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An Integrated Approach to Chlorophyll Monitoring in Surface Freshwater: The Case Study of Lake Albano (Central Italy)

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Abstract: Inland freshwaters are of great importance for human health and activities, but major stressors such as nutrient pollution, deforestation, and urbanization are compromising their status. Water quality degradation and freshwater ecosystem preservation are current issues worldwide requiring frequent and efficient monitoring protocols. The increasing need for large amounts of data to comply with national and international regulations on water quality monitoring highlights traditional procedures limits. Therefore, the purpose of the present study is to investigate the potential of alternative and rapid methods for chlorophyll concentration surveys in freshwaters. The Phyto-PAM (pulse amplitude-modulated) instrument and the Case-2 Regional Coast Colour (C2RCC) satellite image processor were selected to estimate chlorophyll concentration in the surface waters of Lake Albano (Central Italy), selected as a pilot area for the project BLOOWATER (Water JPI 2018 Joint Call Closing the Water Cycle Gap). The correlation tests' results indicate significant relations with chlorophyll data measured spectrophotometrically, confirming the suitability of both methods for chlorophyll retrieval. However, the relatively low strength of the correlation between remotely sensed and spectrophotometric data (r = 0.57, $p < 2.2 \times 10^{-16}$) was not as satisfactory as with Phyto-PAM values (r = 0.97, $p = 1.2 \times 10^{-4}$). Even though the techniques in this study proved to be promising in the water body under investigation, their current limitations suggest the need for further calibration and integration with other systems (e.g., unmanned aerial vehicles).

Keywords: water quality; phytoplankton; monitoring; water management; Phyto-PAM; C2RCC; remote sensing; volcanic lake

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1. Introduction

Besides playing a key role in the global carbon and nutrient cycles and sustaining high levels of biodiversity, inland waters provide valuable ecosystem services [1,2]. Despite their importance, multiple interacting stressors are threatening inland waters worldwide, resulting in an overall decrease in water quality [1,3], which led to the adoption in Europe of the Water Framework Directive (European Commission, 2000).

The growing need for high spatial coverage and temporal frequency of freshwater monitoring stresses the constraints of conventional field methods, which provide accurate measurements but are also time, cost, and labour-demanding. Such limitations are inadequate for monitoring programmes requiring frequent sampling at high spatial and temporal scales. Therefore, the integration of low-cost, portable, and remote systems into the development of more cost-effective and prompt sampling protocols is currently a major monitoring programming interest. Adaptive monitoring is an urgent necessity for efficient data collection that delivers sustainable decision-support for effective natural resources management [4].

Fluorescence based continuous monitoring of phytoplankton biomass has been carried out for decades using in vivo chlorophyll-a (Chl-a) fluorometers [5]. Chl fluorescence parameters have been widely used to evaluate growth and photosynthetic efficiency of phytoplankton. The pulse amplitude-modulated (PAM) fluorometry technique allows retrieving quantitative information on photosynthetic organisms by measuring Chl fluorescence [6,7]. The Phyto-PAM (Multiple Excitation Wavelength Phytoplankton and Photosynthesis Analyzer, Heinz Walz GmbH, Effeltrich, Germany) provides non-destructive and rapid measurements based on in vivo chlorophyll measurements, which do not require sample treatments before carrying out the analysis. Indeed, Phyto-PAM devices are portable and designed to be operational not only in laboratory measurement, but also in the field and for oceanographic studies, thus potentially making them a convenient instrument for routine freshwater monitoring [8–10].

The limits of conventional point sampling to effectively characterize water quality in space and time can be complemented efficiently by Earth-observation (EO) missions. Indeed, satellite remote sensing (RS) offers wide regional coverage and high-frequency data and represents a cost-effective solution, especially for those areas where monitoring activities are scarce or insufficient. Coastal and inland waters are referred to as case-2 waters [11] and are optically complex water bodies subject to potentially large and independent variations of Chl, non-algal particles (NAP), and coloured dissolved organic matter concentrations (CDOM). This optical complexity has caused a time lag for the remote-sensing applications to freshwaters compared to similar studies on oceans [12]. Furthermore, until recently, remote sensing of lake water quality has been mainly carried out with airborne sensors or limited to large water bodies due to the coarse spatial resolution of ocean colour sensors, such as the Medium Resolution Imaging Spectrometer (MERIS) or Moderate Resolution Imaging Spectroradiometer (MODIS) [13]. Unlike ocean colour ones, Sentinel-2 satellites have not been designed specifically for water observation, but even so, they proved to be promising for small lake monitoring [14–17]. Indeed, the Multispectral Instrument (MSI) onboard Sentinel-2 satellites provides frequent (2–5 days), high spatial-resolution (10–60 m) data, offering new opportunities to investigate inland water quality.

Water quality monitoring of small lakes represents a conservation and management priority since they constitute the majority of lakes on Earth [18], and the volcanic lake district in Central Italy makes no exception to this global ratio. Amongst such lakes, Lake Albano is currently the one of greatest concern, with evidence of progressive deterioration over time [19]. Because of its inclusion in European water quality monitoring programmes network and susceptibility to intense algal blooms, Lake Albano was selected as a pilot area for the integrated monitoring of the BLOOWATER Water Joint Programming Initiative (JPI) project [20].

Finally, regardless of the fact that Central Italy volcanic lakes play a key ecologic and economic role for the region and their water quality attracts attention from the European Union, no research implementing innovative monitoring procedures in such lakes has been published so far.

This study aims to investigate the benefits and limitations of integrating alternative methodological approaches to current monitoring procedures of Chl concentration in freshwater bodies. To address this, comparisons between Chl measurements obtained by means of standard and non-conventional procedures were performed. Specifically, we evaluated the (i) total Chl measurements performed with the Phyto-PAM instrument and spectrophotometric analysis, and (ii) Chl-a concentrations calculated through the implementation of a neural network processor for RS image processing and data provided by spectrophotometry.

2. Materials and Methods

2.1. Study Area

Lake Albano is a warm monomictic lake in Latium, Italy, resulting from the Quaternary volcanic activity that affected the region [21] (Table 1). It is characterized by long water renewal times (47 years), which can represent an additional risk factor if deterioration phenomena occur. Indeed, the increase of anthropic disturbance during the last century has led to a decline in water quality, mostly due to water uptake, urbanization, and recreational use of the water body [19]. These pressures hinder the shift from a meso-eutrophic to a mesotrophic condition [22]. Lake Albano (SCI-IT6030038) is located in a crater of the Alban Hills caldera (the "Vulcano Laziale"), located about 25 km South-East of Rome in "Castelli Romani" Regional Park [23,24]. The crater results from several hydro-magmatic explosions that have occurred since ca. 50,000 years ago [25]. Lake Albano has a surface of 6 km^2, a maximum depth of 175 m (which is the greatest of the Italian crater lakes), and is funnel-shaped [19,26,27], which would suggest fluids circulation related to residual anomalies associated with past volcanic activities [28] (further description of the study area is available in Supplementary Materials Section S1, Table S1).

Table 1. Main morphometric and hydrological features of Lake Albano.

Attribute	Value
Location (Lat., Lon.)	41°45′0′′ N 12°39′54′′ E
Maximum depth (m)	175
Mean Elevation (m a.s.l.)	293
Surface Area (km^2)	6.0
Volume (106 m^3)	464
Renewal Time (year)	47.6

2.2. In Situ Data

For the present study, a total of 11 sampling campaigns were carried out from March to October 2019. Samples were collected in five sampling sites across the investigated lake: one located in the centre and four other peripheral ones (Figure 1).

Sampling site coordinates were recorded using a Global Positioning System (GPS) device. Due to boat drift and GPS positional inaccuracies, geolocation uncertainties were taken into account when processing satellite data (3 × 3 pixel windows, see Section 2.4). At each sampling site, epilimnetic water temperature, pH, salinity, and dissolved oxygen (DO) concentration were recorded using a Hach HQ40d portable multi-parameter meter. Furthermore, water transparency was estimated via the Secchi Disk depth (z_{SD}) method, using a standard 30 cm-diameter circular white disk. z_{SD} measurements were carried out from the shaded side of the boat to avoid any disturbance from direct sunlight reflections (Supplementary Materials S1).

Two-litre-water samples were collected from the upper layer of the lake surface (approx. 50 cm depth) with a Van Dorn water sampler (SCUBLA S.r.l., 33047 Remanzacco (Ud), Italy) to estimate Chl concentration (in accordance with previous literature, [16,29–36]. During the transfer from the Van Dorn bottle to the individual sample containers, water samples passed through a 200 μm mesh filter to avoid zooplankton grazing. All samples were transported on ice to the laboratory in sterile, light-reflective containers (to prevent pigment degradation). Furthermore, small aliquots of water samples were used for spectrophotometric analysis of nitrites (NO_2^-), nitrates (NO_3^-), ammonium (NH_4^+), and phosphates (PO_4^-) concentration.

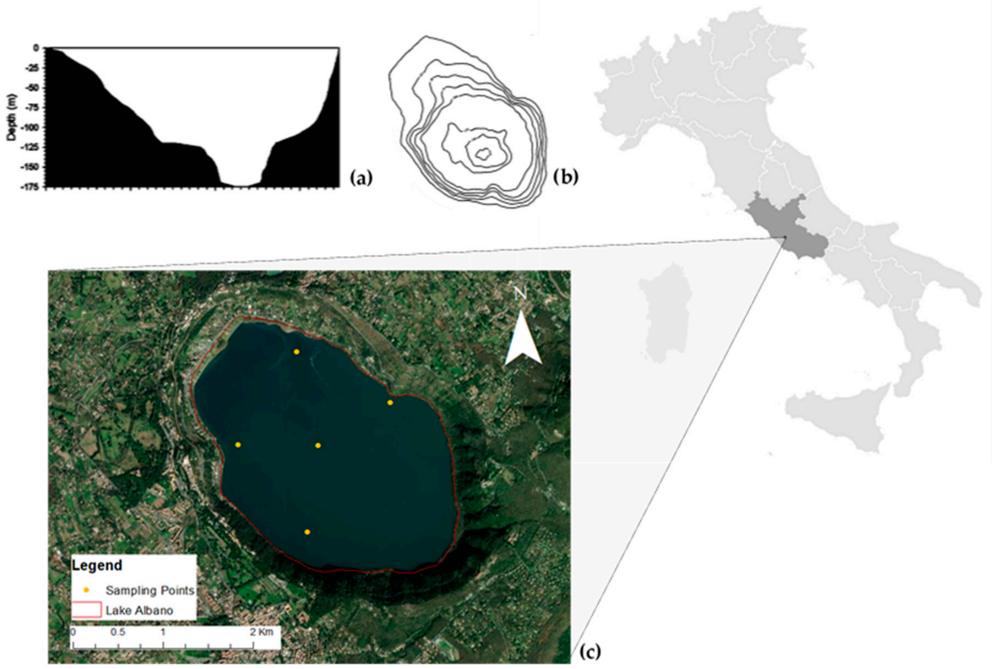

Figure 1. (**a**) Cross-section of Lake Albano's profile and (**b**) bathymetry map [22]; (**c**) True color composite (red, green and blue, RGB) image of Lake Albano with sampling points' location (**c**); the red line indicates the perimeter of the lake before the water level reduction.

2.3. Chlorophyll Measurements

Measurements of Chl content of water samples were carried out within 24 h after collection. Spectrophotometric analysis of Chl concentration was carried out according to the CNR-IRSA (Consiglio Nazionale delle Ricerche—Istituto Nazionale di Ricerca sulle Acque) protocol. After water samples filtration using Whatman (Maidstone, UK) GF/F glass microfiber filters (47 mm diameter, 0.7 µm pore size) under vacuum pressure, filters were put in 90% acetone solution for Chl extraction and homogenised for 1 min at 500 rpm. Sample extracts were stored at 4 °C in the dark for 24 h for pigment extraction. Clarification of extracts was carried out by centrifugation (3500 rpm, 12 min). Triplicate measurements of samples' absorbance were carried out using a dual-beam Beckman Coulter (Brea, CA, USA) DU 800 spectrophotometer. Readings at 630 nm, 647 nm, 664 nm, and 750 nm wavelength were performed (this latter for testing water turbidity). The resulting absorbance values were used to calculate Chl-a, Chl-b, and Chl-c (i.e., the sum of c1 and c2) concentrations by applying the equations of Jeffrey and Humphrey (1975) [37]. Total Chl concentration was calculated as the sum of Chl-a, Chl-b, and Chl-c values.

Fluorescence analysis was undertaken using the Walz PHYTO-PAM-ED system (Heinz Walz GmbH, Effeltrich, Germany) with PHYTO-WIN software version 1.45. The functioning of the PHYTO-PAM fluorimeter is based on the selective amplification of a fluorescence signal, measured with the help of very short (10 µs) pulses of measuring light at a high repetition rate (1200 Hz). These light pulses are provided by an array of light-emitting diodes (LED) at four wavelengths (470, 520, 645, and 665 nm). A photomultiplier in conjunction with a pulse amplifier is used as a detector for fluorescence at wavelengths above 710 nm. The quasi-simultaneous excitation of Chl at the four wavelengths allows discriminating phytoplanktonic group composition relying on the fluorescence characteristics of light-harvesting pigments of selected algal groups. Three measurements were carried out for each water sample, preceded by a "Zero offset" (Zoff) determination to remove other fluorescing substances' contribution to the signal (e.g., humic acids). This operation involves measuring the fluorescence of a 2 mL filtrate of the sample (using a 0.2 µm millipore filter that retains all phytoplankton). After this procedure, the software automatically subtracts the Zoff fluorescence value from the subsequent sample readings.

2.4. Sentinel-2 Data Processing

Sentinel-2 MSI non-atmospherically corrected (L1C) products were downloaded from the Copernicus Open Access Hub (https://scihub.copernicus.eu, accessed on 24 October 2019). A maximum of 8 days difference between Supplementary Materials S2 MSI overpass and in-situ measurements was allowed for matchups, provided that weather conditions were stable between image acquisitions and in situ samplings. Such a time window was defined to allow sufficient matchups between ground data and Sentinel-2 imagery. Due to cloud coverage, no satellite data are available for two sampling dates.

Sentinel-2 Toolbox (S2TBX) version 7.0.0 in the Sentinels Application Platform (SNAP) version 7.0.0 on Windows 10 (64 bit) was used for image processing. This application comes equipped with the Case-2 Regional CoastColour processor (C2RCC). C2RCC constitutes an improvement of the Case 2 Coastal water processor developed by Doerffer and Schiller [38,39] for the Medium Resolution Imaging Spectrometer (MERIS). Such a processor has been updated with a set of additional neural networks trained to cover extreme ranges of scattering and absorption, and is applicable to ocean colour sensors as well as Sentinel 2 [40]. C2RCC functioning is based on a set of neural networks trained to perform the determination of the water leaving radiance from the top of atmosphere radiances (i.e., atmospheric correction), as well as the retrieval of inherent optical properties (IOPs, i.e., absorption and scattering coefficients) of the water body. The IOPs are then converted into Chl-a and total suspended matter (TSM) concentrations using arithmetic conversion factors (e.g., CHL = iop_apig^CHLexp × CHLfac; more detailed information are available in the Help section in SNAP). Among the processing parameters, elevation, salinity, and temperature were adjusted according to values registered during each sampling campaign. The median value of a 3 × 3 pixel window centred at each sampling point was extracted by using the Pixel Extraction tool, to minimize errors due to boat drift and GPS positional uncertainties.

2.5. Statistical Analysis

For the statistical analysis of data, the "R" x64 v. 3.6.1 software was used (R Core Team, 2019). As we assumed a positive linear relationship between Chl concentration measured spectrophotometrically and Phyto-PAM values, a correlation test was performed on the two sets of data. The same test was performed between Chl-a concentration and C2RCC values, to assess the strength and significance of their relation.

3. Results

We explored the potential of two tools to improve space and time resolution of phytoplankton monitoring. Fluorescence-based Chl measures obtained using the Phyto-PAM instrument and Chl-a measures derived from Sentinel-2 images were compared with lab-derived, spectrophotometric measurements of Chl and Chl-a concentrations from water samples collected at 0.5m from 5 fixed location monitoring sites (Figure 2, Table 2).

Table 2. Statistical description (mean, standard deviation, minimum, and maximum) of concentration measurements (pooled results across all sites and dates): total chlorophyll concentration measured with the Phyto-PAM instrument (n = 42), total chlorophyll and chlorophyll-a measured spectrophotometrically (n = 27), chlorophyll-a measured with the Case-2 Regional CoastColour (C2RCC) processor (n = 32). The complete dataset can be found in Table S2, Supplementary Materials.

	Mean (μg/L)	SD (μg/L)	Min. (μg/L)	Max. (μg/L)
Chl_PAM	9.01	9.84	2.03	39.95
Chl_sp	5.97	5.27	1.27	20.21
Chla_sp	5.20	4.93	1.12	19.12
Chla_C2RCC	1.34	1.07	0.23	5.45

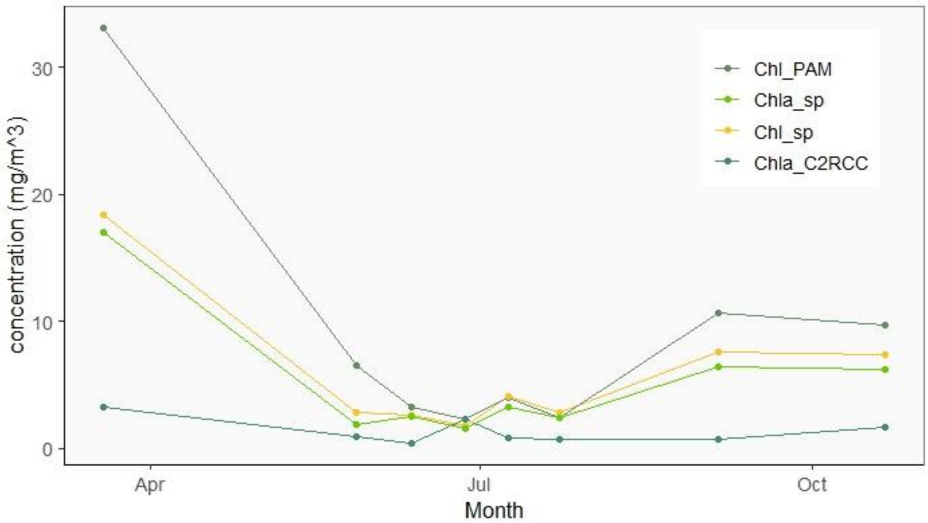

Figure 2. Time-series plot of the concentration values measured (daily mean of the five sampling sites).

The absorbance values measured spectrophotometrically were used to calculate total Chl and Chl-a concentrations from 8 sampling dates. The maximum values of both concentrations were measured in early spring (20.20 µg/L for Chl_{tot} and 19.12 µg/L for Chl-a). Phyto-PAM-measured Chl concentration showed a peak in early spring (39.95 µg/L) due to a cyanobacterial bloom, followed by a quick drop in concentration values. Over the remainder of the sampling period, quite constant Chl concentration values were observed, with a relative maximum recorded in September (12.53 µg/L). Chlorophyll variation over the sampling period showed highly matching trends with the ones determined spectrophotometrically, although spectrophotometric values are overall lower.

The correlation test performed on total Chl concentration estimated by Phyto-PAM and through spectrophotometric measurements exhibited a significant and high association (r = 0.97) between the two sets of values (Figure 3).

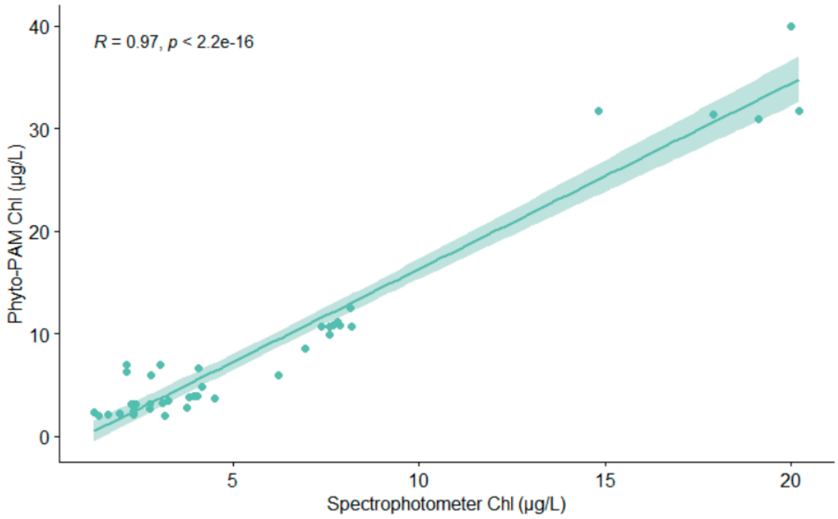

Figure 3. Correlation plot between Phyto-PAM-measured Chl concentration and spectrophotometric measurements (with confidence bands).

The test carried out on Chl-a concentration measured spectrophotometrically and through the C2RCC processor indicated a significant correlation, although not particularly strong (r = 0.57, Figure 4), based on our limited comparisons in this exploratory analysis.

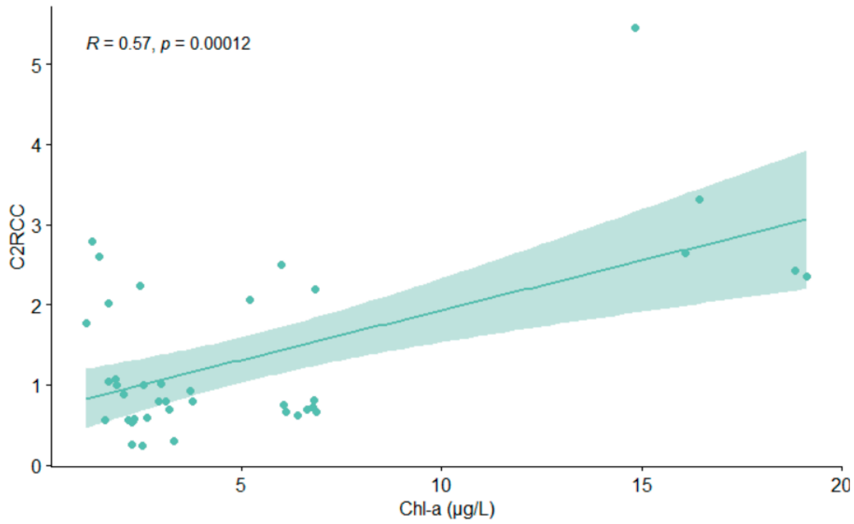

Figure 4. Correlation plot between C2RCC Chl-a concentration estimates and spectrophotometric measurements (with confidence bands).

4. Discussion

Spectrophotometric analysis is a standard method for pigment quantification in water samples [41]. However, it involves expensive, time-consuming procedures that can hinder compliance with current national and international regulatory requirements on water quality monitoring. Furthermore, the limited frequency and spatial-representativity of traditional sampling methods do not allow rapid change observation. Thus, this paper aimed at assessing the efficiency of the Phyto-PAM instrument and C2RCC image processor as rapid methods for Chl concentration measurement in lake surface waters. To do so, spectrophotometric pigment measurements were compared to concentration data obtained by applying the two suggested methods.

Chl concentration values were consistent with previous data indicating the occurrence of algal blooms in winter and early spring [22], with *Planktothrix rubescens* as dominant species and microcystin concentrations above the World Health Organisation threshold [23,42]. Therefore, constant monitoring and early detection of such phenomena are essential for recovering and preserving the lake ecosystem's well-being.

The Phyto-PAM instrument was chosen for this study as it allows rapid, non-destructive measurements that can be carried out in situ during sampling campaigns [43]. A further benefit of this instrument is that it can provide data on the photochemical efficiency of Photosystem II (PS II) reaction centers and group composition of the algal community under investigation. However, Chl concentration assessment depends on instrument calibration undertaken with reference algal cultures with the most common species in the water body of interest. Indeed, Chl estimation reliability can be impacted by the reabsorption of the emitted fluorescence by Chl, fluorescence from other fluorescing compounds, light-condition-adaptations of accessory pigments, and variability of PS II/PS I ratios [44]. Considering the high correlation between total Chl concentration results of Phyto-PAM and spectrophotometric analysis, both methods seem suitable to assess Chl content in this study's conditions, even with no previous calibration of the Phyto-PAM instrument. Even though the values provided by the two instruments have similar trends, their absolute values differ significantly, as observed in previous studies [44]. This could be related to the intrinsic differences between the two optical techniques used in this study. Indeed, while the spectrophotometric measurements are based on the absorbance of extracted Chl, Phyto-PAM measures pigment fluorescence in vivo. Furthermore, the discrepancy between the two sets of values can be explained by the interference of accessory pigments present in the light-harvesting complex, thus contributing to the fluorescence signal.

The C2RCC processor ready availability through the SNAP software and its ease of use make it a preferable option for Chl-a estimation based on Sentinel-2 and ocean colour sensor's data. Its neural network architecture has been trained with a large dataset of simulated water-leaving reflectance and top-of-atmosphere (TOA) radiance. Such simulations are based on a wide set of optical data and water constituent measurements, thus enabling the C2RCC processor to cover extreme ranges of scattering and absorption and making it suitable for a wide variety of water bodies [38,40]. Moreover, IOPs retrieval is made more accurate by the possibility to adjust processing parameters depending on the study area's conditions. On the one hand, the significance of our correlation test confirms the suitability of the C2RCC processor for Sentinel-2 image analysis. However, the absence of a strong correlation with spectrophotometric data could be affected by multiple factors. The natural variability of phytoplankton populations in space and time when water samples and satellite images were allowed to be matched up to eight days apart in this exploratory analysis introduces ecologically-based uncertainty into the evaluation of the RS-based method. The variance in the relationship between the two sets of data may also suggest that there is a technical influence on the assessment, as the additive nature of IOPs could result in Chl-a retrieval errors and, thus, different proportions of optically-active water constituents can lead to similar reflectance values [45,46]. Furthermore, the processor's effectiveness in solving IOPs is strongly dependent on the training dataset, which affects its performance when investigating values beyond training ranges [12,47,48]. On the other hand, the strength of the correlation between Chl-a concentration measured spectrophotometrically and through the C2RCC processor could be affected by the Sentinel-2 MSI radiometric accuracy in terms of signal-to-noise Ratio (SNR). Indeed, MSI spectral bands' SNR values are lower than those required from ocean colour sensors for sensing Chl-a in marine waters [49,50]. Therefore, the sensed signal could be affected by a higher error when pigment concentration in the water body is low [51].

To overcome space-born RS limitations due to adverse atmospheric conditions and sensors' characteristics, airborne remote sensing could represent a valid solution. However, manned aircraft overflights can be prohibitively costly for regular monitoring. Over the past few years, unmanned aerial vehicles (UAVs) have become a viable and cost-effective alternative method for airborne surveys. UAVs deliver fine spatial resolution data at temporal resolutions defined by the user, with data acquisition free of cloud coverage restrictions [52,53]. However, their application is limited by data consistency amongst sensors (requiring empirical radiometric calibration) and flight stability, that can affect data quality. Therefore, the synergetic use of drones and satellite remote sensing could help overcome the limitations of the single systems, thus allowing the increase of data acquisition in terms of temporal, spectral, and spatial resolution.

The positive correlation between results from the laboratory-derived and satellite-based Chl-a measures suggests good potential to successfully integrate satellite-based Chl-a tracking at high spatial resolution for Lake Albano. The integration of satellite-based Chl measurements will be a cost effective and efficient method for improving water quality status and trend evaluations using data with better spatial and temporal resolution than the existing point sampling monitoring approach. Since uncertainty levels are high in our initial assessment, point sampling will be important to provide good quality assurance in future satellite-based assessments. Further tuning of the method is needed to reduce inaccuracy in the satellite-based measurements.

5. Conclusions

This study aimed at assessing the Phyto-PAM instrument and C2RCC image processor potential and limits for estimating Chl concentration in Lake Albano. The efficiency of the pulse-amplitude-modulated fluorometric technique was confirmed by comparison with standard spectrophotometric analysis, thus endorsing the use of the Phyto-PAM instrument for measuring Chl concentration in the study area. Even if it did not prove necessary in this case, a preliminary instrument calibration could provide more accurate information in terms of algal composition and photosynthetic activity of the population under study. Moreover, the significant relation between C2RCC and spectrophotometric data shows the potential of Sentinel-2 MSI images for inland water remote sensing. However, the strength of such a relation points out that further improvements of the technique are needed for this study area. Therefore, the results presented in this study provide a valid feedback for the research activities with which the BLOOWATER project is aiming to improve measurements and increase the efficiency of an integrated system for the water quality monitoring of Lake Albano. The BLOOWATER project will test the possibility of integrating remote-sensing technology and in situ data analysis with standard and non-standard methods. Therefore, the acquisition of Sentinel-2 satellite data and sampling campaigns are planned for weekly data collection in the different seasons. To overcome the current limits of non-commercial space-borne remote sensing, it could be integrated with UAVs. In this perspective, the BLOOWATER project intends to propose innovative technological solutions that aim to develop a methodological approach based on the integration of the treatment of water affected by toxic algal blooms and monitoring techniques including UAV remote sensing. The use of UAVs in BLOOWATER will allow integrating the unique wavelength signals and understanding the algal bloom dynamic into an effective red, green and blue (RGB) detection platform. In this way, it will be possible to achieve an optimal information acquisition efficacy for early and timely algal bloom detection.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13091253/s1, Section S1: Further description of the Study Area, Section S2: Chlorophyll Concentration Results, Table S1: In situ data ranges, mean values, and standard deviation over the sampling period: Secchi Disk depth (z_{SD}), epilimnic water temperature (T), salinity (Sal), pH, dissolved oxygen (DO) concentration, nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+), and phosphates (PO_4^-) concentrations, Table S2: Chlorophyll concentration results.

Author Contributions: Conceptualization, M.S. (Maria Sighicelli), M.P. and M.S. (Massimiliano Scalici); methodology, M.P., F.L. and M.M.; validation, M.S. (Maria Sighicelli), M.P. and M.S. (Massimiliano Scalici); formal analysis, M.S. (Maria Sighicelli) and M.P.; investigation, M.S. (Maria Sighicelli), M.P. and F.L.; resources, M.S. (Maria Sighicelli), M.M. and M.S. (Massimiliano Scalici); data curation, M.S. (Maria Sighicelli) and M.P.; writing—original draft preparation, M.P.; writing—review and editing, M.S. (Maria Sighicelli), M.P. and M.S. (Massimiliano Scalici); visualization, M.P.; supervision, M.S. (Maria Sighicelli) and M.S. (Massimiliano Scalici); project administration, M.S. (Maria Sighicelli) and M.S. (Massimiliano Scalici); funding acquisition, M.S. (Massimiliano Scalici). All authors have read and agreed to the published version of the manuscript.

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