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EVALUATION OF LASER IN SITU SCATTERING INSTRUMENT FOR MEASURING CONCENTRATION OF PHYTOPLANKTON, PURPLE SULFUR BACTERIA, AND SUSPENDED INORGANIC SEDIMENTS IN LAKES

By Teresa Serra¹, Jordi Colomer², Xavier P. Cristina³, Xavier Vila⁴, Juan B. Arellano⁵, and Xavier Casamitjana⁶

ABSTRACT: A laser in situ scattering and transmissometry (Lisst-100) probe has been used for estimating the particle-size distribution of phytoplankton, purple photosynthetic sulphur bacteria (*Chromatiaceae*), and suspended inorganic sediments in different lakes. Results from Lisst-100 have been compared to laboratory measurements, such as those obtained by using a Galai laser size analyzer (GL), an optical microscope (OM), and a flow cytometer (FC). Although all of these instruments were shown to provide reliable values of the particle number concentration for the given populations, the Lisst-100 was the fastest and most reliable instrument because it did not require manipulation of the samples—which is not the case of GL, OM and FC instruments—and avoided the tedious procedure of microscopic counts. The total particle volume concentration results obtained with Lisst-100 differed from those obtained with GL for populations with large and porous aggregates, such as phytoplankton cells. The difference was attributed to the breakage of fragile algal aggregates resulting from the measuring procedure used by GL. Although for suspended sediment particles both instruments gave the same results for the total particle volume concentration, the particle-size distribution obtained with GL was found always shifted to smaller diameters than with Lisst-100, probably because inorganic sediment particles present compact aggregates. When these aggregates break, they split into a high number of small particles that contribute the same to the total volume concentration as the previous aggregates. Finally, results of the total particle volume concentration with Lisst-100 were in accordance with those obtained with GL for the *Chromatiaceae* population, because cells remained in a dispersed phase. A good correlation was found between the total particle volume concentration of *Chromatiaceae* measured with Lisst-100 and the concentration of bacteriochlorophyll *a* (BChl *a*), which is the parameter habitually used to estimate the concentration of *Chromatiaceae*. Therefore, Lisst-100 was found to be a reliable instrument to estimate the *Chromatiaceae* concentration in aquatic ecosystems.

INTRODUCTION

Particles are ubiquitous in several aquatic systems—oceans, lakes, reservoirs, wetlands, rivers, etc.—These particles can present a broad range of sizes, from the colloid category size (smaller than 1 μm) to the suspended category size (larger than 1 μm), as have been traditionally classified (Elimelech et al. 1995). Measurements of the concentration and sizes of suspended organic and inorganic particles in water systems are highly important for different ecological implications. Among them there are the seasonal production of phytoplankton and bacterioplankton (Vrede et al. 1999) and the formation of aggregate accumulations (marine snow) in ocean midwaters (Honeyman and Santschi 1989; Macintyre et al. 1995; Levi 1999). Also, some contaminants have affinity to suspended colloidal particles, which determine their spatial and temporal distribution (Krishnappan et al., personal communication; Santschi et al. 1997) or the mass transport in water and waste water treatment plants (Dymaczewski 1997). Processes such as coagulation and breakup of particles might determine the diameter of suspended particles (Serra et al. 1997; Serra and Casamitjana 1998). During sedimentation, aggregates scavenge colloidal particles that are suspended in the water column, therefore enhancing transport to the bottom (Hill and Nowell 1990; Serra and Logan 1999). Scavenging of dissolved colloidal material has been shown to control the sorption of metals by suspended particles and therefore their transport throughout the water column (Honeyman and Santschi 1989).

¹ Dept. of Phys., Campus Montilivi, Univ. of Girona, 17071-Girona, Spain

² Dept. of Phys., Campus Montilivi, Univ. of Girona, 17071-Girona, Spain.

³ Inst. of Aquatic Ecology, Campus Montilivi, Univ. of Girona, 17071-Girona, Spain.

⁴ Inst. of Aquatic Ecology, Campus Montilivi, Univ. of Girona, 17071-Girona, Spain.

⁵ Inst. of Aquatic Ecology, Campus Montilivi, Univ. of Girona, 17071-Girona, Spain.

⁶ Dept. of Phys., Campus Montilivi, Univ. of Girona, 17071-Girona, Spain.

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Numerous instruments have traditionally been used from long ago to measure the size distribution of suspended particles (both organic and inorganic) in aquatic systems, such as Coulter-counters (Jonasz and Prandke 1986; Tsai et al. 1987; Stramski and Mobley 1997; Huisman 1999), microscopes (Monfort and Baleaux 1992), flow cytometers (Cavender-Bares et al. 1998; Robertson et al. 1998; Cristina et al. 2001), laboratory laser particle-size analyzers (Edelvang and Austen 1997), etc. However, when these instruments are used, a previous handling and treatment of samples is always needed, which can damage the suspended aggregates modifying the size distribution and thus the results (Alldredge et al. 1990; Del Giorgio et al. 1996). In contrast, optical sensors (Pottsmith and Bhogal 1995) and submersible cameras are in situ field instruments that provide nonintrusive sampling and therefore more realistic results (Alldredge and Gotschalk 1988; Knowles and Wells 1998). In these cases, the drawback of floc breakage associated with traditional methods of sediment sampling and analysis are overcome. Traditional optical sensors can only measure the total concentration of particles, but they cannot differentiate among populations of different sizes. Submersible cameras provide information of the shape, size, and structure of the suspended aggregates, but they do not perform a large enough number of measurements to obtain confident particle counts, especially for the larger particles that occur in lower concentrations. Moreover, the range of measurable particles with cameras is usually $\geq 20 \mu\text{m}$, in which case small particles such as bacteria ($\sim 5 \mu\text{m}$) or fine sediments cannot be resolved. In this study, the Lisst-100 (Laser in situ Scattering and Transmissometry) probe designed by Sequoia Scientific was used to obtain the particle-size distribution in different aquatic environments. The instrument used here is a new version of Lisst-100, which is able to measure smaller particles ($1.2\text{--}250 \mu\text{m}$) with more resolution than those measured with its previous version ($5\text{--}500 \mu\text{m}$). The previous version of Lisst-100 has already been evaluated and found appropriate for measuring natural sediments (Traykovski et al. 1999). In the present work, Lisst-100 has been also used to measure organic particles, such as photosynthetic sulphur bacteria (*Chromatiaceae*) and phytoplankton, and inorganic particles such as suspended sediments. Results will be compared with those obtained with a laboratory laser particle-size analyzer (GL) and other conventional laboratory instruments, such as an optical microscope (OM), flow cytometer (FC), and HPLC in order to evaluate the validity of the nonintrusive method (Lisst-100) as compared with the intrusive methods (GL, OM, and FC and HPLC photosynthetic pigments concentration). Complementary chemical information—such as oxygen, redox potential, conductivity, and temperature—was also measured.

LOCATIONS

Measurements of algal, bacterial microorganisms and inorganic particles were carried out in four different lakes (Montcortés, Banyoles, Sisó, and Vilar) and in one reservoir (Boadella), with the purpose of obtaining a broad range of results. Lake Montcortés is situated in Northwest Catalonia, in the Northeast of Spain. Water samples were harvested at different depths from April 1998 to November 1998 in order to study the population of *Chromatiaceae* (bacteriochlorophyll a-containing microorganisms) that it is known to thrive between 18 and 21 m depth (Vila et al. 2001). Lake Banyoles is a karstic lake situated in Northeast Catalonia. In this lake, the warmer groundwater generates a convective plume in the southern lobe of the lake (Serra et al. 2000; Colomer et al. 2001), which carries a suspension of inorganic particles from the bottom of the lake upwards until the level of neutral buoyancy ($\sim 15 \text{ m}$). Also, in the northern lobe of the lake, populations of phytoplankton and photosynthetic bacteria are known to be present at $\sim 10 \text{ m}$ and $\sim 20 \text{ m}$, respectively (Guerrero et al. 1987; Garcia-Gil and Abella 1992; Vila et al. 1993; Figueras et al. 1993). Grab samples of particle suspensions were taken from the northern lobe of the lake at 20 m depth in April 1998 and from the southern lobe of the lake in July 1998 in order to study the bacterioplankton population and the inorganic particles of the plume, respectively. Lake Sisó is situated in the lacustrine area of Banyoles (Spain) and is characterized by the presence of phytoplankton populations at $\sim 1 \text{ m}$ depth and also of a complex photosynthetic bacterial community from 1 to 6 m depth (Guerrero et al. 1987; Vila et al. 1993). Algal populations were also measured in Lake Vilar, a small eutrophic lake situated in the lacustrine area of Banyoles (Vila et al. 1993). Boadella reservoir is situated on the northeast of Catalonia (Spain); this reservoir presented a dense algal community from April to August of 1998 that was located at the epilimnion of the lake.

METHODS

Lisst-100 (Sequoia Scientific, Inc., Redmond, Washington) consists of a laser beam, an array of 32 detector rings to analyze the light received, a data storage unit, and a battery system [Fig. 1(a)]. Lisst-100 measures the particle volume concentration (n_{vi}) of particles for 64 size classes logarithmically spaced in the range $d_i = 1.2\text{--}200 \mu\text{m}$, by using a procedure based on the laser diffraction theory. By integrating over the whole spectra of size classes,

one can obtain the total particle volume concentration of the suspension of particles ($V_T = \sum_j n_{vj}$). The particle number concentration (n_j) and the area concentration can be calculated from the particle volume concentration (n_{vj}) by using $n_j = n_{vj} / d_j^3$ and $n_{Aj} = n_{vj} / d_j$. Therefore, both the total number (N_T) and area (A_T) concentrations were determined by integrating over the whole spectra of size classes $N_T = \sum_j n_j$ and $A_T = \sum_j n_{Aj}$, respectively. The water depth, with a resolution of 5 cm, was also recorded by a pressure sensor on the instrument.

FIG. 1. (a) Lisst-100 Instrument. Together with Schematic Diagram of Sampling Volume and Optical Path (Photograph Courtesy of Sequoia Scientific, Inc.); (b) Schematic Diagram of Double-Conus-Shaped End Device

Laboratory samples were collected at different depths in the aquatic environments described in the location section, with a double cone-shaped end device [Fig. 1(b)], ensuring low shear rates (Jørgensen et al. 1979). These samples were fixed in the field with formaldehyde (4%) and stored in a freezer at 4°C until they were measured. GL is a laboratory laser particle size analyzer that has been used to measure the particle-size distribution of samples harvested at different depths [Fig. 1(c)]. The instrument consists of a rotating laser beam that is used to scan the samples; the interaction of the laser beam and the particle gives a pulse, and the width of this pulse is directly proportionate to the particle size (this is known as the "time of transition theory"). The range of measurable particle sizes with GL is from 0.5 μm to 150 μm . Samples must be kept in movement by means of a magnetic stirrer or a continuous flow in order to be measured. For this reason, depending on the strength of particles this measuring procedure might cause their breakup. Because of these limitations, this instrument might only be properly applied to dispersed populations (Edel-vang and Austen 1997) and to strong aggregates. In order to determine that both instruments give the same results using Lisst-100 and GL, they have been calibrated together in the laboratory with latex particles of 2 μm , giving differences smaller than 1%.

A FACScalibur (Becton Dickinson, Oxford, U.K.) FC equipped with a 15 mW, air-cooled argon-ion laser as the excitation source (488 nm) has been used for this study [Fig. 1(d)]. FC has been successfully applied in the analysis of picoplankton from the sea (Peters et al. 1998; Gasol et al. 1999; Trouselier et al. 1999). In order to obtain absolute counts, a known concentration of a bead solution was added to samples (Cantineaux et al. 1993). Polystyrene yellow-green fluorescent microspheres (1 μm diameter; Molecular Probes, Inc., Eugene, Oregon) were found to be the most suitable. The concentration of the microspheres in the sample was in the range of $5.6\text{--}6.6 \times 10^5 \text{ mL}^{-1}$, and they were always counted under optical microscopy (OM) before the analysis of a sample set. Counts of bacteria under OM were done on polycarbonate filters (0.22- μm -pores size; 25 mm; MSI) after staining with 4',6-diamidino-2-phenylindole (DAPI; $2.5 \text{ } (\mu\text{g } \mu\text{L}^{-1})$) for 10 min. At least 20 fields containing >400 cells were counted for each sample with a Zeiss Axioskop microscope (50 W mercury lamp, filter-set AF 01).

Finally, a multiparametric probe (Hydrolab, Hydrolab Corp.) provided vertical profiles of dissolved oxygen concentration (accuracy = 0.2 mg/L and resolution = 0.01 mg/L), redox potential (accuracy = 20 mV and resolution = 1 mV), conductivity (accuracy = 1%), and temperature (accuracy = 0.1°C and resolution = 0.01°C).

General Identification of Populations Suspended in Water Column of a Lake. Some populations of microorganisms can be recognized when analyzing particle volume concentration, conductivity, redox potential, and temperature profiles. This is the case of the algal and bacterial populations found in the water column of Lake Banyoles. The former population, detected by the total particle volume concentration profiles obtained with Liss-100 [Fig. 2(a)], showed subsurface a maximum of $\sim 5 \mu\text{L/L}$, centered at the thermocline at 10 m depth [Fig. 2(b)]. The peak was coincident with a peak of dissolved oxygen concentration of 11 mg/L [Fig. 2(c)]. The latter population was detected in the hypolimnion with a concentration of $\sim 1 \mu\text{L/L}$ [Fig. 2(a)] and was constant down to 19 m. From here to the bottom, the total particle volume concentration increased to values of $\sim 2 \mu\text{L/L}$. At this level, an increase of the conductivity [Fig. 2(d)] and a decrease of the redox potential [Fig. 2(e)] was observed, suggesting the presence

of a photosynthetic bacterial population (Guerrero et al. 1987; Garcia-Gil and Abella 1992; Vila et al. 1993). The presence of both algal and bacterial populations was later corroborated by observation under OM.

FIG. 2. Total Particle Volume Concentration Obtained with Liss-100 (a), Temperature (b), Dissolved Oxygen Concentration (c), Conductivity (d), and Redox Potential (e) (Profiles Obtained by Hydrolab Probe: Data Sampled in April 1998 in North Lobe of Lake Banyoles)

Specific Identification of Chromatiaceae Population in Water Column of Lake Montcortés. Samples of water were harvested from different depths in Lake Montcortés from April 1998 to November 1998 in order to study the population of *Chromatiaceae* that is known to thrive between 18 and 21 m depth (Vila et al. 2001). From June through November, observations under OM showed the presence of *Chromatiaceae* population, with a diameter ranging from 4 to 8 μm and with an oval-shaped morphology. They were flagellated and contained photosynthetic pigments in intracytoplasmic membrane systems. In November, another population of nonflagellated *Chromatiaceae*, with a diameter ranging from 6 to 20 μm , was also observed under OM. In June, the Liss-100 detected a peak of the particle volume concentration with a maximum of 0.5 $\mu\text{L/L}$ centered on a diameter of 4 μm in the range 2–8 μm ; it was related to the *Chromatiaceae* population [Fig. 3(a)], in accordance with observations under OM. Small secondary peaks of particles with diameter $>10 \mu\text{m}$ were also measured with Liss-100 and were attributed to sparse algal cells, also observed under OM. In November, the particle-size distribution showed three main peaks centered on 3 μm , 10 μm , and 40 μm , corresponding to different populations [Fig. 3(b)]. In order to identify the nature of the peaks in the particle distributions, particle volume concentration measurements for each of these peaks were calculated and plotted together with BChl *a* profiles [Figs. 4(a and b)] analyzed by HPLC. In June, a peak of the particle volume concentration, for particles with $d < 10 \mu\text{m}$, together with a peak of BChl *a* were found at the same depth (~18–21 m) [Fig. 4(a)]. On the contrary, no peak of the particle volume concentration was found for particles with $d > 10 \mu\text{m}$ [Fig. 4(a)]. Therefore, only the peak of particles centered at 4 μm diameter observed at this depth [Figs. 3(a)] could be related to the *Chromatiaceae* population. In Fig. 4(b), the particle volume concentration has been split in particles with diameter $<6.47 \mu\text{m}$ to account for particles with the peak centered on 3 μm , particles with diameter between 6.47 and 20 μm to account for particles with diameter centered on 10 μm , and particles with diameter $>20 \mu\text{m}$ to account for particles with diameter centered on 40 μm . This partition of the volume concentration has been done in order to account for all the size classes in the vicinity of the peaks. In November, particle volume concentration profiles corresponding to particles with peaks centered at diameters of 3 and 10 μm coincided with a peak of BChl *a* at ~17–25 m depth [Fig. 4(b)]. OM measurements reveal that these peaks were related to different *Chromatiaceae* populations. In contrast, no peak in particle volume concentration was evident for particles centered at 40 μm ; therefore this population was attributed to an algal population, which was also confirmed by OM observations.

The total particle volume concentration measured with Liss-100 was related to the BChl *a*, which is habitually used to estimate the concentration of *Chromatiaceae*, for measurements carried out in June, September, October, and November in Lake Montcortés at the depths where this bacterial population was found [Fig. 5(a)]. A fairly good correlation was found between the BChl *a* and the particle volume concentration obtained with the Liss-100 [$R^2 = 0.92$, Fig. 5(a)]. Lower correlations were found between BChl *a* and the total area concentration [$R^2 = 0.76$, Fig. 5(b)] and the total number concentration [$R^2 = 0.67$, Fig. 5(c)]. This indicates that pigments were more strongly

related with the bacterial volume than with their surface area or their numbers, which is in trn in accordance with the fact that photosynthetic pigments in *Chromatiaceae* are located in intracytoplasmatic membrane systems (Staley et al. 1989). The con-elation of both parameters indicates that the Lisst-100 measurements give a good estimate of the *Chromatiaceae* concentration present in lakes.

FIG. 3. Particle-Size Distributions at Different Depths Obtained with Lisst-100 in Lake Montcortés in June 1998 (a) and November 1998 (b)

FIG. 4. Profiles of Particle Volume Concentration, Obtained with Lisst-100, and BChl *a*, Obtained by HPLC on Grab Samples, in Lake Montcortés in June 1998 (a) and November 1998 (b)

General Comparison between Lisst-100 and OM, FC and GL. The total particle number concentration results (mL^{-1}) obtained with Lisst-100 were compared with those obtained by using GL, FC, and OM. A profile of the total particle number concentration (mL^{-1}) obtained with these instruments in Lake Montcortés is presented in Fig. 6. Similar results were obtained with the Lisst-100, PC, and OM. However, GL was found to overestimate the particle number concentration by a factor of ~ 2 . This difference may be caused by the measuring procedure used by GL, as

will be discussed later in the text.

From the data obtained in June 1998, the ratio (r) between the total particle number concentration (mL^{-1}) of *Chromatiaceae* obtained with Liss-100 to that obtained with GL, FC, and OM were calculated by means of a linear regression forced through the origin (Table 1). Although R^2 was found to be statistically significant at 99% confidence level using a one-sided t-test, the best agreement was obtained between Liss100 and FC, with the highest value of R^2 ($R^2 = 0.95$). Slightly smaller values of the particle number concentration were recorded with FC than those obtained with Liss-100, noted by the concentration ratio Liss-100/FC = 1.4 (Table 1). This difference might be caused because of the fact that the range of measurable particles is smaller with the FC ($0.5\text{--}50\text{ }\mu\text{m}$) than with the Liss-100 ($1.2\text{--}200\text{ }\mu\text{m}$). The total particle number concentration obtained with Liss-100 also differed from that obtained with OM. However, it is known that the reliability of counts with OM is subject to the number of fields counted, while GL, Liss-100, and FC undertake measurements over a large number of particles, providing statistically reliable values of the total particle number concentration. The largest difference in the results was found between the GL and Liss-100. GL overestimates the number of particles by a factor of 2 (GL/Liss-100 = 2.2, Table 1). The reason for this difference can be explained by the fact that GL uses a magnetic stirrer to maintain particles, which might produce a high shear stress on the aggregates causing their breakage and therefore increase their number in the suspension.

FIG. 5. Relationship between BChl a Concentration and Particle Volume Concentration (a), Particle Area Concentration (b), and Particle Number Concentration (c) for Measurements of Bacterial Population in Lake Montcortés

FIG. 6. Particle Number Concentration (mL^{-1}) Profiles Obtained with Different Instruments [OM ($\circ$), FC ($\bullet$), GL ($\blacksquare$), and Lisst-100 (Δ)] in Lake Montcortés in June 1998 at Depth Where Bacterial Population Was Found

TABLE 1. Correlation between Results Obtained with Lisst-100 and Other Methods Used: FC, GL, and OM in Lake Montcortés (R^2 = Value of Correlation Coefficient; r = Slope Obtained in Adjustment; n = Sample Size; SE = Sample Error; Percentage of Statistical Significance Based on 1-Sided T-Test Also Presented)

	Lisst-100/EM	Lisst-100/FC	Lisst-100/GL
r	0.6 ± 0.1	1.4 ± 0.1	2.2 ± 0.4
R^2	0.63	0.95	0.60
Significance (%)	99	99	99
n	9	9	10
SE	0.001	0.001	0.016

The particle-size distributions obtained with Lisst-100 and GL are compared in Fig. 7 for particle distributions taken in Lake Montcortés [Fig. 7(a)] and Lake Banyoles [Figs. 7(b and c)]. In Lake Montcortés the maximum value of $\sim 0.6 \mu\text{L/L}$ that was centered on $4 \mu\text{m}$ was identified as the *Chromatiaceae* population described previously [Fig. 3(a)]. This peak was equally resolved by GL and Lisst-100 [Fig. 7(a)]. However, secondary peaks attributed to few algal cells, also observed under OM, were measured with Lisst-100 and GL but without any accordance between them. The secondary peak for GL occurred at $\sim 20 \mu\text{m}$ and the secondary peak for the Lisst-100 occurred at $\sim 50 \mu\text{m}$. The shift from 50 (measured with the nonintrusive method) to $20 \mu\text{m}$ (measured with the intrusive method) can be explained by the fact that aggregates can break easily using the handling procedure of the GL method (Alldredge et al. 1990). Algal populations can aggregate, presenting large and highly porous structures (Logan and Wilkinson 1990). When an aggregate breaks, it splits into a relatively small number of particles of smaller sizes. Since particle-size analyzers interpret broken flocs as compact particles, the total volume of the small particles is smaller than the volume of the original aggregates [Fig. 7(a)]. This is also observed with the algal population obtained from Lake Banyoles [Fig. 7(b)]. However, aggregates of inorganic sediment particles present highly compact structures. When an inorganic aggregate fractures, it splits into a large number of small particles. The total number of these particles contributes significantly to the total volume concentration and gives the same concentration as the initial distribution of inorganic aggregates [Fig. 7(c)].

FIG. 7. Particle Volume Distributions Obtained with GL (—) and Lisst-100 (●) for Different Populations [Bacterial Population in Lake Montcortés (a), Algae Population in North Lobe of Lake Banyoles (b), and Inorganic Particles in Lake Banyoles (c)]

Comparison between Lisst-100 and GL. The total particle number concentration and the total particle volume concentration obtained with Lisst-100 and GL, for all of the measurements carried out in all locations, are compared in Fig. 8(a) and Fig. 8(b), respectively. Despite the scatter, the overall total particle number concentration for the bacterial, algal, and inorganic populations measured with the Lisst-100 is of the same order as that measured with the GL [Fig. 8(a)]. In contrast, the total particle volume concentration measured with the Lisst-100 deviates from that measured with the GL depending on the particle nature. Similar values of the total particle volume concentration were found with both instruments (Lisst-100 and GL) for bacteria and inorganic particles. However, the total particle volume concentration for algae obtained with the GL was one order of magnitude higher than that measured with the Lisst-100 [with an offset of ~ 11 , Fig. 8(b)]. In that case, two main tendencies are more appropriate to describe the behavior of particles, one for the algal populations and the other for inorganic and bacterial populations. Again, this difference can be explained by the fact that algal aggregates break with the measuring procedure of GL. The increase in the total particle number concentration due to the breakage of these aggregates is negligible compared with the large particle number concentration usually present in the samples. Thus, the total particle number concentration, but not volume concentration, of algae gives similar results when it is measured with both instruments (GL and Lisst-100). Further, the contribution of these small particles to the total particle volume concentration is considerably smaller than the total particle volume concentration of the algal aggregates.

FIG. 8. Relationship between Lisst-100 and GL Data with Respect to Particle Number Concentrations (a) and to Particle Volume Concentration (b) for 3 Different Populations Studied: Algae (Δ), Inorganic Particles ($\square$), and Bacteria ($\circ$)

CONCLUSIONS

In this study, an in situ laser particle-size analyzer was used to nonintrusively measure suspended particles at different depths and results were compared with laboratory instruments, including an optical microscope, a flow cytometer, and a laboratory laser size analyzer. The in situ sensor provides fast and reliable particle counts and is more appropriate for sampling a range of particle types, giving more confident results than the other instruments. However, this instrument cannot be used to fully identify the particle population. For this reason it is necessary to first identify samples using the microscope or the analysis of photosynthetic pigments. After this, the Lisst-100 can be used alone to describe the temporal evolution and dynamics of the identified population.

Following this procedure, a good correlation was obtained between the particle volume concentration (obtained with Lisst-100) and BChl *a* (obtained by HPLC) of a *Chromatiaceae* population, which corroborates that the Lisst-100 can be properly used as a technique to estimate the concentration of *Chromatiaceae*, illustrating the usefulness of the sensor for biological applications.

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