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CYCLIC VOLTAMMETRY ARRAY REQUIREMENTS TO CORRELATE SATELLITE OBSERVATIONS OF ALGAL BLOOM SEVERITY WITH BLOOM-GENERATED HYPOXIA

Cimpoiașu Vily Marius[1], Popa Radu[2], Popa Răzvan Dan[3], Ungureanu Laurenția[4], Tumanova Daria[5], Gherman Vasile Daniel[6*]

[1] University of Craiova, Biology and Environmental Engineering Department, Craiova
[2] National University, Los Angeles CA, USA
[3] Personal, Craiova,
[4] State University of Moldova, Chisinau, Moldova
[5] State University of Moldova, Chisinau, Moldova
[6*] University Politehnica of Timișoara, Department of Hydrotechnics, Timișoara
* *Correspondence author. E-mail*: vasile.gherman@upt.ro

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ABSTRACT

Aerial and spaceborne instruments have increased the ability to monitor algal blooms frequently and in large water bodies. But such remote sensing methods cannot inform about toxic outcomes of severe algal blooms such as hypoxia, ammonia, hydrogen sulphide and phycotoxins. We developed a robust and low-cost cyclic voltammetry multielectrode instrument (called SPEAR) for automated monitoring of redox parameters. This instrument produces information about the evolution of oxic/anoxic and oxic/sulphidic transition zones in the water/sediment column. We present requirements and calibration of a SPEAR64-based array with 300 m XY resolution, similar to the OLCI instruments from Sentinel-3 satellites. We discuss how SPEAR array results can enhance the interpretation of spectral-based algal blooms analyses, with regards to predicting hypoxia and hydrogen sulphide in water.

INTRODUCTION

In aquatic ecosystems, algal blooms (ABs) are the fastest ecological response to eutrophy and climate warming, and in the last decades, Severe Algal Blooms (SABs) have become more frequent and stronger (Brenckman et al. 2025). Hence, studying toxic consequences of SABs, such as hypoxia, hydrogen sulfide, ammonia and phycotoxins, has become a necessary branch of anthropogenic ecology (Sinha et al. 2017). In lakes and ponds, the day/night (diurnal) cycle produces fluctuations in temperature (T), pH and dissolved oxygen (dO_2). In plankton, the diurnal vertical migration caused by hypolimnetic anoxia influences the migration of invertebrates (Doubek et al. 2018). In theory, such chemical changes can be monitored with probes making multiple measurements at various depths. Similar stratification and dynamics can be measured in sediments, albeit at sharper resolution. Oxygen availability and hydrogen sulfide (H_2S) are key controllers of the vertical distribution of biota and its physiological activity (Sass et al. 1997; Brune et al. 2000; Berninger and Epstein 1995). It is generally assumed that the oxic/anoxic and oxic/sulfide transition zones in water and sediments (Fig. 1) migrate down during

the day due to oxygenic photosynthesis, and rise at night as aerobic respiration and fermentation become dominant (Jorgensen & Postgate 1982). These vertical excursions can be modeled to a point (Katsev et al. 2006; Geng et al. 2024), but modelling alone is not a substitute for actual measurements and many factors (including ABs) influence such changes. For example, predicting bloom-derived toxicity needs direct chemical measurements.

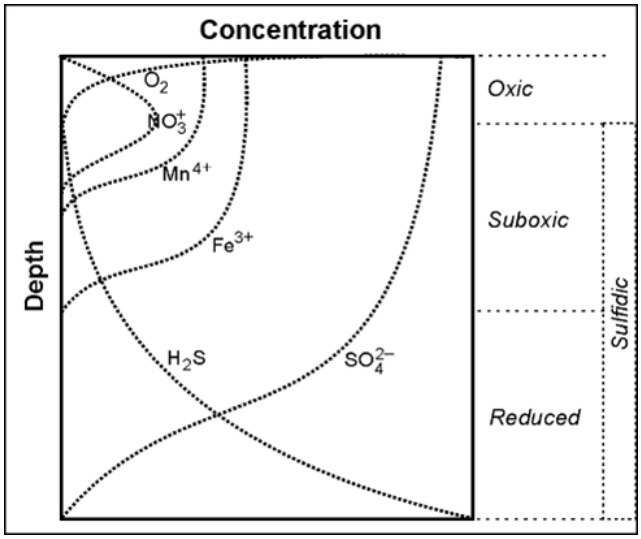

Figure 1. Redox gradients in sediments are complex and combine the stratification of many oxidized and reduced chemical species (after Kristensen 2000; Glud 2008). When studying their vertical dynamics by cyclic voltammetry (CV) techniques, such complex profiles can be reduced to three domains: oxic, suboxic and reduced, where the suboxic and reduced layers may be sulfidic as well. This stratification is common in sediments, but also applies to water, though planktonic dynamics (with convection, waves, flow currents, etc.) makes vertical gradients more variable, broader and shorter-lived than in sediments.

Two methods are generally used to describe the vertical dO_2 and H_2S profiles as well as redox transition zones (e.g., oxic/anoxic and oxic/sulfidic) in sediments. The 1st method employs electrochemical probes moved up or down across chemical interfaces with manipulators (Li et al. 2023). This method can produce remarkably sharp sediment profiles, although it is best suited for softer and more permeable substrates that can reduce probe breakage. Most such measurements are momentous snapshots, and cannot be repeated at the same exact location because the up-down movement of the probes disrupts the sediment, biasing future measurements. This method is not suitable to describe the evolution of the various chemical layers shown in Figure 1. A 2nd method is to implant 2 or more probes at various depths, not move them while making many successive measurements and then interpret quantitative changes and putative vertical dynamics (Cornuejols et al. 2025). This method eliminates the need for active manipulators during measurements, and allows repeated measurements that are, this way, not mechanically disrupted by earlier measurements. Combining data from multiple probes can help describe changes in the vertical position of chemical

interfaces. This method also works with heavy sediments, such as sandy and clastic, because the mechanically-sensitive probes are immobile during measurements. However, using many electrodes and many electronic instruments raises costs, increases data variability, decreases spatial resolution, while the ability to describe the vertical migration chemical interfaces is limited by the number of probes that can be deployed in each station. E.g., commercial multielectrode CV systems (such as Gamry; PineResearch; and Keithley), are expensive and costs range from $1,000 to $12,000 per working electrode (WE). Also, the commercial liquid-based reference electrodes (REs) break easily and cannot be fully submerged for long (Bard & Faulkner 2000).

Adding to the above-mentioned difficulties, changes in dO_2 and H_2S produced by SABs have to be monitored in real-time and at relevant vertical resolution, but also horizontal spatial resolution is needed that is comparable to satellite-based observations ABs. The resolution of the OLCI instrument from the Sentinel-3 satellites is 300 m. This means that an array of 44 measuring stations has to be deployed per km^2. Assuming an average 3 m water depth and 50% of the electrochemical probes will be placed at smaller intervals in the water sediment interface, an instrument with 64 working electrodes should be able to monitor the water column at 10 cm intervals and a 30 cm deep sediment transition zone at 1 cm intervals. Cost and the management of such a complex array are key factors in its feasibility.

We have developed a multielectrode instrument called SPEAR (Solitary Probe for Electrochemical Analysis and Reporting) (Cimpoiasu et al. 2020, 2022; Popa et al. 2022). This instrument has advanced to a third generation carrying up to 64 or 128 WEs, ≤ 8 solid state REs, and ≤ 8 CEs. SPEARs are cyclic voltammetry (CV) instruments for observing redox changes in water and thus showing how oxic/anoxic, O_2/H_2S and Eh interfaces evolve. This data can be combined with data on the severity of ABs observed by pigment spectroscopy to predict two toxic outcomes of SABs: hypoxia and H_2S. The scope of this work is to describe data produced by SPEAR64 and SPEAR128 instruments and to show how an array of 16 SPEAR64 instruments is deployed and calibrated for automated monitoring of electrochemical profiles in water and sediments. The ultimate aim of this work is to make electrochemical data produced by SPEAR arrays compatible with Sentinel-3-based observations which will increase the ability to predict the SAB/toxicity relationship.

MATERIAL AND METHODS

The architecture of a SPEAR instrument is shown in Figure 2. We have constructed three generations of SPEARs, each with improvements toward lower weight and price, and at achieving remote programmable unassisted function under in-field conditions, as well as automated data reporting within a locally established WiFi network with station-to-station signal hopping. In this instrument series, the CV potentiostats (Figure 2(A)) have evolved from a 4 kg Keithley 2450 (in the 1st generation SPEAR), 280 g WaveNow PineResearch (2nd generation) and 100 g for a custom made potentiostat based on the LMP91000 and Arduino board unit (3rd generation SPEAR). The channel selector (Figure 2(B)) was custom made based on MAX4781EUE CMOS (i.e., Complementary Metal Oxide Semiconductor) analogical multiplex commuters selected for low voltage, electrical resistance and noise/signal ratio. The number of WEs in the multi-electrode probes (Figure 2(C)) is a multiple of 8 and has reached ≤ 128. The SPEAR instrument can work with solid-state REs that are easy to replace, long-lived and cannot be fractured by rough handling or in

turbulent field conditions; these REs are produced by electrodepositing polypyrrole on a Pt wire (Ghilane et al. 2006).

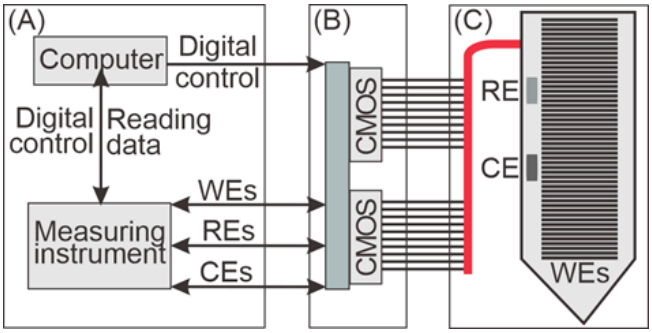

Figure 2. The general architecture of a SPEAR instrument. (A) Control, measure and data storage system with a CV potentiostat as the measuring instrument. (B) CMOS-based channel selector. (C) Multi-electrode probe. Working Electrodes (WEs). RE = Reference Electrode. CE = Counter Electrode.

During construction, the graphite WEs were installed on a 3D-printed plastic plate with parallel trenches to receive up to 128 round graphite rods (0.75 mm in diameter; 30 mm long). Electrical connection between the WEs and the instrument circuitry was made via a Pt wire and graphite/toluene-based conductive paste. The electrode-carrying plate was embedded in a resin block and polished on the WE side to expose approximately 75-100 % from the maximum width of each graphite rod. When necessary, based on the level of graphite surface pitting and/or fouling we have analysed the WEs function based on conductivity and impedance spectroscopy (Kus et al. 2005). For WE restoration, the surface of the electrodes was polished along with the resin using a sequence of wet 320, 400 and 600 grit sandpaper, wiped with 1 mM HCl to remove metal oxides and carbonates, soft-polished with buffing wheel, washed with water to remove the polishing debris and wiped with acetone and isopropanol to remove organics.

RESULTS AND DISCUSSIONS

The main purpose of a SPEAR instrument is to perform various types of CV measurements at many depths targeting O_2 and H_2S redox transformations. In CV, the redox potentials for the oxygen system are complex because oxygen participates in multiple electron transfer reactions, rather than a single reversible step. The redox potential of the HS^-/S^o couple ($H_2S \Leftrightarrow S^o + H^+ + 2e^-$) in pH 7 water is ~+0.17V. Yet again, many S-active redox species exist (both inorganic and organic) in water and sediments. Moreover, CV is better at measuring redox potentials than quantifying chemical species. Hence, SPEARs are not suitable to measure the concentration of dissolved [dO_2] and [H_2S], but to indicate their presence and relative abundance. This method can show when and where various water layers have become oxic, anoxic or sulphidic to identify oxic/anoxic and oxic/sulfidic transition zones. It allows studying how redox interfaces migrate up-and-down through plankton and sediments. The Figure 3 results were produced with a 1st generation SPEAR64. A full 64-WEs analysis takes 0.5-2 hrs depending on the measurement conditions (see

next). To describe the vertical migration of the redox interfaces, successive measurements have to be combined.

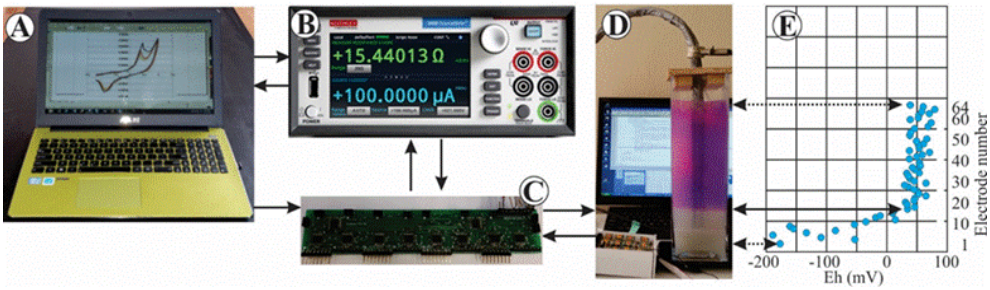

Figure 3. Laboratory setup to show how a 1st generation SPEAR64 instrument can be used to monitor the location and evolution of an oxic/anoxic O_2/H_2S interface (modified after Cimpoiasu et al. 2022). (A) Data recording computer. (B) Measuring instrument (Keithley 2450). (C) 64x channel selector. (D) Multi-electrode probe with 64 WEs. The WEs are 3 mm in diameter, spread vertically over 20 cm. The probe was introduced in a gradient gel with 0.5-0.7% agar on top (with no H_2S added) and 1.5-1.7% agar in a bottom plug (spiked with H_2S) yielding a relatively stable oxic/anoxic O_2/H_2S interface. The bottom of the light gray gel area (panel D) contains ≤ 1 mM H_2S (derived from a 50 mM Na_2S solution titrated to pH 7 in anaerobic conditions. The pink/purple colored above gel was exposed to air and contained dO_2. The pink/purple color is due to 0.1% resazurin (a redox indicator) to help visualize the transition from the reducing area to the oxidized area. (E) Plot compiling Eh data from all 64 WEs after one full series of measurements. The dotted arrows between the (D) and (E) indicate the position of the uppermost and lowermost WEs respectively. The continuous arrow between (D) and (E) indicates the position of the O_2/H_2S redox interface.

Next, we present the protocol to set-up an array and to calibrate SPEAR64s in the field.

‣ Identify local factors that can interfere with the SPEAR stations, e.g., strong water currents, tides, traffic, etc. Verify electromagnetic interferences that may disrupt communication. Identify the approximate position of the oxic/anoxic or oxic/sulfidic interface in the sediment.

‣ Characterize the sediment' composition and texture (e.g. clastic, sandy, muddy, detrital, granulation, penetrable depth; density and settling time) and the water depth in each station. This work may take > 48 hrs. of labor and is needed to adjust the length of the penetrable tip as well as the submerged length of each SPEAR probe.

‣ Determine water temperature and light penetration in the water column, such as Secchi depth or noon light intensity in water at various depths.

‣ Take a water/sediment core sample for ex-situ electrochemical measurements.

‣ Perform a set of single electrode profiles (O_2, H_2S, Eh, pH and Conductivity).

‣ Mark the position of the sediment/water interface and the depth and depth extent of greatest interest in the sediment with regards to the oxic/anoxic and O_2/H_2S interface and make predictions about their expected vertical diel migration. This is needed to position the SPEAR probe correctly, at the best possible depth for

observing the dynamics of chemical gradients (i.e., water sediment interface and the CV-based redox transition zones).

‣ DC conductivity and impedance spectroscopy measurements of the SPEAR electrodes when submerged in filtered field water. This is needed to record the state of the WEs prior to deployment and also to select a small set of external resistances to be used during future calibrations (see next).

‣ Ex-situ tests of CV functions with aerated and degassed (boiled and N_2 purged) field water to verify correct instrument functioning and graphical appearance under oxic vs. anoxic non-sulphidic conditions.

‣ Identify the optimal sampling frequency (SF), the minimum acceptable SF and the number of full SPEAR cycles per day.

‣ Establish the optimal reading conditions:

- The CV cycle to be recorded (it is most commonly the 3rd);

- The reading range, i.e., as broad as 625 mV to –625 mV in the 3rd generation SPEARs;

- Step voltage; 1-2 mV/step measurements are unnecessarily detailed; 3-6 mV/step is most common; 10-20 mV/step readings are too distant for analyzing some CV graph parts;

- Calculate the number of steps per CV cycle; the SPEAR software requires modifications for the size of data packages. E.g., 500 steps/cycle is a good number but it may vary, most commonly between 250 and 1,000.

- The rate of voltage change can be 100 mV/sec or more, and as low as 50 mV/sec. Slower rates will extend the duration of measurements and produce less reading cycles per day;

- Use water salinity, impedance measurements of electrodes and earlier trials to select a range for the external resistance values under a fixed LMP = 1 value. E.g., in water with 0.1-0.3 % salinity) we have tested external resistances between 0.068 and 4.7 kOhm. For each SPEAR-64 probe, select an external resistance that will produce the largest overall signals (uA), and no saw-like features in any of the CV graphs.

- Verify the effect of increasing the LMP values for the 2-3 external resistances that show the best graph results.

- Select the best combination of external resistance and LMP (i.e. the optimal Ohm/LMP pair for each specific water body), to achieve the best signal/noise ratio and to avoid saw-like plots. The rule of thumb is to find the least applied external resistance and largest LMP value where a majority of electrodes produce the best quality CV plots.

- For the best Ohm/LMP pair identified, test verify various unit t rates (mV/sec) between 50 and 250 on a selected number of electrodes (e.g. 10-20). Compare the quality of the CV plots, and calculate the duration of a full SPEAR64 series. As the unit t rate increases the SPEAR64 reads become shorter, but the quality of the reads will decrease. Establish the slowest t rate where readings have acceptable quality across the entire WEs probe.

- Identify problem-electrodes, remove them from measurements and re-adjust the waiting and start times to synchronize the reading frequency across the SPEAR grid.

- Establish how many electrodes, and which electrodes, will be read during a full SPEAR instrument to achieve a desirable SF value.

‣ Implant the probes in the sediment in each station, marking down the GPS coordinates. Stabilize the probes in each station to avoid tipping, drifting and up/down sliding during measurements.

‣ Allow time for the sediment to settle around the sediment-inserted probe (this may take 4-24 hrs.)

‣ Do a fast test of the functioning of all SPEAR stations post-deployment.

‣ Apply a 24 hrs. run of the entire array and make synchronization corrections.

‣ Monitor the evolution of key parameters near the sediment water interface pH, O_2, T, salinity, light intensity in a reference station.

‣ Prepare the software for data analysis, because once the data stream begins, the volume of information will be to too large to catch-up by manual processing.

‣ Periodic sampling of the data stream, and make corrections as necessary; especially regarding the external resistance, LMP and the CV rate.

‣ Produce a series of at least 2-3 days of continuous measurements to obtain information about the diel redox migration patterns.

‣ At the end of a reading campaign, extract the probes from the sediment, wash the WEs surface and repeat the conductivity and spectral impedance measurements to verify signs of electrical drift (commonly caused by graphite corrosion or surface fouling). If needed make corrections to the data stream to account for changes in electrode resistance.

Examples of data generated by a 3rd generation SPEAR-128 instrument are shown in Figure 4.

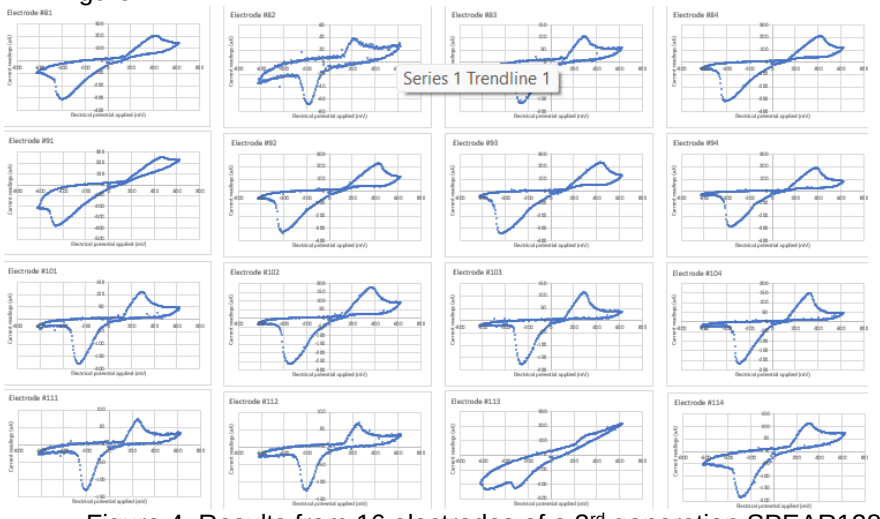

Figure 4. Results from 16 electrodes of a 3rd generation SPEAR128 instrument analyzing a water/sediment column (0.5 g/L salinity). The measurement specifications were: LMP gain 1; 3rd CV cycle saved; voltage range -625 mV to 625 mV; 5 mV/step; 500 steps per CV cycle; rate of 50 mV/sec and 1 kOhm added external resistance. A complete instrument cycle lasted 377 min.

To correlate the SPEAR array results with satellite spectral data we begin with identifying suitable ecosystems and proceed toward planning the array specs. The XY resolution of the Ocean and Land Colour Instrument (OLCI) from the

Sentinel-3 satellites is 300 m. In order to obtain at least one clean pixel of free water chlorophyll analysis (i.e. an OLCI data point with no shore effects or emergent vegetation interference) the smallest cross section of a lake's free water area is 600 m (= 0.36 km^2). Based on these the number of SPEAR stations per array has to be determined. E.g., if a lake has a surface area of 0.36 km^2 and SPEAR stations are placed at 150 m distance from each other, the number of SPEAR stations needed is 360,000 m^2 / 22,500 m^2/station = 16. Such a large number of instruments is a major constraint when using commercial equipment and was the driver behind the development of the 3rd generation SPEARs, which presently cost < $100 per SPEAR64 instrument and < $150 per station.

Fig 5 shows the map-matching between a satellite-based chlorophyll imaging and the deployment of a 16X-SPEAR64 grid in a selected lake. In this example we used Copernicus/Sentinel-3 OLCI instrument and OTCI (standing for Ocean and Land Colour Instrument Terrestrial Chlorophyll Index) analysis. In this example the target ecosystem is Gorgova Lake (approx. 13.8 km^2) from the Danube Delta biome. This lake's large size produced at least 50 areas (i.e., Sentinel-3 OLCI pixels) of open water unhindered by shoreline effects.

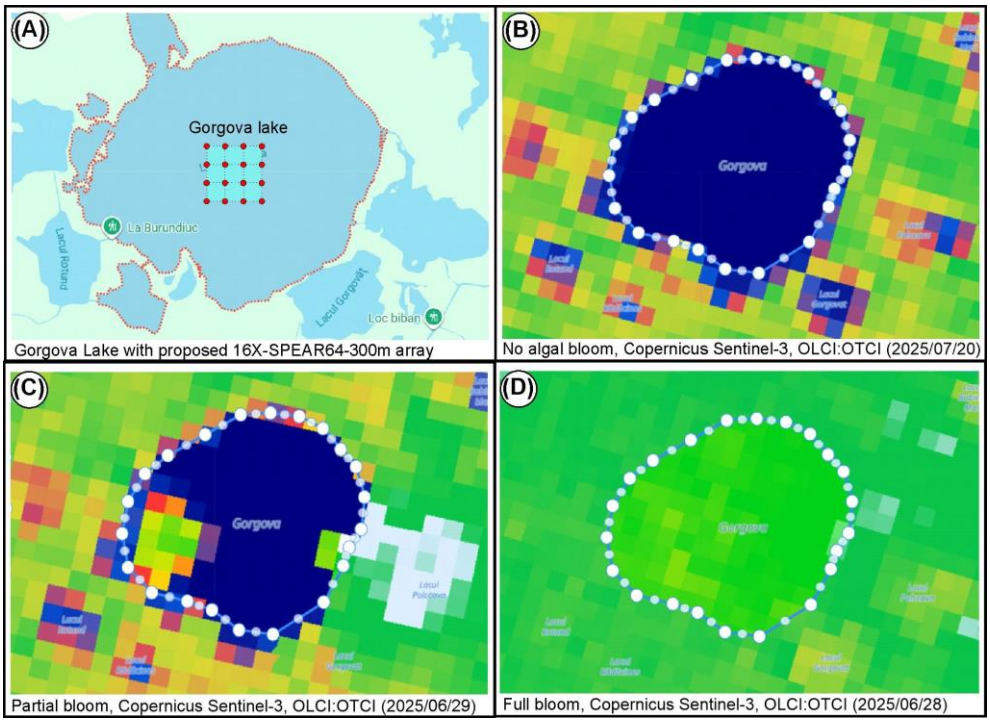

Figure 5. (A) Proposed positioning of an array with 16 SPEAR64 instruments in Gorgova Lake (Romania) to achieve an XY resolution similar to the OLCI instrument from the Copernicus Sentinel-3 satellites. (B) Spectral image of Gorgova Lake processed by the OTCI script for chlorophyll (image taken on July 20 2025). Blue represents chlorophyll free water, Green represents chlorophyll. The dotted contour represents OLCI pixels of open water unhindered by seashore and emersed vegetation. (C) Partial bloom in Gorgova Lake observed on the West shore on June 29 2025. (D) Algal bloom covering the entire surface of Gorgova

CONCLUSIONS

We have analyzed the technical requirements to develop an array of 3rd generation SPEAR instruments compatible with Sentinel-3 satellite data on freshwater algal blooms. Our results indicate that using a SPEAR instrument with 128 WEs is redundant for only a few meters deep lakes. We recommend using SPEAR64s with 64 WEs, one solid-state RE and one CE. Results also showed that an array with 300 m XY resolution, which is similar to the Sentinel-3/OLCI will require 16 SPEARs covering a surface area of 2.25 km^2 of open water. Each node (i.e., SPEAR64 instrument) in this array can provide a vertical (0Y) resolution of 10 cm in 3 m deep water, and 1 cm in a water/sediment transition zone that is 30 cm deep (though these numbers will vary from station to station). After approximately 4-7 days of preparation (for deployment and calibrations) this array can produce a string of month-long 24/7 electrochemical data regarding the location and vertical migration of oxic/anoxic, oxic/sulphidic, and Eh interfaces in plankton and sediments. These data are compatible for correlations with the satellite spectral data regarding the severity of algal blooms. Our analysis also showed that the SPEAR64 array will have a production cost of < $150 per station. In freshwater, the regeneration of the electrode surfaces (due to graphite pitting and surface fouling) is recommended at > 30 - day intervals. However, this is likely to vary based on the level of electrode usage and water chemistry. The voltage applied range in a 3rd generation SPEAR is -625 / 625 mV, which is less than the 1st and 2nd generation SPEARs, but sufficient to observe both O_2 caused oxidations and sulphide redox processes. The measurement frequency can be 12-48 sets of measurements per day per station. A great asset of a SPEAR array with floating buoys, solar panels and energy storage is the ability to make unassisted measurements and communicate results for extended periods of time. Data generated by SPEAR arrays will allow predicting hypoxic and sulfidic toxicity of SAB observed by satellite means. This technological development will enhance the ability to understand the risks posed by SABs in aquatic ecosystems.

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AUTHOR CONTRIBUTIONS

Radu Popa: concept instrument development, experiments, manuscript preparation. Vily M. Cimpoiasu: concept development of the SPEAR instruments, construction of the instruments, software writing, instrument tests and manuscript preparation. Razvan D. Popa: blueprints and field deployment logistics. Vasile D. Gherman: concept development, methodology, data analysis, satellite data interpretation, manuscript preparation. Laurentia Ungureanu and Daria Tumanova: concept development for use of the SPEAR system and correlations with ecological analyses.

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