

SUPPLEMENTARY MATERIAL 1

Methodological techniques for physicochemical and biological data described in "A Latin American network of microbial observatories for monitoring aquatic ecosystems"

The methods are taken from the Latin American Microbial Observatory Network's Protocols Book (Latin American Microbial Observatory Network 2024). Here, we present the variables used to characterize the observatories in this work. The other standardized variables for the observatories' progress are described in the Protocol Book.

Sampling

Observatory-sites measurements were conducted during the morning hours, in the second fortnight, targeting the photic layer of the water column. Precipitation events and/or strong winds were avoided to maximize the representativity of the major temporal patterns at each site. Lack of accessibility or other circumstances that made it not feasible to follow the complete synchronized sampling scheme were considered as well. For instance, in the context of glacial streams (BL and N sites), the sampling scheme concentrates on periods before, during, and after melting. Subsurface water samples for physicochemical and biological variables were collected in 5-L acid-washed bottles for subsequent determination of physical, chemical, and biological parameters.

Chlorophyll-a

Samples for planktonic chlorophyll-*a* (Chl-*a*) analyses were filtered through 0.7 µm size pore filters within the same day they were taken, and filters were preserved at -4°C. Concentrations of Chl-*a* (corrected for phaeopigments by HCl 1N acidification) were determined by spectrophotometry, using ethanol or acetone, depending the site, as an extraction solvent for overnight incubation. The equations published by Marker et al. (1980) were used to calculate Chl-*a* concentrations.

$$[\text{Chl-}a \text{ corrected for phaeopigments}] = F [(\text{Abs}_{1665} - \text{Abs}_{1750}) - (\text{Abs}_{2665} - \text{Abs}_{2750})] k v$$

Where Chl-*a* corrected for phaeopigments is expressed in µg/l;

Abs1 = absorbance before acidification;

Abs2 = absorbance after acidification;

F = correction factor to match the reduction in absorbance with the initial Chl-*a* (2.43 for ethanol and 2.43 for acetone);

k = specific absorption coefficient (11.2 for ethanol and 10.48 for acetone);

v = volume of extract in ml / (sample volume in liters x cuvette thickness in cm).

Periphyton was obtained by scraping the individual stones with a brush and rinsing them carefully with distilled water on the same day of sampling. The final volume obtained was adjusted to a constant volume (100 ml); the area (cm²) scraped on the stone was estimated from the lengths of the three main axes (Graham and others 1987). To determine the Chl-*a* concentration, a 1-ml aliquot of the periphyton suspension was filtered through glass fiber filters (GF/F, Whatman, Maidstone, UK). After the filtration step, Chl-*a* was extracted in hot ethanol (Nusch 1980) and measured with a fluorometer previously calibrated against spectrophotometric measurements.

Bacterial abundance

Microorganisms such as heterotrophic bacteria, picocyanobacteria, and picoeukaryotes were identified by flow cytometry (Dubelaar et al. 2007; Schiaffino et al. 2013; Gasol and Morán 2016). Water samples were fixed with P+G (paraformaldehyde and glutaraldehyde) and GlyTE (glycerol and TE buffer), frozen in liquid nitrogen, and stored at -80°C until analysis. Heterotrophic bacteria were stained with SYBRGreen. Subsamples were run through different flow cytometers available on the net, equipped with blue and red lasers, and cytometric diversity analysis was performed with FlowDiv R package (Wanderley et al. 2019). FL1-SSC and FL3-FL1 biplots were used to quantify heterotrophic bacterial abundance.

Light measurements

Evaluation of the vertical extinction and spectral characteristics of light in natural waters is commonly accomplished *in situ* with underwater quantum sensors. The Secchi disk, a simple tool, can also approximate water transparency. It consists of a white or black-and-white disk, typically 20 cm in diameter, lowered into the water until it is no longer visible. The depth at which the disk disappears, known as the Secchi depth, indicates the water's clarity and light penetration. The greater the Secchi depth, the clearer the water (Wetzel 2001).

Light extinction and related variables, such as turbidity, total suspended solids (TSS), and the attenuation coefficient (K_d), help assess the optical properties of water bodies. The light attenuation coefficient (K_d) measures how quickly light diminishes with depth. It is calculated using light intensity measurements at different depths, with a higher K_d indicating greater light attenuation. TSS quantifies the amount of suspended particles (like silt, clay, and organic matter) in water. It is determined by filtering a water sample, drying, and weighing the particles. High TSS levels indicate more particles, affecting light penetration. Turbidity measures the cloudiness of water caused by suspended particles and is often measured in Nephelometric Turbidity Units (NTU).

The relationships between these measurements provide insights into water quality and light penetration. Secchi depth is inversely related to turbidity: as turbidity increases, Secchi depth decreases due to reduced transparency. Similarly, higher TSS levels generally result in a lower Secchi depth. The attenuation coefficient (K_d) increases with higher turbidity levels, as more turbidity leads to more light being absorbed and scattered, reducing penetration. There is also a positive correlation between K_d and TSS, as more suspended solids cause greater light attenuation. Following the interrelationship between variables we plot only K_d .

The formula of light attenuation coefficient (K_d) is:

$$K_d = \frac{1}{Z} \ln \ln \left(\frac{I_0}{I_z} \right)$$

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