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Mixing layer running incubator (MIRI): an instrument for incubating samples while moving vertically in the mixing layer

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Abstract

Research on the impact of natural radiation on aquatic biota requires novel instruments and methods enabling realistic tests of the effects of changing radiation on communities. This article describes an instrument devised to expose samples maintained in incubation flasks to the variable conditions experienced by the water masses in motion in the mixing layer. This apparatus can be useful to measure the biological processes occurring in the photic zone of aquatic ecosystems avoiding the bias due to the incubation at fixed depths. Here an example of its use is presented, comparing the effect of photosynthetically active radiation (PAR) and ultraviolet radiation (UV-R) in samples incubated at fixed depths and using MIRI (MIxing layer Running Incubator).

Biological processes occurring in the photic zone of lakes are often measured in confined systems exposed at fixed depths (Steemann Nielsen 1952) or at constant light levels (Parsons et al. 1984). Although the in situ incubation at fixed depths is widely accepted as the nearest approximation to natural field conditions, the measurements thus obtained could be biased because of the mixing exclusion. The dynamics of the wind-mixed layer in lakes is complex (Imboden and Wüest 1995), and thus generalizations are difficult due to the numerous interactions between the morphology of the enclosed basins and the meteorological forcing variables. Nevertheless, as a first approximation the turbulent mixing layer, in the pelagic zone of a deep lake, can be assumed as extending down to a depth limit set by thermal stratification and by the surface wind stress (Wüest and Lorke 2003). The mixing layer can exceed the photic zone or can be included in it, according to the termocline depth. In each case, the water masses in motion in the mixing layer and the planktonic organisms living there are exposed to a variable radiation that is a function of vertical mixing velocity and the thickness of the photic zone affected by mixing.

There have been several attempts to overcome the mixing exclusion when performing in situ incubation experiments. For instance, Behrendt (1989) used a bottles-lift to investigate the influence of vertical water movements on primary production. Although this device allowed the regulation of the lifting velocity in a convenient range (0.08 to 3 cm s^{-1}), its use was limited to 15 m depth by the winch size and manageability. For turbulence simulation, Zagarese et al. (1998) used a wheel rotating perpendicularly to water surface plane and carrying the incubation vials to make the samples moving through the subsuperficial layers. However, the operation depth is limited to 80 cm. Helbling et al. (1994) and Gocke and Lenz (2004) designed a deck incubator where the vertical change in light is simulated by moving the incubation vials in a water bath covered by a glass lid of neutral optical density filters. The use of a fixed set of optical filters cannot be easily adapted to reflect the huge variety of underwater radiation ranges that can be found in lakes. The different wavebands have different extinction coefficients. This holds true for UV-B, UV-A, and PAR. But also within PAR itself, the different wavelengths exhibit different extinctions coefficients due to radiation absorption by lake water with its dissolved and particulate matter content (Kirk 1994). Neutral filters fail to simulate the natural conditions unless adjustment is applied to match any particular situation of a given lake and weather condition. The naturally occurring vertical variations in light climate within the mixing layer are not negligible and are likely to affect the phytoplankton primary production measures and the UV-R impact on aquatic organisms. To further improve the approximation to natural in situ conditions, we devised an easy to handle instrument, which can move up and down in the mixing layer, through a water column extending up to 30 m, at a preset speed. It carries incubation flasks, and the samples are

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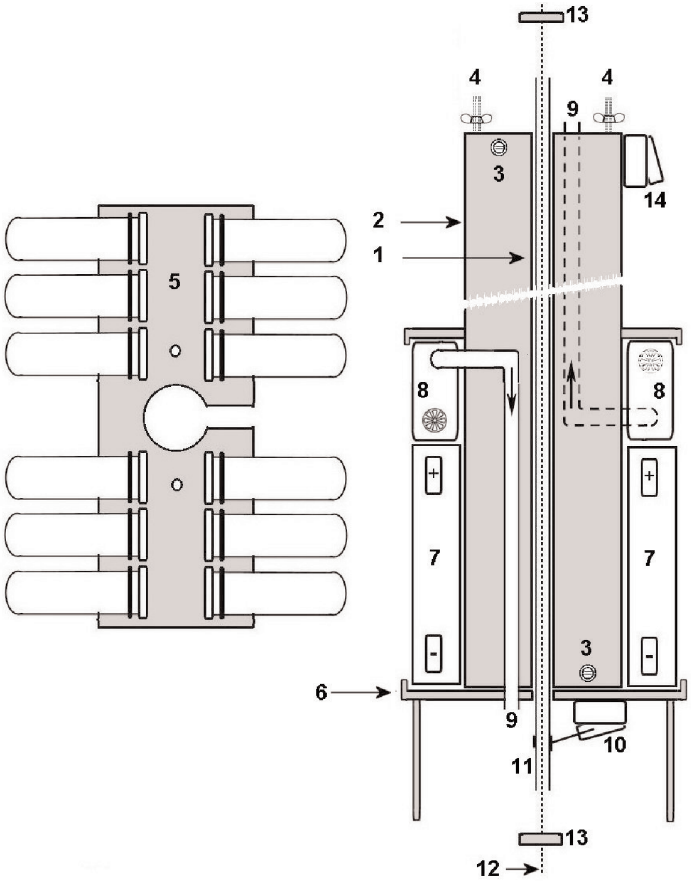

Fig. 1. Schematic drawing of the instrument. *See* explanations in *General design of apparatus*.

thus exposed to the underwater radiation in a way that better approaches the variable conditions existing in the mixing layer. As the operating depths can be easily fixed to match any desired working depth range, also the effect of vertical mixing due to particular phenomena (e.g., Lagmuir circulation) can be studied using MIRI. However, the nearness of MIRI approximation to real lake conditions decreases as much as the actual trajectory of the "water parcels" departs from the simple vertical displacement and/or change in velocity during the incubation time (Wüest and Lorke 2003).

Materials and procedures

Principle of operation—A cylindrical buoy with a central tube for a rope to pass through holds batteries that are operating two submersible pumps. At the upper edge of the buoy, there is a frame holding the incubation flasks. The device is adjustable for neutral buoyancy. The pumps are set to eject water—one upward and the other downward—thus pushing the instrument. They are put into operation alternatively by a switching system. This is activated when the instrument, sliding along a driving rope, reaches the upper or lower stopping point. Thus the pump pushing downward and that pushing upward are energized in succession. The stopping points are Perspex™ disks fixed on the mooring rope in positions corresponding with the upper and lower border of the mixing layer.

General design of apparatus—A schematic drawing of the instrument is presented in Fig. 1. The cylindrical buoy with the central passage for the driving rope is made out of two acrylic (Perspex) tubes (prototype dimensions: tubes thickness 5 mm, height 60 cm, outer tube diameter 10 cm, inner tube internal diameter 1.5 cm). The tube with the smaller diameter [1] is placed inside the larger [2], and the resulting chamber is sealed at both ends with two Perspex disks (diameter 10 cm) with a central hole of 1.5 cm in diameter. The resulting buoy has taps [3] at both ends to allow water to enter until the device is adjusted for neutral buoyancy. The upper disk is provided with two screws [4] to hold the flasks incubation frame [5]. A frame [6] fixed to the lower disk by screws can accommodate up to four sealed batteries [7] of 12V 2.2Ah (size 3.5 × 6 × 17.5 cm). Above the two batteries are accommodated two submersible pumps [8] (12V 5W, Reich), which are connected to the batteries through an adjustable voltage regulator and a switching system. They are arranged such that the outlet of one is facing upward and the outlet of the other downward. The pump outlets are attached as close as possible to the respective edge of the buoy axis by a length of silicon tube [9]. The switching system is a rocker switch [10] fixed at the bottom of the device. Its rocker actuator is tightly fixed to a rigid plastic tube [11], which can slide through the central passage of the buoy and inside which the driving rope [12] is passing. Two plastic discs (ca. 5 cm in diameter [13]) fixed to the driving rope make the upper and lower stopping point. In low conductivity waters (ca. 100 μS cm^{-1}), waterproofing of wiring is not critical and common switches can be used. To perform experiments moving the incubation flask in the mixing layer, the instrument is moored as shown in Fig. 2, with the driving rope passing through the inner central passage of the instrument. A convenient weight [9] placed at the bottom of the rope keeps the incubator vertical. The plastic disc [8] fixed close to the weight acts as inferior brake by limiting the downward run of the instrument. Then the incubation flasks holder is set in place and the upper brake [7] limiting the upward run is fixed to the rope. The main switch (Fig. 1, [14]) is now closed, activating the pump, which is pushing downward. Then the MIRI starts its trip downward until the bottom stop is reached and vice versa.

The instrument equipped with four batteries and two pumps of the above-mentioned size can run up to 7 h, which is exceeding the exposition time normally used in primary production experiments.

The speed of the device can be regulated by means of a voltage regulator to match the expected vertical current velocity of the mixing layer. This could be empirically determined with an Acoustic Doppler Current Profiler (Gordon et al. 2000). As the empirical approach is not always possible, a first, rough approximation of the time scale of mixing in a thermally homogeneous epilimnetic layer could be calculated as follows:

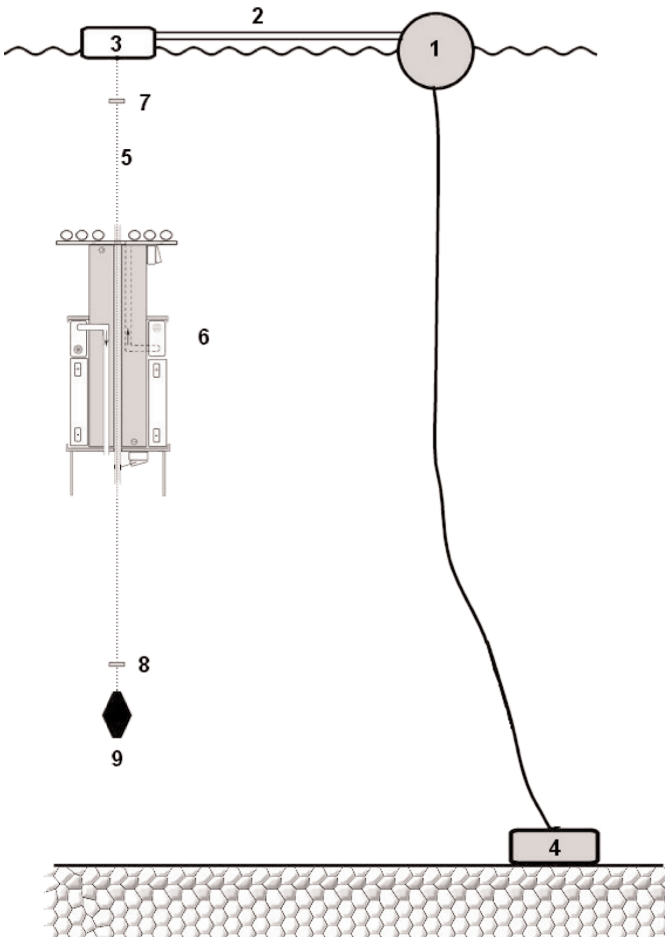

Fig. 2. Mooring of the instrument. 1, main floater; 2, rigid connection; 3, transparent buoy; 4, anchor weight; 5, driving rope; 6, MIRI; upper (7) and lower (8) run limiters; 9, MIRI driving rope weight.

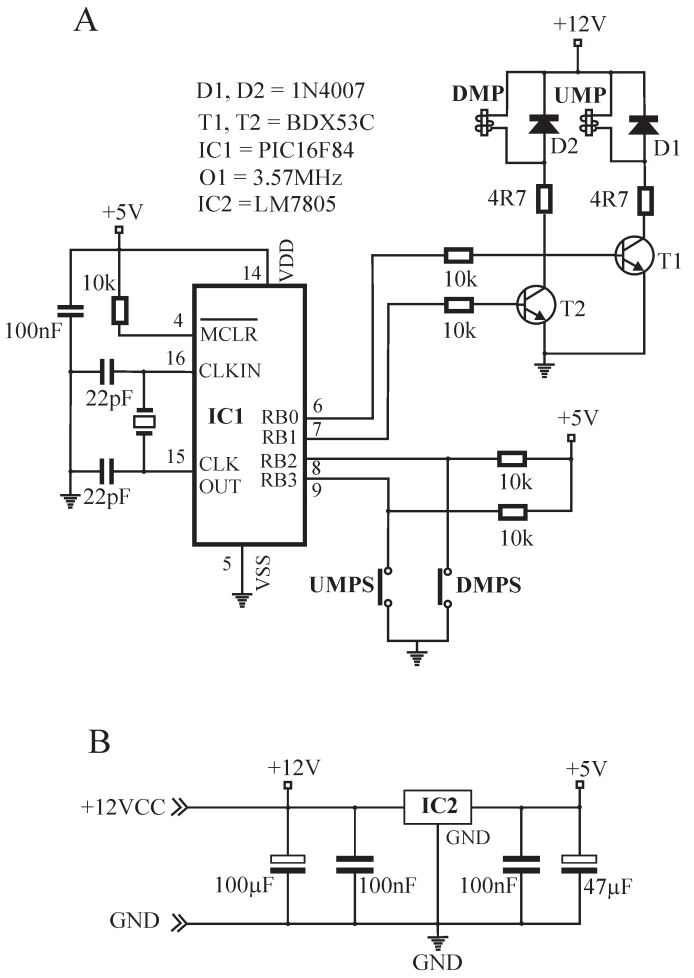

Fig. 3. Schematic diagram of the electronic switching system. A is microcontroller and B is power supply regulator. DMP, downward moving pump; UMP, upward moving pump; DMPS, switch of the downward moving pump; UMPS, switch of the upward moving pump.

$$t = h/u$$

where t is time (in seconds), h the thickness (in m) of the upper layer considered, and u the mixing velocity (in m s^{-1}), which can be estimated as

$$u = sqr\ (da/dw \times 0.0013 \times U^2)$$

where U is the wind velocity in $m\ s^{-1}$, and da and dw are the air and water densities (in kg dm^{-3}), respectively (J. Catalan, pers. comm. unref.).

Instead of the electromechanical switching system previously described, an electronic one was tested. This one needs less force to be actuated; it is worth using when MIRI is running at low speed (<3 cm s^{-1}). The electronic switching system diagram is shown in Fig. 3. In the electronic version, the rocker switch is substituted by two magnetic proximity switches (MK3-1A71C, Meder Electronic) fixed one at upper and the other at the lower edge of the central passage of the buoy. They are activated in succession by a couple of magnets making up the lower and upper brake limiting the run of MIRI, as [7] and [8] in Fig. 2. The magnetic proximity switches drives the switching circuit, which is made up by a micro-controller (PIC16F84, Microchip Technology Inc.) programmed to energize the downward moving pump and the upward moving pump in sequence when the appropriate switch is activated. In addition, the program loaded in the micro-controller memory (in assembly for RISC Microchip processors, PIC16 series, *see* Web appendix 1) energize the upward moving pump first when the electronic switching system is set to "on". This assures the correct start-up of the incubation and avoids an unwanted downward run of the instrument. A further feature of the program is the checking for the accidental switching of the last activated magnetic proximity switch, which can possibly occur in heavily wavy condition. The board with the electronic of the switching system is placed in a small cylin-

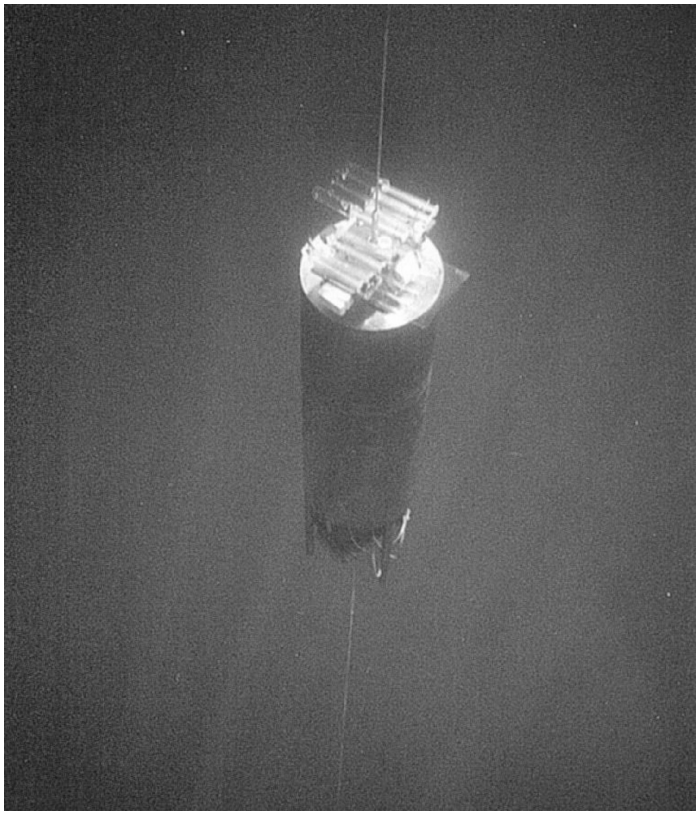

Fig. 4. Picture showing the MIRI during its operation in Lago Moreno waters, carrying a frame holding the incubation tubes. The top of the cylindrical buoy was made white for visibility.

drical (60 mm diameter by 60 mm height) waterproof aluminum box tightened to the body of MIRI buoy.

The prototype we tested, shown in Fig. 4, has a weight of approximately 8 kg and a height of 80 cm. It was moored and deployed from a small (4 m length) inflatable boat, and it can be easily operated in the field by two persons. In all cases, the device worked well and never failed while running during 4 h incubation in the field under calm and rough conditions (wind velocity up to 40 km h^{-1}). The cost of the spare parts used to build the prototype, including the electronic switching option, was approximately of 300 US$.

Test of the instrument in the field

The instrument was first tested in Lago Moreno (Northern Patagonia, Argentina), which is particularly suitable for this kind of evaluation because it is a very clear ultraoligotrophic lake (Modenutti et al 2000). The underwater light regime of Lago Moreno is summarized in Table 1. The summer K_d values from vertical profiles were obtained with a radiometer (mod. PUV 500, Biospherical Instruments).

According to the above formulas, with a typical late summer air temperature of 20°C, water temperature of 17°C, a mixing layer depth (*h*) of 15 m, and a wind velocity (*U*) of 10 to 15 m s^{-1} (quite usual in Northern Patagonia), we can expect a mixing time (t) of about 10 min.

Table 1. Vertical extinction coefficients (K_d) and depth (m) reached by 1% of surface irradiance ($z_{1\%}$) for UV-R and PAR in Lago Moreno, 2 February 2004 (Southern hemisphere)

	305 nm	320 nm	340 nm	380 nm	PAR
K_d	0.752	0.610	0.471	0.294	0.166
$z_{1\%}$	6.12	7.55	9.78	15.66	27.80

To test the instrument, we used an integrated sample from subsurface to 15 m, the lower limit of the mixing layer of Lago Moreno in February 2004. The light regime affecting samples incubated at fixed depths (subsurface and 10 m) and moving along the mixing layer from 0 to 15 m at a speed close to that of mixing water masses is presented in Fig. 5. The graphs are from three measurement series with PUV 500 starting at noon and lasting 15 min each. Fig. 5 clearly shows the difference in radiation regime to which samples incubated at fixed depth or with MIRI are exposed. The moving samples undergo high radiation and low radiation in turns. Thus photoinhibition followed by photorepair can occur. The subsurface sample is permanently exposed to extremely high radiation (and possibly photoinhibited) while the 10 m sample is permanently protected from high radiation. In addition, the MIRI exposed samples do not simply receive a radiation approaching that of

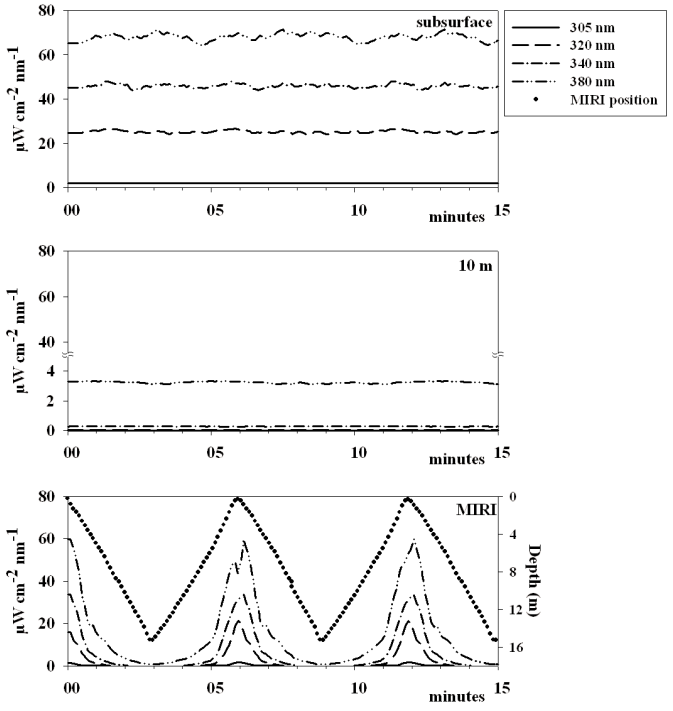

Fig. 5. Radiation regime of UV-R for subsurface, 10 m and MIRI-incubated vials. PAR (not reported in the graph) at the same time was 1460 and 140 µmol photons m^{-2} s^{-1} at subsurface and 10 m, respectively.

Table 2. Surface (I_0) and 10 m (I_{10}) depth irradiance and mean irradiance for MIRI (I_{MIRI}), and percentage of surface incident radiation reaching the vials incubated at 10 m fixed depth and using MIRI*

	305 nm	320 nm	340 nm	380 nm	PAR
Fixed					
I_0	3.484	37.38	63.67	91.31	1705.3
I_{10}	0.002	0.08	0.57	4.82	325.4
% 10 m	0.054	0.22	0.90	5.28	19.1
Moving					
I_{MIRI}	0.308	4.08	9.00	20.45	629.1
% MIRI	8.861	10.92	14.14	22.39	36.9

*Data from Lago Moreno, 2 February 2004. The bands 305, 320, 340, and 380 nm are in μW cm^{-2} nm^{-1}. PAR is in μmol photon m^{-2} s^{-1}

the intermediate depth but rather an amount depending on the specific K_d of different wavelengths. Table 2 shows the percentage of radiation at each wavelength with respect to the surface (100%) measured in Lago Moreno during the test of MIRI, running up and down between subsurface and 15 m depth. MIRI mean irradiance (I_{MIRI}) was calculated according to I_m of Helbling et al. (1994):

$$I_m = I_0 \cdot \frac{1 - e^{(-K_d \cdot z)}}{K_d \cdot z}$$

where I_0 is the irradiance at the surface, K_d is the diffuse attenuation coefficient (for the corresponding wavelength band), and z is the depth of the mixed layer.

Here are presented the results of an experiment of measurement of net primary production (NPP) in Lago Moreno by the ^{14}C technique (Steeman Nielsen 1951, 1952). Subsamples from a 0- to 15- m integrated sample were incubated (3 replicates) at two fixed depths (subsurface and 10 m) and through a MIRI set to run back and forth from subsurface to 15 m, i.e., in the mixing layer at the time of the experiment. The incubation, in quartz vials, lasted 4 h starting at 11:00 am. Dark bottle measurements were substituted by the "time 0" organic ^{14}C by adding the isotope to a dark bottle and immediately filtering and analyzing (Fahnenstiel et al. 1994). The results obtained are synthesized in Table 3.

The NPP from MIRI samples has an intermediate value between the exposed (subsurface) and protected (10 m) sample, the former possibly suffering photoinhibition and the second taking advantage from a permanent radiation protection in this very transparent lake. Some field results achievable with MIRI are presented in more details in Modenutti et al. (in press).

The NPP from conventional in situ incubation should thus be regarded as suffering of a methodological drawback. On the contrary, the NPP from MIRI samples, undergoing the variable radiation regime of water mass moving in the mixing layer, are likely to be a best approximation to the real NPP in the whole mixing layer. The observed NPP pattern would obviously vary according to the composition of phototroph assemblages, depending also on the underwater light climate. The light climate changes along the water column as a function of the lake water optical characteristics and of the season. In fixed depth incubation, a particular light climate is continuously affecting the organisms under study and does not allow them to recover from possible photoinhibition or UV-R damage through displacement to a less harmful zone. The instruments subjecting the organisms to variable light intensities through rotating neutral filters to some extent overcome the fixed depth incubation problems. However, this approach underestimates the underwater light climate variation as all the wavelengths are equally attenuated. The advantage of MIRI is that the experiments can be run under the natural varying light conditions, allowing the organisms temporarily exposed to subsurface high light to recover from photoinhibition or UV-R damage while the device is deep in the epilimnion.

Table 3. Average (±SD) of three replicates of NPP at fixed depths and using MIRI*

	mgC m^{-3} h^{-1}	±SD
Subsurface	2.55	0.27
10 m	7.70	0.74
MIRI	4.80	0.89

*Lago Moreno, February 2004 (Southern Hemisphere).

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