensortype is affected with an obstructed, incomplete field of view, caused in fact by the electric output connection. The sensor is positioned (in the flask) in such a way that this less sensitive direction is orientated in the dark neck of the flask to minimize the influence of this imperfection. By this the influence is negligible, although the restriction remains.

The sensors should be kept in good condition by storing them always mounted in an empty flask so that the sensors are kept free from dirt, scratches
and/or other damages.

With respect to the neutral density filterlayers the only requirement is in fact the spectral neutrality which is, as stated before, sufficient for reliable irradiance control with the spectral response of fig.4.
The only imperfection in the application in the ICES incubator is actually the absorption due to which part of the available light is lost (in general, local light attenuation may be achieved more efficiently by light scattering instead of absorption, see discussion).

Changing the filterflasks partly by clear ones will influence the average irradiance more or less, which holds for changing every absorbing and/or reflecting element in or even around the incubator.
For completeness, a general error resulting from spectral differences between the incubator lightsource and the actual daylight (underwater) spectrum, actually the radiation effectiveness for phytoplankton primary production, should be mentioned. The TL33-spectrum is expected to be less effective compared with most natural spectral situations. The choise of the TL33-type fluorescent tubes is therefore questionable. The available daylight types of fluorescent tubes will be generally more effective.

IRRADIANCE MEASUREMENTS IN FOUR INCUBATORS

Four complete incubators were compared with respect to the irradiance in the filter/flask series during revolution in front of the TL- illuminationsets. For convenience the measurements were carried out in one

incubator, using four separate TL-illuminationsets and four separate filter/flask series. First a registration was made of the (spatial)
irradiance distribution during one revolution for different illumination conditions. The irradiance is measured in absolute values (expressed in uEinst.*sec-1*m-2) with the mini sensor positioned in the centre of the filterflask 1-11 and all the other filterflasks in position, each one filled with 55 ml water. The same measurement for one illumination condition was carried out with all the twelve flasks clear (uncoated). Top-position of the flask containing the sensor, during revolution, is indicated as zero-position and clockwise increasing to 360 degr. which is top-position again.
At zero-position the transmission sequence of the four filter/flask series was measured, and the result plotted in fig.2.

Fig.7 is the registration of the irradiance distribution with the coated flasks and the polystyrene foam-layer with respectivily 2,4,6,8 and 10 fluorescent tubes switched on in a pattern as indicated below:

Fluorescent tubes switched on (O):

2 ----------------------- : XXOXXXXOXX
4 ----------------------- : XOXOXXOXOX
6 ----------------------- : XOXOOOOXOX
8 ----------------------- : XOOOOOOOOX
10 ----------------------- : OOOOOOOOOO

Maximum irradiance is reached when the flask passes the middle of the fluorescent tube(s) where the illuminance of the tube is maximum. This characteristic holds for most of the the curves measured. The pattern for six fluorescent tubes switched on results in the most uniform irradiance distribution. It was found that the small spikes on the maxima of the curves are caused by reflection from the coolingpipe situated in the corners of the water tank on the opposite side of the illuminationset.

Fig.7. >>

Figs.8 and 9 show the comparison of the four illuminationsets with (fig.8) and without (fig.9) the polystyrene foam-layer used and with coated flasks.
No big difference exists in the output and spatial distribution of the four different sets. The obvious effectiveness of the polystyrene foam-layer is demonstrated, although uniformity of the distribution certainly is not improved as one would expect at first.

Fig.8. >>
Fig.9. >>

Fig.10 shows the irradiance distribution when dual-side illumination is used.
With the tube direction of both sets in crossed position the irradiance becomes 50% higher, with the advantage of better uniformity, compared with the single-side illumination with the polystyrene foam-layer in position.

Fig.10. >>

Fig.11 demonstrates a 50% irradiance gain when (12) clear flasks are used instead of (12) coated flasks (compare fig.11 with figs.8 and 9).
The effectiveness of the foam-layer is obvious again. The maximum irradiance is only 10% lower in comparison with dual-side illumination with parallel tube direction (compare fig.11 with fig.10).

In table 3, the statistics for the measured irradiance distributions have been listed; all statistical values refer to one complete revolution comprising 360 samples. Table 4, is a specification of the average absolute irradiance in the four different filter/flask series. These values result from the averages specified in table 3, multiplied by the easured relative transmission factors, determined in the flask zero-position for the different filter/flask series (see fig.2 and table 1).

Fig.11. >>

DISCUSSION AND RECOMMENDATION

Within certain limits the aimed light differentiation and control is achieved. A general restriction concerning the lightlevel remains. When only one illuminationset is used the maximum averaged absolute irradiance is rather low, even with the polystyrene foam-layer in position. The coated flasks absorb part of the available light and due to the 0paque sides there is also a spatial limitation of the light-input in the flasks (see table 3, influence of flask-coating). However, the opaque sides were necessary for a good control of the transmissionfactors of the transmission sequence in the filter/flask series. In this way the transmission is well defined by the absorbing front- and backside filterlayers only. For practical reasons the thickness of the filterlayers should be increased. These filters are not yet standard available. Probably a more efficient use of the available light is possible by coating the flasks with a neutral diffuse white epoxy-coating.
Attenuation then is mainly achieved by diffuse scattering/reflection instead of absorption. As a consequence the transmissionfactor then is difficult to predict and to control, whereas a well defined transmission sequence then is hardly to achieve. Moreover, the now available neutral (diffuse) white epoxy is less neutral than the NEUTRAL black epoxy which is used now. Possibly also a well protected metallic coating could be used as a more efficient one.
Another point of view is to leave all the flasks clear with the advantage that one can more easily dispose them. It is recommended (in an eventually revised model of the incubator with a transmission/irradiance sequence) to mount the filters on two filterwheels on both sides of the clear flasks. Furthermore, more compactly mounted circular fluorescent tubes could be used dual-side for more effective and uniform illumination.
Even more than one concentric circular tube (or spiral tubes) could be effectively used.
In general, an optimal spectral characteristic of the fluorescent tube(s) with respect to the effectiveness for primary production is recommended.
The available daylight types of fluorescent tubes are expected to be more effective than the TL33-type. J. de Keijzer.

SUMMARY

As a result of some special developments, irradiance differentiation and control in the ICES incubator, needed in order to be able to accomplish reliable P-I relation measurements with respect to phytoplankton primary production studies, is achieved. Due to these developments neutral density epoxy filterlayers could be made on four identical filter/flask series.
Besides that, it was possible to construct some mini spherical irradiance sensors, small enough to measure the irradiance inside the flasks. The filters and sensors proved to have good optical qualifications.
The maximum averaged absolute irradiance is rather low when single-side illumination is used. Therefore, when an incubator with an irradiance sequence is needed for P-I relation measurements, a more effective and uniform dual-side circular TL-illumination is proposed with the flasks between two filterwheels.

ACKNOWLEDGEMENT

The author would like to thank Mr. L.P.M.J. Wetsteyn for useful comments on the manuscript.

REFERENCE

Colijn, F., G.W. Kraay, R.N.M. Duin & M.J.W. Veldhuis, 1993. Design and tests of a novel Pmax incubator to be used for measuring the phytoplankton primary production in ICES monitoring studies. Annex 5 from Report of the Working Group on phytoplankton and the management of their effects. ICES C.M.1993/ENV:7 Ref.:L.

Tables 1, 2, 3 and 4 >>>>>.

Experts to add tables