Next Generation of Advanced Laser Fluorescence Technology for Characterization of Natural Aquatic Environments

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INTRODUCTION

The project research addresses our long-term goal to develop the Advanced Laser Fluorescence (ALF) methods and instruments to improve our capacity for characterization of aquatic environments. The ALF technique (Chekalyuk and Hafez 2008, 2013a) uniquely combines spectral and temporal measurements of laser-stimulated emission (LSE) to provide assessments of key variables, including chlorophyll *a* (Chl), chromophoric dissolved organic matter (CDOM), and phycobiliprotein-containing phytoplankton and cyanobacteria. The measurements of variable fluorescence, F_v/F_m, yield assessments of phytoplankton photophysiological status. ALF measurements in diverse water types have demonstrated its utility for aquatic research and observations. The ALF has been integrated into the major oceanographic programs, including the California Current Ecosystem Long Term Ecological Research (CCE LTER, NSF) and California Cooperative Oceanic Fisheries Investigations (CalCOFI, NOAA).

OBJECTIVES

The goal of the project was to develop the next generation, commercial ALF sensors for oceanographic research, environmental monitoring, and validation of satellite ocean color data. The objectives were:

1. To develop the Aquatic Laser Fluorescence Analyzer (ALFA) for laboratory and field applications.
2. To develop a prototype of the ALF In Situ (ALFIS) fiber-probe sensor.

3. To integrate, test and deploy the new instruments on the research cruises.
4. To initiate operational use of the new ALF instruments for ecological and biogeochemical measurements, and validation of ocean color remote sensing.

APPROACH

The project work was conducted in collaboration between Lamont-Doherty Earth Observatory (LDEO) of Columbia University, WET Labs, Inc., and Scripps Institution of Oceanography (SIO). The basic suite of optical and electronic modules and software was selected and designed to integrate in the ALF sensors. The ALF analytical algorithms were refined in laboratory and field tests and integrated in the ALF software. The ALF sensors have been calibrated, validated, tested, and operationally deployed in coastal and offshore areas of the Pacific and Atlantic Oceans, Mediterranean and Bering Seas, Gulf of Mexico, California Current, Amazon River Plume, and Chesapeake Bay.

Project Team. The PI, Dr. Alexander Chekalyuk, specializes in laser fluorescence, bioenvironmental monitoring, and oceanographic research. Dr. Andrew Barnard, a CoI, is a WET Labs Vice President of Research and Development. Dr. Mati Kahru, a CoI from Scripps Institution of Oceanography (SIO) is a lead expert in satellite remote sensing. Dr. Andrew Juhl (LDEO), a biological oceanographer, assisted this project by maintaining phytoplankton cultures for tests and calibrations. Mr. Mark Hafez, Staff Associate, LDEO of Columbia University, has contributed on developing the ALF software, laboratory and field measurements, and data analysis.

WORK COMPLETED

We conducted the project work in a series of Tasks outlined in the Work Plan of the original proposal:

- Development of Aquatic Laser Fluorescence Analyzer (ALFA)
- Development of ALF In Situ (ALFIS) sensor
- Refinement of ALF analytical algorithms
- ALFA field tests and operational deployments
- Validation of ocean color remote sensing

A brief description of the project results and accumplishments can be found in the “Results” section.

RESULTS

Development of Aquatic Laser Fluorescence Analyzer (ALFA). The lightweight, compact, portable flow-through ALFA instrument (Fig. 1A) provides high-resolution underway shipboard measurements and analysis of discrete water samples. Several prototypes of the commercial benchtop ALFA instrument were designed, built, calibrated, tested in laboratory and field conditions, and operationally used for oceanographic studies [5,6,9,12,14]. The ALFA operational and analytical software was developed, field-tested, and integrated with the shipboard data acquisition systems. Improvements have been made to simplify the system design, maintain optical alignment, and improve serviceability. The ALFA instrument can be customized for single or multi-wavelength fluorescence excitation (Fig. 1C,D), as well as spectrally and/or temporally resolved measurements.

A new type of green laser emitting at 514 nm was successfully tested and integrated as a source of fluorescence excitation for both ALFA and ALFIS instrument configurations [5,6]. The new ”T” optical design allows single- or multi-wavelength excitation of laser-stimulated emission (LSE), provides optimized signal collection, and incorporates swappable modules for flow-through and small-volume sample measurements [5]. The basic instrument configuration (Fig. 1C) uses 510 nm laser excitation for assessments of chlorophyll-a, phycobiliprotein pigments, variable fluorescence (Fv/Fm) and chromophoric dissolved organic matter (CDOM) in CDOM-rich waters. The three-laser instrument configuration (Fig. 1D) eqiupped with 375, 405, and 514 nm lasers provides additional

Fv/Fm measurements with 405 nm excitation, CDOM assessments in a broad concentration range, and potential for spectral discrimination between oil and CDOM fluorescence. The new measurement protocols, analytical algorithms and examples of laboratory and field measurements are discussed in detail in [5] and reflected in the recent patent application [14]. The three-laser ALF-T instrument was eqiupped with 375, 405, and 514 nm lasers and deployed in 2012 and 2013 in the Gulf of Mexico to assess consequences and bio-envornmental impacts of the Deepwater Horizon oil spill of 2010 and support NASA field program for sea-truthing future geo-stationary satellite (Fig. 13)

The various versions of the ALFA instrument were extensively tested and refined in a series of field deployments in the Bering Sea[6], Gulf of Mexico, Amazon River plume [7], and California Current [3-6]. The new ALFA instrument was successfully demonstrated at the AGU/ASLO Ocean Sciences meeting (Salt Lake City, 2012; Fig. 1B) [9], Ocean Optics XXI conference (Glasgow, 2012), and is planned to be presented at the AGU/ASLO Ocean Sciences meeting (Honolulu, Hawaii, 2014) [12]. Two ALFA instruments were build to order from Scripps Institution of Oceanography (San Diego, CA) and State Key Laboratory of Marine Environmental Science, Xiamen University (China) in 2011 and 2013, respectively. The former instrument has been operationally integrated into the California Current Ecosystem Long-Term Ecological Research (CCE LTER) program (NSF) and extensively used for their studies since 2011 [3,4,11]. The LDEO and WET Labs ALF teams have recently conducted on request of the Xiamen University training of the technical personnel repsonsible for the ALFA maintanance and deloyments.

Development of ALF In Situ (ALFIS) sensor. Development of the in situ version of the ALF instrument (ALFIS) was a technological challenge. It required new solutions, and careful component selection to design a compact, robust, low power instrument capable of long-term autonomous operation in the chemically and biologically aggressive seawater under pressure. The LDEO ALF team has developed the breadboard prototype of the ALFIS instrument, based on the 514 nm laser, fiber-probe optics, and dichroic beamsplitter. The key design solutions (Fig. 2) are described in our recent patent application [14], including the 514 nm laser excitation [5,6], the dual-leg fiber probe to transmit the excitation and the laser-stimulated emission back to the instrument sensors, and the dichroic filter splitting the signal for temporal and spectral measurements [1,5]. The ALFIS breadboard prototype (Fig. 2) includes a miniature spectrometer, 14-bit waveform digitizer, and a small low power computer. Fig. 2 shows an example of spectral and temporal measurements with a signal-to-noise ratio comparable to the benchtop ALFA instrument. The WET Labs and LDEO ALF teams are currently working on two submersible ALFIS prototype instruments (Fig. 2) based on the ALFIS breadboard design. After the initial tests at stationary settings (the SIO pier, WHOI dock, etc.), the Moving Vehicle Profiler and SeaSoar towed vehicles (Fig. 3), as well as Wave Glider (Liquid Robotics) are considered as prospective platforms for the ALFIS instrument.

Refinement of ALF analytical algorithms. The ALF analytical algorithms were further refined, including improved fluorescence assessment of chlorophyll concentration [2, 3], extension of the ALF analytical capabilities for assessment of phytoplankton biomass and community composition [3], as well as assessments of the key aquatic fluorescence constituents, and phytoplankton photo-physiological parameters [2-8]. Photo-physiological variability of in vivo chlorophyll fluorescence (CF) per unit of chlorophyll concentration (CC) was analyzed using a biophysical model to improve the accuracy of CC assessments [2]. ALF field measurements of CF and photosystem II (PSII) photochemical yield (PY) in the Delaware and Chesapeake Bays were analyzed vs. HPLC CC retrievals. It was shown that isolation from ambient light, PSII saturating excitation, optimized phytoplankton exposure to excitation, and phytoplankton dark adaptation can improve the accuracy of in vivo CC fluorescence measurements. The four-step measurment protocol was sucessfully tested later in the Gulf of Mexico and at other locations. It provides the accuracy of CC fluorescence measurments comparable to the accuracy of commonly accepted preparatory methods [2]. For in situ or flow-through measurements, it is possible to use the concurrent PY measurements to adjust for CF non-photochemical quenching (NPQ). Field evaluation has shown the NPQ-invariance of CF/PY and $CF(PY^{-1}-1)$ parameters and their high correlation with HPLC CC retrievals [2] (Fig. 7). The correction of the ALF spectral measurements for the instrument spectral response was included in the ALF analytical algorithms [5]. A new, instrument-independent set of spectral components was developed [5]. These algorithm refinements were used to convert the high-resolution underway ALF CF measurements in diverse marine emvironments into the CC distributions (Fig. 11A). The ALF spectral deconvolution (SDC) algorithms were further updated [5] to analyze the emission spectra stimulated with the 514 nm and 375 nm excitation.

Field measurements of chlorophyll-a (Chl), phycoerythrin (PE), chromophoric dissolved organic matter (CDOM), and variable fluorescence (Fv/Fm) in the California Current, Mediterranean Sea and Gulf of Mexico using 375, 405, 510 and 532 nm laser excitation wavelengths (EW) were analyzed [6]. All EWs were found suitable for accurate Chl assessments (Fig. 5). Both EW=532 and 510 nm can be also used to efficiently stimulate PE fluorescence for structural characterization of phytoplankton communities (Fig. 5). EW=375 nm and 405 nm provide best results for CDOM assessments in offshore oceanic waters; the green EWs can be also used for CDOM measurements in fresh and estuarine water types in conjunction with spectral discrimination between CDOM and PE fluorescence. Both EW=405 and 510 were found suitable for photo-physiological Fv/Fm assessments (Fig. 6), though using EW=405 nm may result in underestimation of PE-containing phytoplankton groups present in mixed phytoplankton assemblages [6].

Field tests and operational deployments. An extensive series of field deployments was conducted in 2010-2013 to test the new instrument design and refine the measurement protocols and analytical algorithms. The various ALFA instrument configurations were deployed in the Bering, Arctic, and Mediterranean Seas, Pacific Ocean, Gulf of Mexico, and Amazon River plume on the ships of opportunity in collaboration with our colleagues from LDEO and other research centers and universities. The rich high-resolution ALF data provided valuable information for characterizing interactions between physical, chemical and biological fields in the ocean (for example, Figs. 9-11; for details, see [3,4,6-8]).

A significant portion of the fieldwork was conducted in the California Current on the CCE LTER Process cruises in collaboration with our SIO colleagues [3-5]. Two new ALF instruments, ALFA and ALF-T, were used for underway transect measurements and discrete sample analysis (for examples, Fig. 4). The Chl and PE underway fluorescence measurements were analyzed [6] to evaluate the efficiency of assaying phytoplankton pigments, photo-physiology, and community composition using EW=375, 405, 510, and 532 nm (Fig. 5). The local CDOM concentration was too low to achieve an acceptable S/N ratio with EW=510 nm. More reliable ALF CDOM fluorescence data from the Gulf of Mexico were analyzed to evaluate the CDOM assessments with EW=375, 405, and 510 nm [6]. The regression analysis of Fv/Fm measured with EW=405 and 510 nm was conducted using ALFA measurements in the Ligurian Sea (Mediterranean) in collaboration with the NATO Undersea Research Center; March 2013) (Fig. 6) [6].

Along with testing the new instrument configurations, we kept deploying the ALFA instruments integrated into the CCE LTER (NSF) and CalCOFI (NOAA) program studies. An example of such measurements across a frontal zone in the California Current is displayed in Fig. 8. Strong frontal changes in phytoplankton pigment biomass, physiological status, community composition, and CDOM concentration were detected by the ALF measurements [3]. The ALFA data from discrete sample analysis were used for regression analysis vs. independent retrievals of the commonly accepted parameters, such as Chl concentration and phytoplankton carbon biomass (e.g.,

Fig. 9) to build the 2D spatial patterns in the euphotic layer (see Fig. 10), as well as the high-resolution distributions and vertical profiles (Fig. 11).

Along with extensive field tests of the ALF analytical capabilities, such field measurements provide valuable information for characterization of the interactions between physical, chemical and biological fields in the ocean. The ALF underway measurements can also be used for validation of the ocean color satellite data (for example, Fig. 12) and general characterization of the surveyed meso- and synoptic-scale areas. A recent (Sept. 2013) example of the ALF multi-variable high-resolution underway measurements over large portion of the Gulf of Mexico in support of the future NASA GEO-CAPE geostationary satellite mission is displayed in Fig. 13.

The recent field deployments have shown that the ALF technique can serve equally well for short-term (days) surveys in the coastal and estuarine areas (Fig. 14) and long-term bioenvironmental studies. For example, the ALFA instrument appeared to be one of the few sensors that flawlessly operated over seven months in the harsh conditions of the Arctic Seas during the research cruise of the French yacht Tara to evaluate the impacts of the climate change in this area. An informative set of the ALFA around-the-Pole transect maps is displayed in Fig. 15.

IMPACT/APPLICATIONS

The project research addresses NOPP BAA subtopics: 2.1 Integration of … in situ … bio-optical sensors on nontraditional or novel sampling platforms; 2.2A Development of the next generation of … bio-optical field sensors to further exploit current "ocean color" satellite data, and/or new observations from ocean color satellite retrievals; 2.2B Development of enhanced or new laboratory instrumentation for ecological or biogeochemical measurements in support of ocean color remote sensing. The ALFA sensor provides high-resolution shipboard underway flow-through measurements and sample analyses over a range of spatial and temporal scales. The ALFIS sensor will be used for deployments from a variety of platforms, including autonomous unmanned vehicles, automatic gliders, vertical and drift profilers, buoys, and moorings, thus contributing to development of the Ocean Observing Systems and other emerging initiatives. Development of the new ALF sensors built upon state-of-the-art scientific and technological advances provides the scientific community and state/local agencies new means for research, observations and environmental monitoring.

RELATED PROJECTS

"Collaborative Research: Advanced Laser Fluorometer (ALF) for in vivo Characterization of Phytoplankton Pigments, Physiology and Community Structure". NSF Ocean Technology and Interdisciplinary Coordination Program, Award # OCE-07-24561, May 2007- April 2011.

"RAPID: Rapid Assessment of Extent and Photophysiological Effects of the Deepwater Horizon Oil Spill"; NSF OCE; Award # OCE-1048482; June 2010 – July 2012;

PUBLICATIONS

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2. Chekalyuk A. and M. Hafez, "Photo-physiological variability in phytoplankton chlorophyll fluorescence and assessment of chlorophyll concentration," Optics Express. **19**(23), 22643–22658 (2011).
3. Chekalyuk, A.M., M.R. Landry, R. Goericke, A.G. Taylor, and M. Hafez. Laser fluorescence analysis of phytoplankton across a frontal zone in the California Current ecosystem. J. Plankton Res., 34(9), 761-777 (2012).

4. Ohman, M.D., Rudnick, D.L., Chekalyuk, A., Davis, R.E., Feely, R.A., Kahru, M., Kim, H.-J., Landry, M.R., Martz, T.R., Sabine, C.L., Send, U. Autonomous ocean measurements in the California Current Ecosystem. Oceanography 18(3), 18-25 (2013).
5. Chekalyuk, A. and M. Hafez. Next Generation Advanced Laser Fluorometry for Characterization of Natural Aquatic Environments: New Instruments. Optics Express. 21, 14181-14201 (2013).
6. Chekalyuk, A. and M. Hafez. Analysis of spectral excitation for measurements of fluorescence constituents in natural waters. Optics Express. **21**, 29255-29268 (2013).
7. Goes, J. I., H. do Rosario Gomes, A. M. Chekalyuk, E. J. Carpenter, J. P. Montoya, V. J. Coles, P. L. Yager, W. M. Berelson, D. G. Capone, R. A. Foster, D. K. Steinberg, A. Subramaniam, and M. A. Hafez, "Influence of Amazon River discharge on the biogeography of phytoplankton communities in the western tropical north Atlantic," Prog. Oceanopgr. (in press) (2014). http://dx.doi.org/10.1016/j.pocean.2013.07.010
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PRESENTATIONS

9. A. Barnard, A. M. Chekalyuk, A. Derr, W. Strubhar, M. A. Hafez, J. Pearson, C. Orrico, and C. Moore, "Aquatic Laser Fluorescence Analyzer (ALFA): a new instrument for characterization of natural aquatic environments," ASLO/AGU 2012 Ocean Sciences meeting, Salt Lake City (2012). http://www.sgmeet.com/osm2012/viewabstract2.asp?AbstractID=11217
10. Chekalyuk, A. "Laser in situ fluorosensing of natural aquatic environments". ASLO 2013 Aquatic Sciences meeting, New Oreans (2013) http://www.sgmeet.com/aslo/neworleans2013/viewabstract2.asp?AbstractID=11177
11. Ohman, M. D., Chekalyuk, A. "Spray glider, moving vessel profiler, and Advanced Laser Fluorometer situate lagrangian experiments in the CCE-LTER site". ASLO 2013 Aquatic Sciences meeting, New Oreans (2013) http://www.sgmeet.com/aslo/neworleans2013/viewabstract2.asp?AbstractID=10882
12. Chekalyuk, A.; Barnard, A.; Hafez, M.; Koch, C.; Strubhar, W. "Advanced laser fluorometry (ALF): new technology for oceanography and environmental monitoring. ASLO/AGU 2014 Ocean Sciences meeting (2014). http://www.sgmeet.com/osm2014/viewabstract.asp?AbstractID=17868

PATENTS

Most of the ALFA methodological and design solutions were included in the below patent applications. Sea-Bird Scientific/WET Labs and Columbia University have signed an Exclusive License Agreement on the ALF technology.

13. A. Chekalyuk, "Spectral and Temporal Laser Fluorescence Analysis such as for Natural Aquatic Environments", Pending patent application US 20120324986 A1 (2010).

https://www.google.com/patents/US20120324986?dq=Spectral+and+Temporal+Laser+Fluorescence+Analysis+such+as+for+Natural+Aquatic+Environments&hl=en&sa=X&ei=_EirUorBNcbNsQSvkoC4AQ&ved=0CD4Q6AEwAQ

14. A. Chekalyuk, "Optical analysis of emissions from stimulated liquids," Pending patent application WO2013116769 A1 (2013). https://www.google.com/patents/WO2013116760A1?cl=en&dq=WO2013116760+A1&hl=en&sa=X&ei=N9FKUsJ4863gA8n8gcgB&ved=0CDkQ6AEwAA

Fig. 1. A: The commercial Aquatic Laser Fluorescence Analyzer (ALFA) developed by the LDEO/WET Labs project team [9,12]. B: The ALFA demonstration at the ASLO/AGU meeting in Salt Lake City in February 2012 [9]. C,D: The ALF-T instrument prototype configured with single laser (510 nm) and three lasers (375, 405, and 510 nm) [5]. E: Instrument calibration at WET Labs optical laboratory. F: Two ALFA instruments were used for underway measurements and discrete sample analysis during the CCE LTER Process cruises in the California Current in 2011 and 2012.

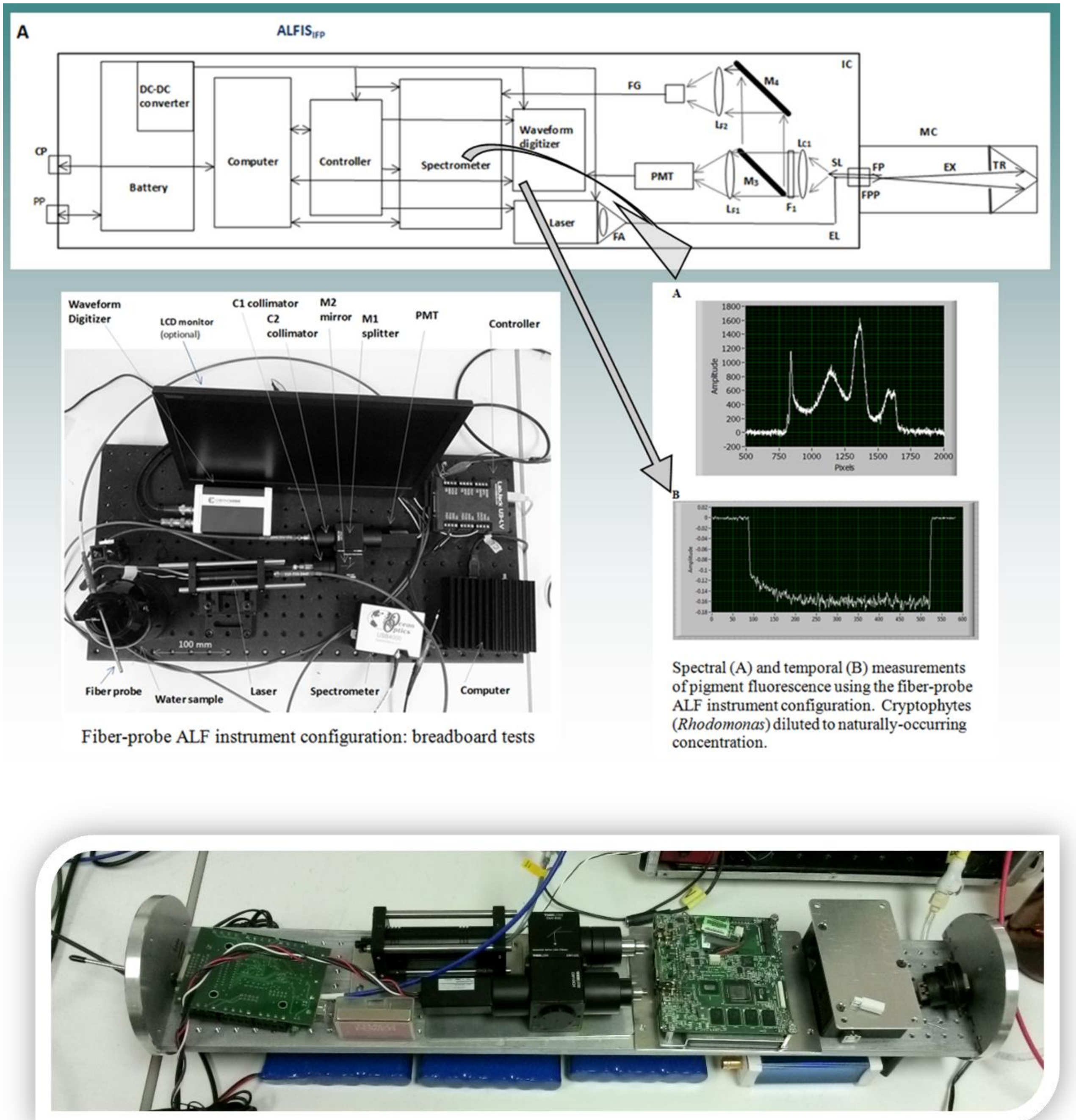

Fig. 2. <u>Upper</u>: The patent-pending design of the fiber-probe ALF In Situ (ALFIS) instrument. <u>Middle</u>: A breadboard test of the fiber-probe ALFIS instrument (left). An example of spectrally (A) and temporally (B) resolved fluorescence measurements with the ALFIS prototype using the phytoplankton culture of Cryptophytes diluted to naturally-occurring concentration (right). <u>Lower photo</u>: The ALFIS prototype built by the LDEO ALF team to autonomously operate up to 12.5 hours in 4.5" diameter aluminum case using the internal rechargeable batteries.

Fig. 3. The Moving Vehicle Profiler (MVP, upper photo) and the SeaSoar (lower photo) towed vehicles are considered, along with the Wave Glider (Liquid Robotics), as prospective platforms for deployments of the next generation ALF In Situ instrument.

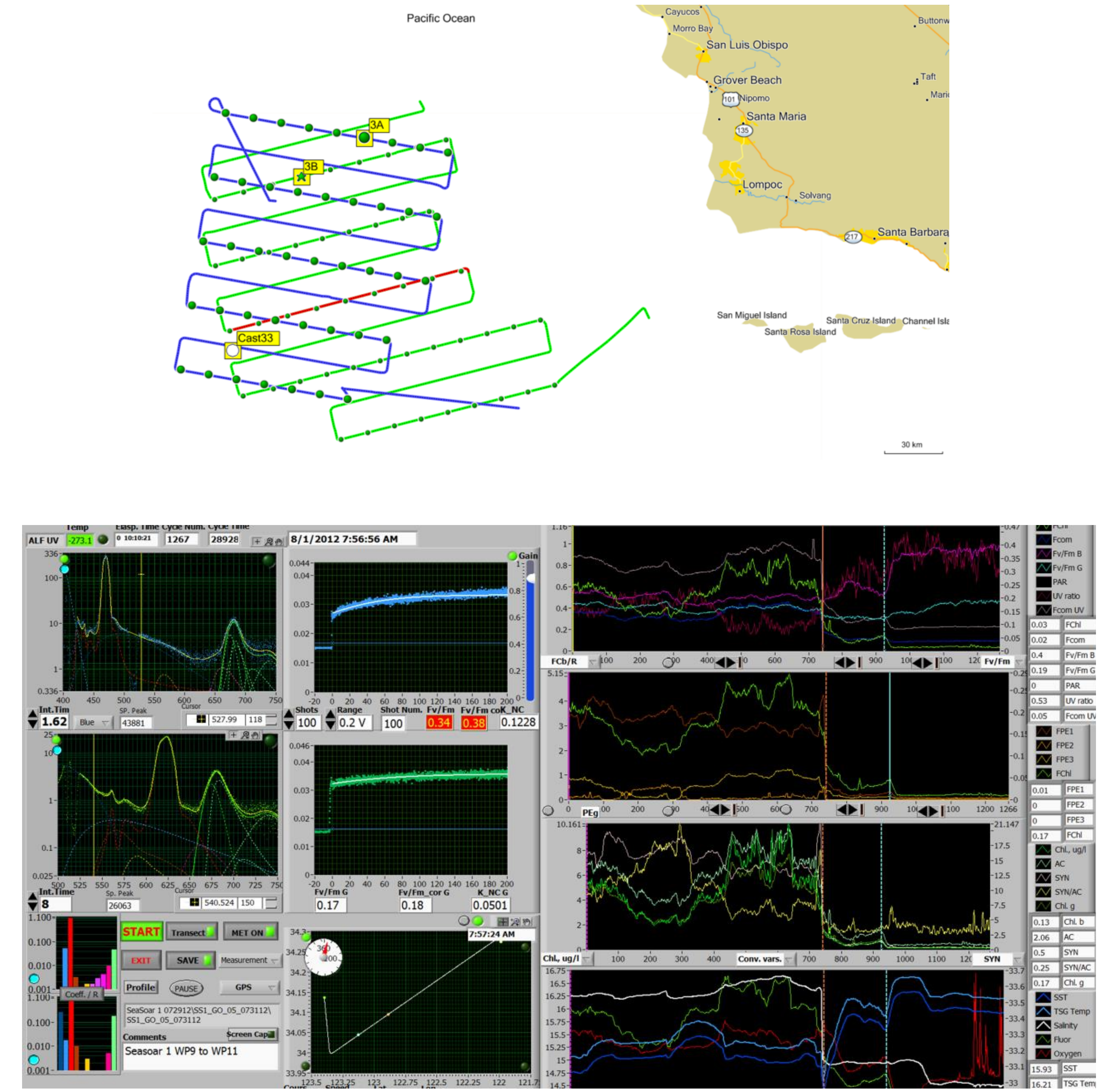

Fig. 4.** **<u>Upper panel</u>: A map of the ALF underway measurements during the CCE LTER Process cruise in the California Current, July-August 2012. Dots indicate locations of the underway sampling used for correlation analysis displayed in Fig. 5. "Cast 33" shows sampling location for the vertical profiles displayed in Fig. 11C,D. <u>Lower panel</u>: A screenshot of the ALF software user interface during the underway transect measurements (the location is marked with red line in the upper panel). The ALFA transect data are displayed in Fig. 11A,B. See [6] for details.

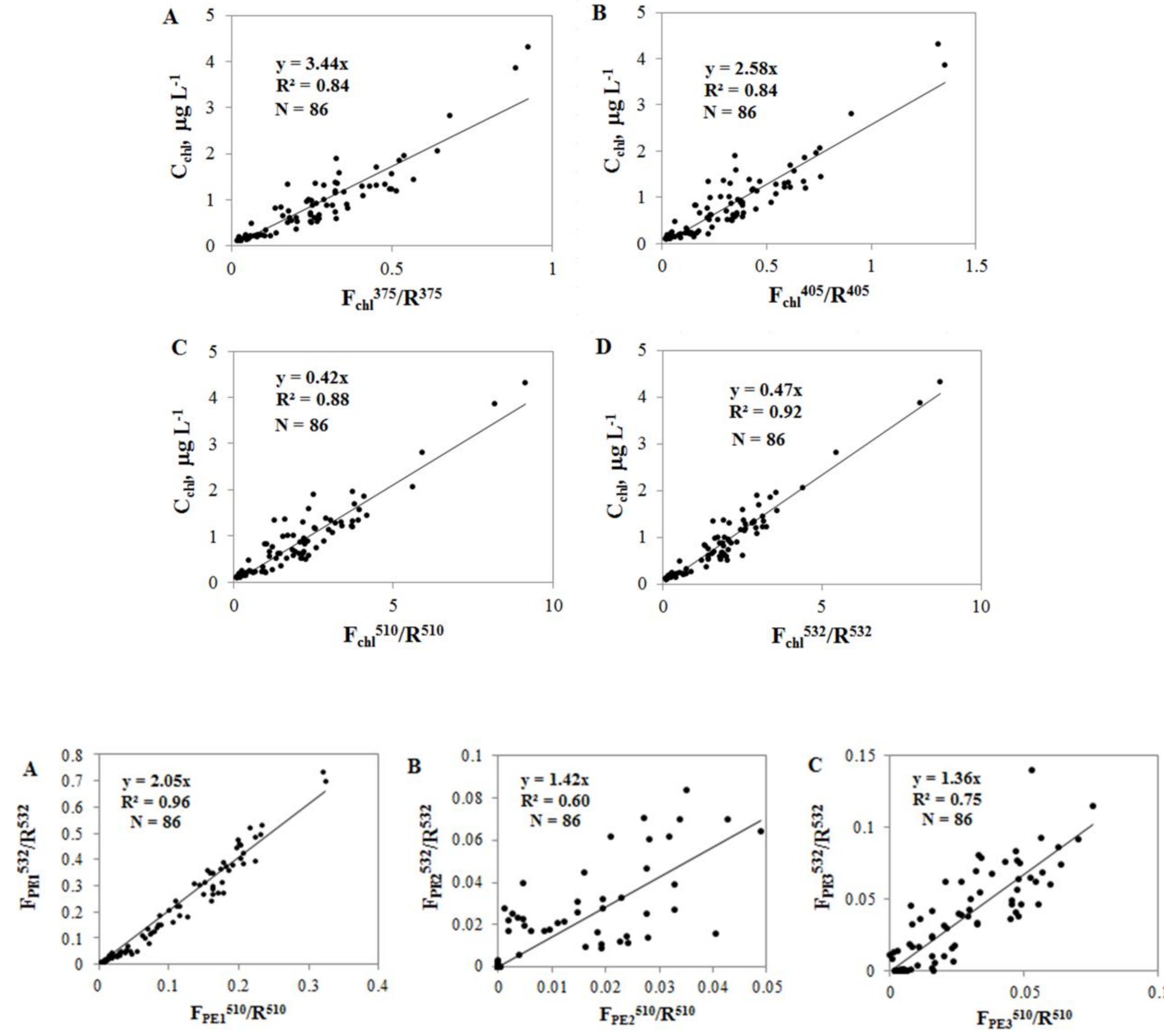

Fig. 5. <u>Upper panels A-D</u>: The high correlation between Chl concentration and Chl fluorescence normalized to water Raman scattering measured with excitation wavelengths EW = 375 (A), 405 (B), 510 (C), and 532 (D) nm (see a sampling map in Fig. 4) indicates that all four EWs can be used for accurate fluorescence measurements of Chl concentration in the coastal and offshore environments. <u>Lower panels A-C</u>: The regression analysis of the ALF measurements of the group-specific phycoerythrin fluorescence suggests that the new 510 nm laser can be used, as well as the older 532 nm laser excitation, for structural analysis of phytoplankton communities (see a sampling map in Fig. 4 and [6] for detailed discussion).

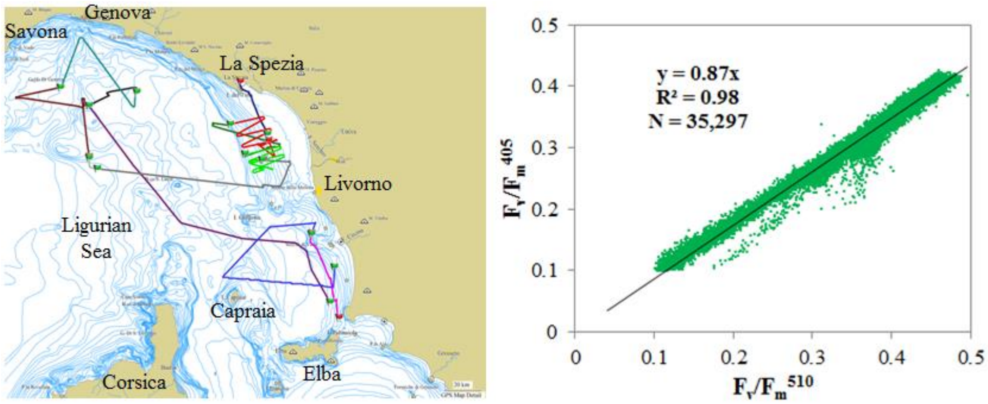

Fig. 6. <u>Left</u>: A transect map of the ALFA underway measurements of phytoplankton photochemical efficiency, Fv/Fm, with 405 and 510 nm excitation in the Ligurian Sea (Mediterranean; March 2013). <u>Right</u>: The high correlation of the two data sets indicates that the new 510 nm laser can be used, as well as the earlier used 405 nm laser excitation, for analysis of phytoplankton photo-physiological state and photosynthetic capacity (see discussion in [6]).

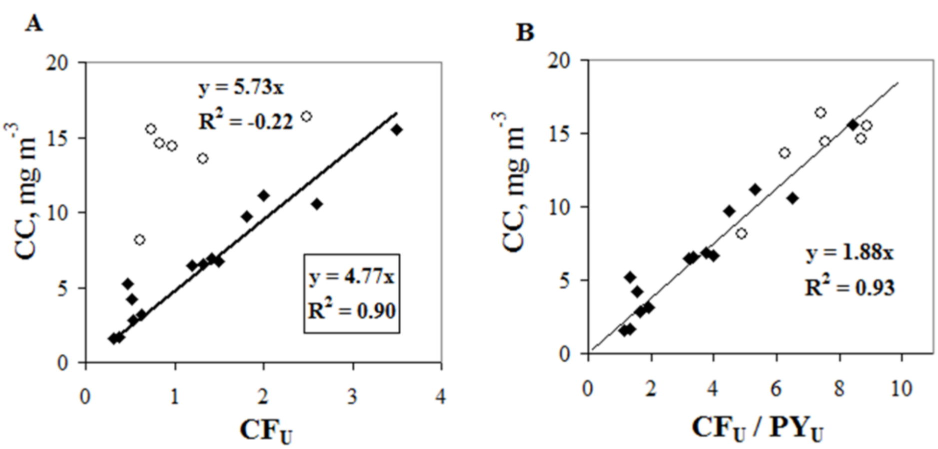

Fig. 7. (A) Correlation between measurements of Chl concentration (CC) in water samples and underway Chl fluorescence measurements (CF_U) in the Delaware and Chesapeake Bays [2]. Black dots and empty circles represent the dark-time and morning measurements, respectively. The framed and unframed regression equations are calculated for the dark-time and entire data sets, respectively. (B): High correlation between CC in water samples and fluorescence parameter CF/PY calculated from the ALF underway flow-through measurements of Chl fluorescence (CF) and PSII photochemical yield (PY) (the same data set as in panel A) indicates that normalizing the CF to F_v/F_m measurements can be used to eliminate the effect of the solar-induced non-photochemical quenching and improve the accuracy of CC fluorescence assessments (see [2]).

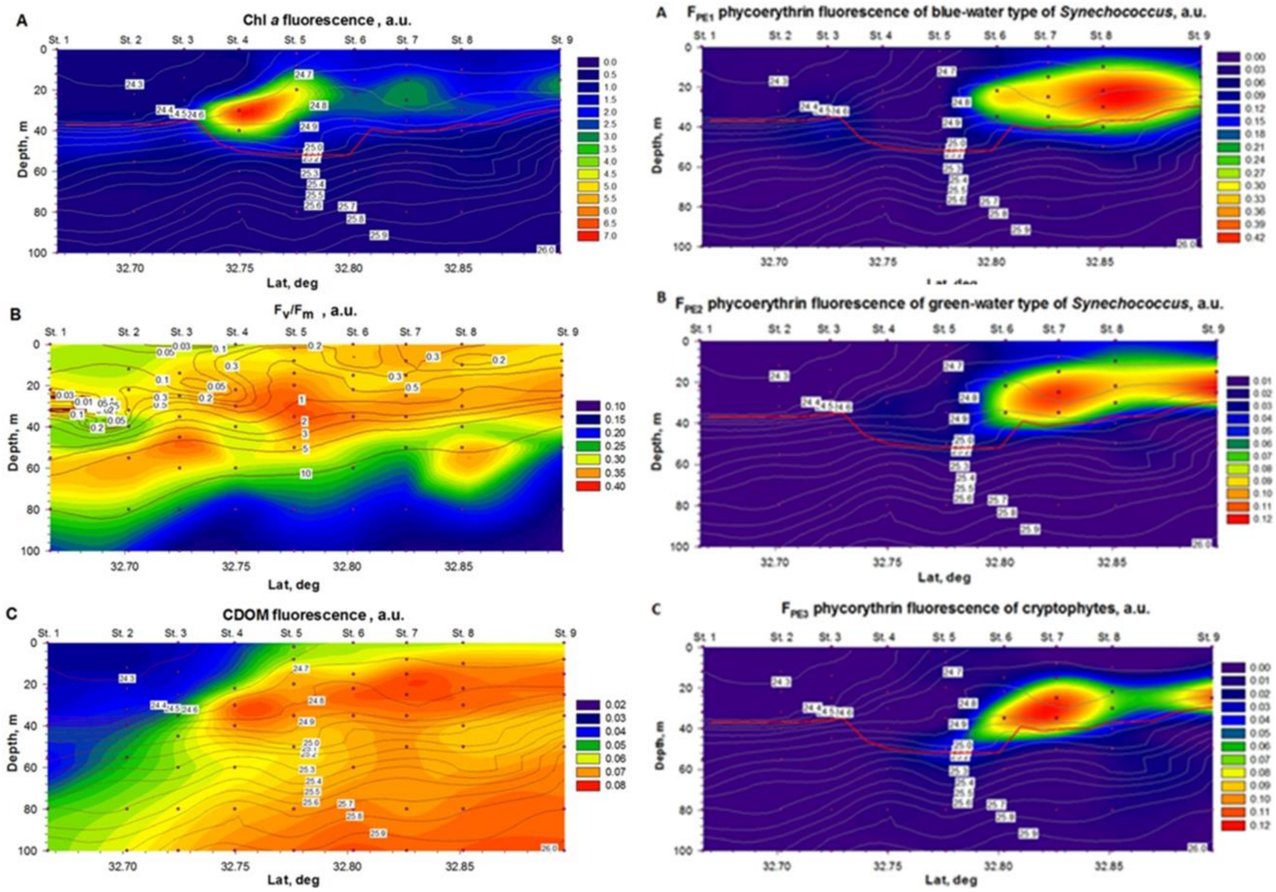

Fig. 8. <u>Left panels</u>: Spatial distributions of Chl a fluorescence (A), variable fluorescence (B), and CDOM fluorescence (C) measured with the ALF instrument in the euphotic layer across the frontal zone in the California Current. <u>Right panels</u>: Spatial distributions of phycoerythrin (PE) fluorescence of blue-water (A) and green-water (B) types of Synechococcus, and cryptophytes (C) measured with the ALF instrument in the euphotic layer across the frontal zone in the California Current. The ALF measurements indicate strong biological responses of phytoplankton community to the frontal variability in physical and chemical properties of water (see [3] for detailed discussion).

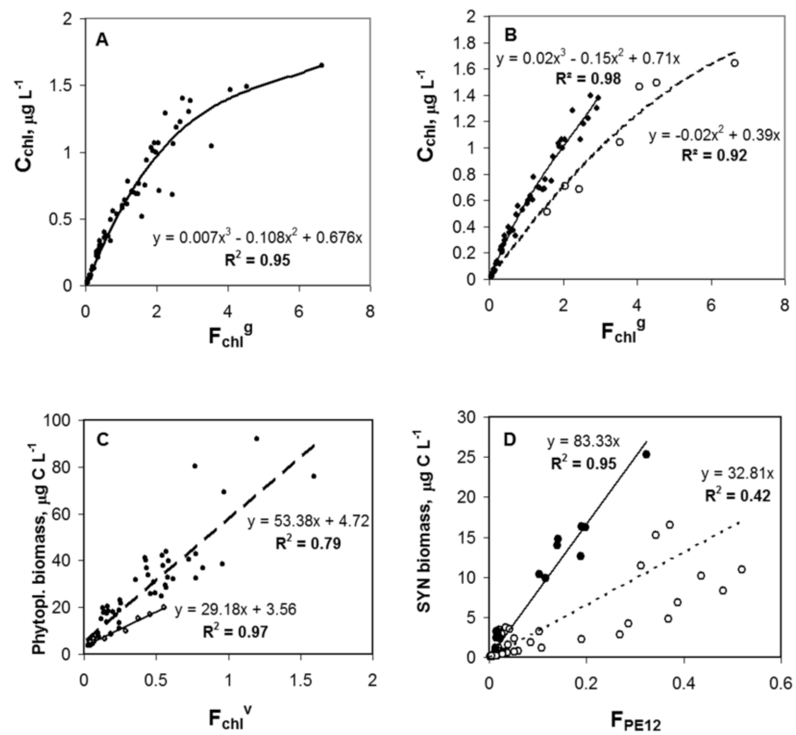

Fig. 9. (A): Correlation between chlorophyll a (Chl a) concentration (C_{chl}) and Chl a fluorescence (F_{chl}) measured with ALF instrument across the frontal zone in the California Current (same data set as displayed in Figs. 8 and 10). (B): Same data as in panel A; a subset of samples from the diatom-dominated Chl a maximum at the front is displayed with empty circles. (C): dashed regression line – correlation between autotrophic carbon biomass (AC) and Chl a fluorescence (F_{chl}) measured with ALF instrument; solid regression line – correlation for a subset of samples collected between 40 m and 80 m in the Southern portion of the patterns displayed in Fig. 8. (D): dashed regression line - correlation between phycoerythrin fluorescence of Synechococcus (F_{PE12}) and their carbon biomass (SYN); solid regression line - correlation for a subset of the samples (filled dots) collected in the subsurface layer above the Chl a maximum. The results of this regression analysis were used to convert the ALF fluorescence units into commonly accepted units of Chl concentration and autotrophic carbon biomass as shown in Figs. 10 and 11 (see [3] for details).

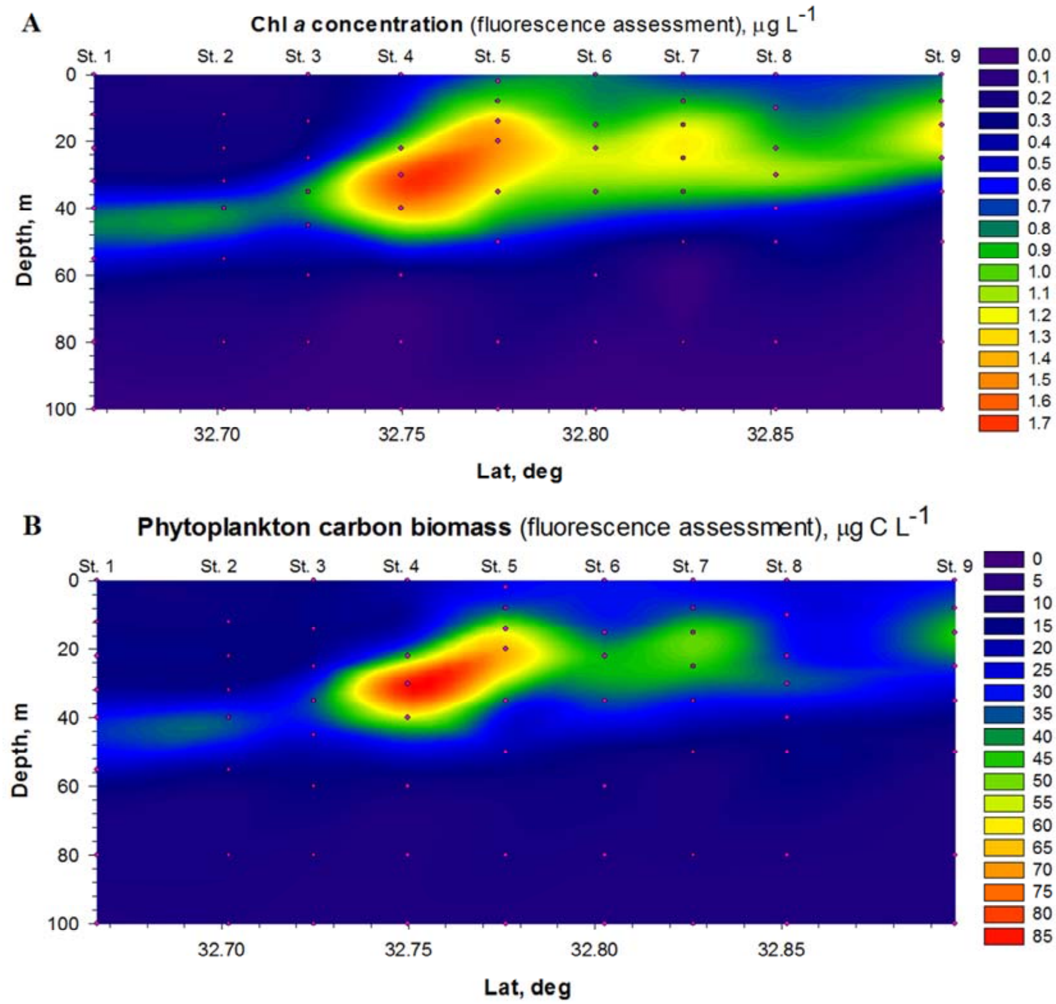

Fig. 10. Distributions of Chl a concentration (A) and carbon biomass of autotrophic phytoplankton (B) in the euphotic layer across the frontal zone calculated using Chl a fluorescence measurements of discrete water samples [3]. The regression relationships displayed in Fig. 9 were used to convert the ALF fluorescence measurements into commonly accepted units of Chl concentration and carbon biomass.

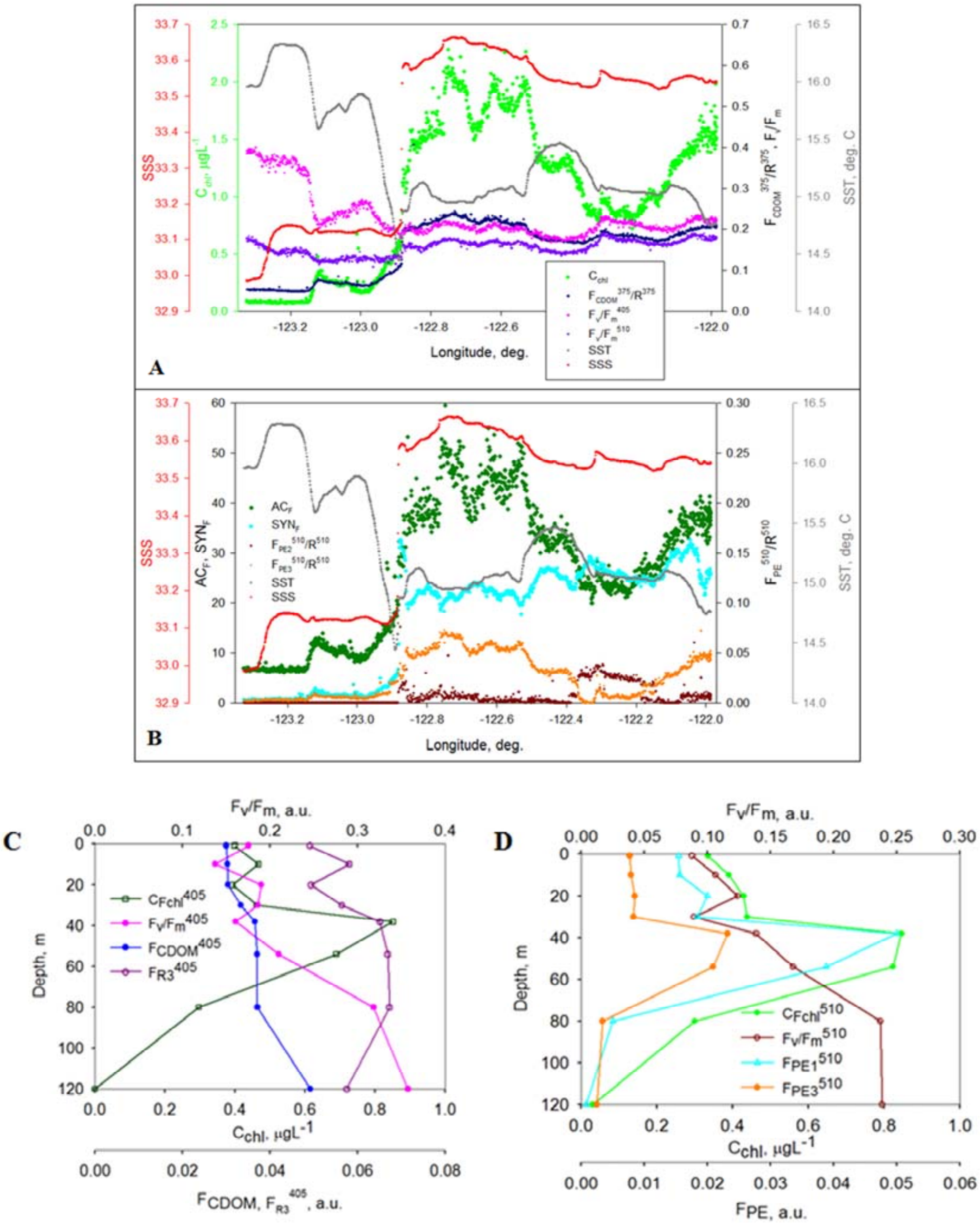

Fig. 11.** ***<u>Upper panels</u>: Surface distributions of the key bio-environmental variables measured with the ALF-T instrument across the frontal zone in the California Current in Aug. 2012. C_{chl}, AC_F, SYN_F, SST, and SSS are abbreviations for Chl concentration, total phytoplankton carbon biomass, carbon biomass of Synechococcus cyanobacteria, seasurface temperature, and seasurface salinity, respectfully. Strong biological responses of phytoplankton community were detected across the frontal zone [6]. <u>Lower panels</u>: Vertical profiles of the key ALF measurements show significant variability that justifies development of the ALFIS instrument for high-resolution in situ measurements (the sampling location is marked as "Cast33" on the map in Fig. 1).

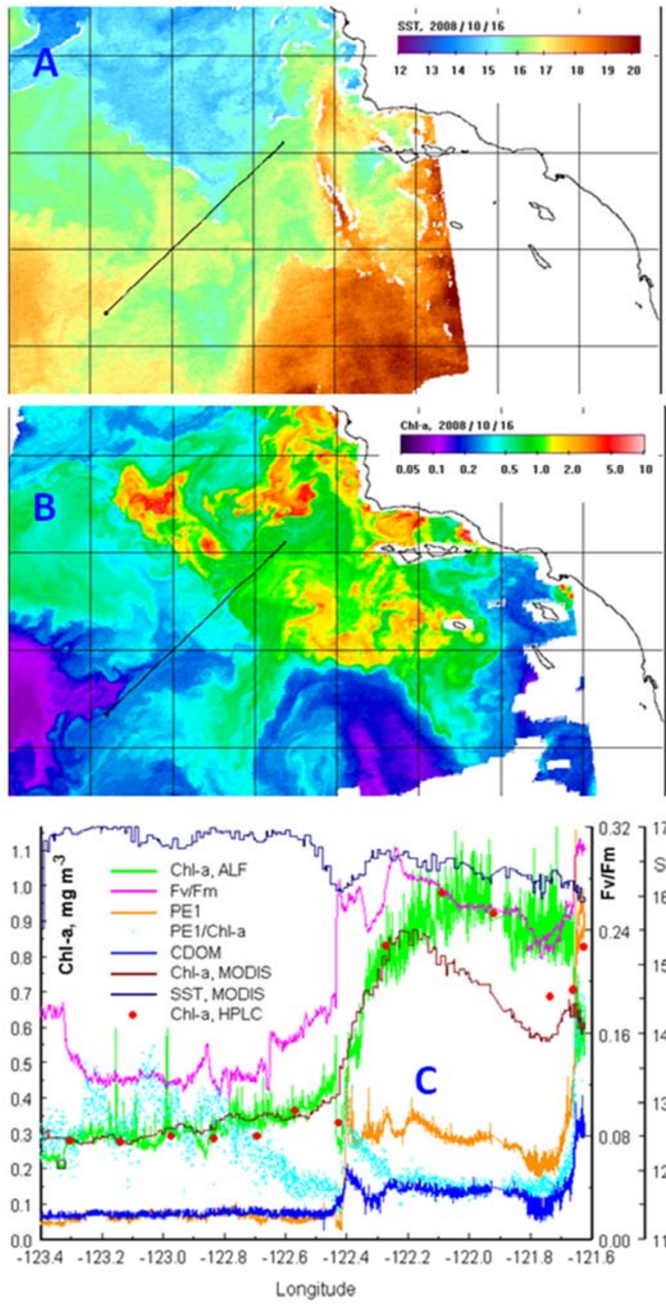

Fig. 12. Matching MODIS Aqua SST (A, C) and Chl-a (B, C) data with ALF multivariable shipboard underway (C) and HPLC Chl a (C) measurements. Ship track: black line in (A) and (B). California Current, CCE LTER Process cruise. The ALF high-resolution (~30 m) measurements can be used for validation of the satellite retrievals, assessment of sources uncertainties, and development of the new ocean color products (e.g. phycoerythrin biomass, phytoplankton functional types, etc.).

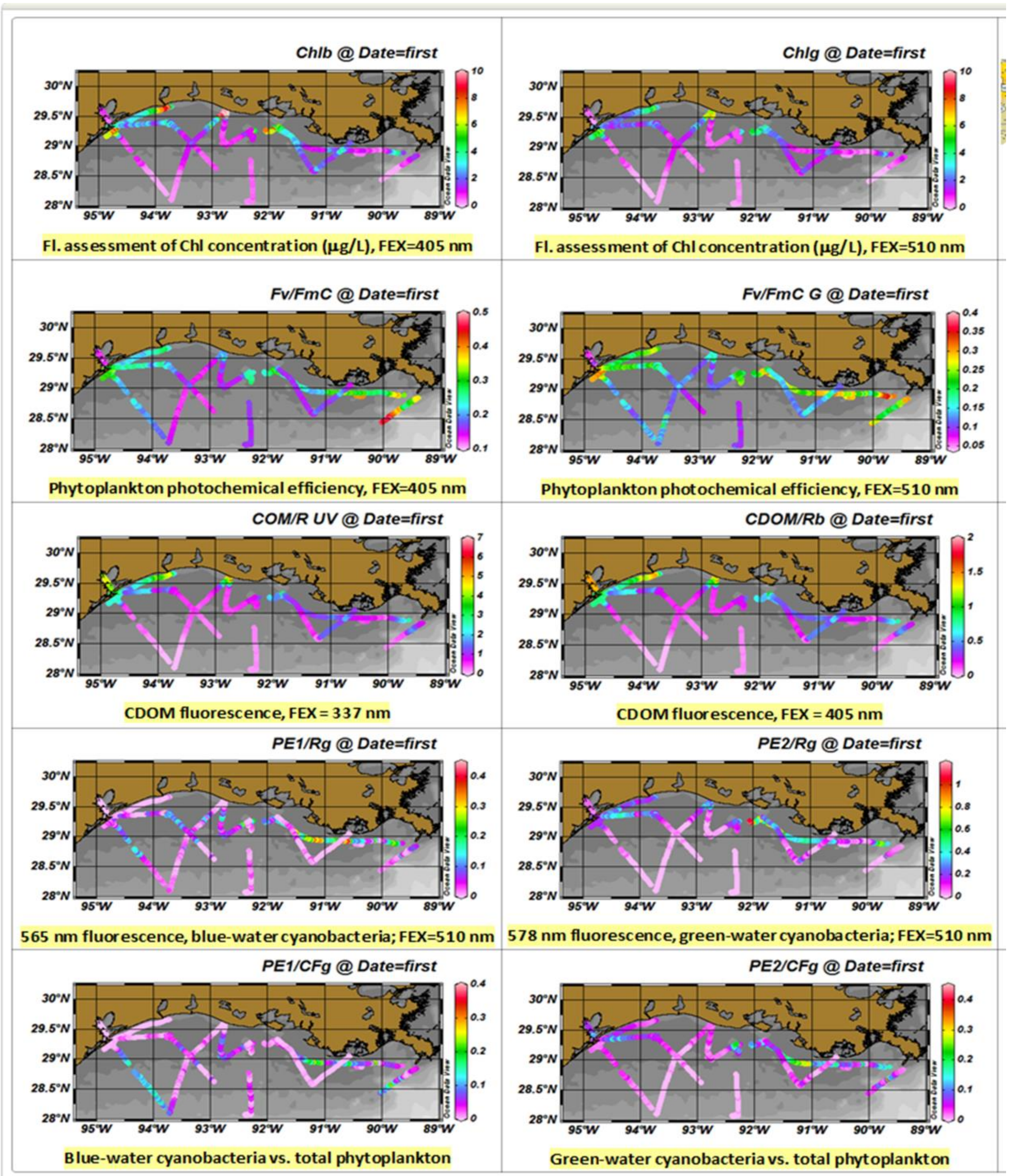

Fig. 13. Underway ALF measurements of the key aquatic bio-environmental characteristics in the Gulf of Mexico in support of the future NASA GEO-CAPE geostationary satellite mission (Sept. 2013). The rich and informative high-resolution ALF measurements can be used for validation of the satellite algorithms and interpretation of the satellite observations.

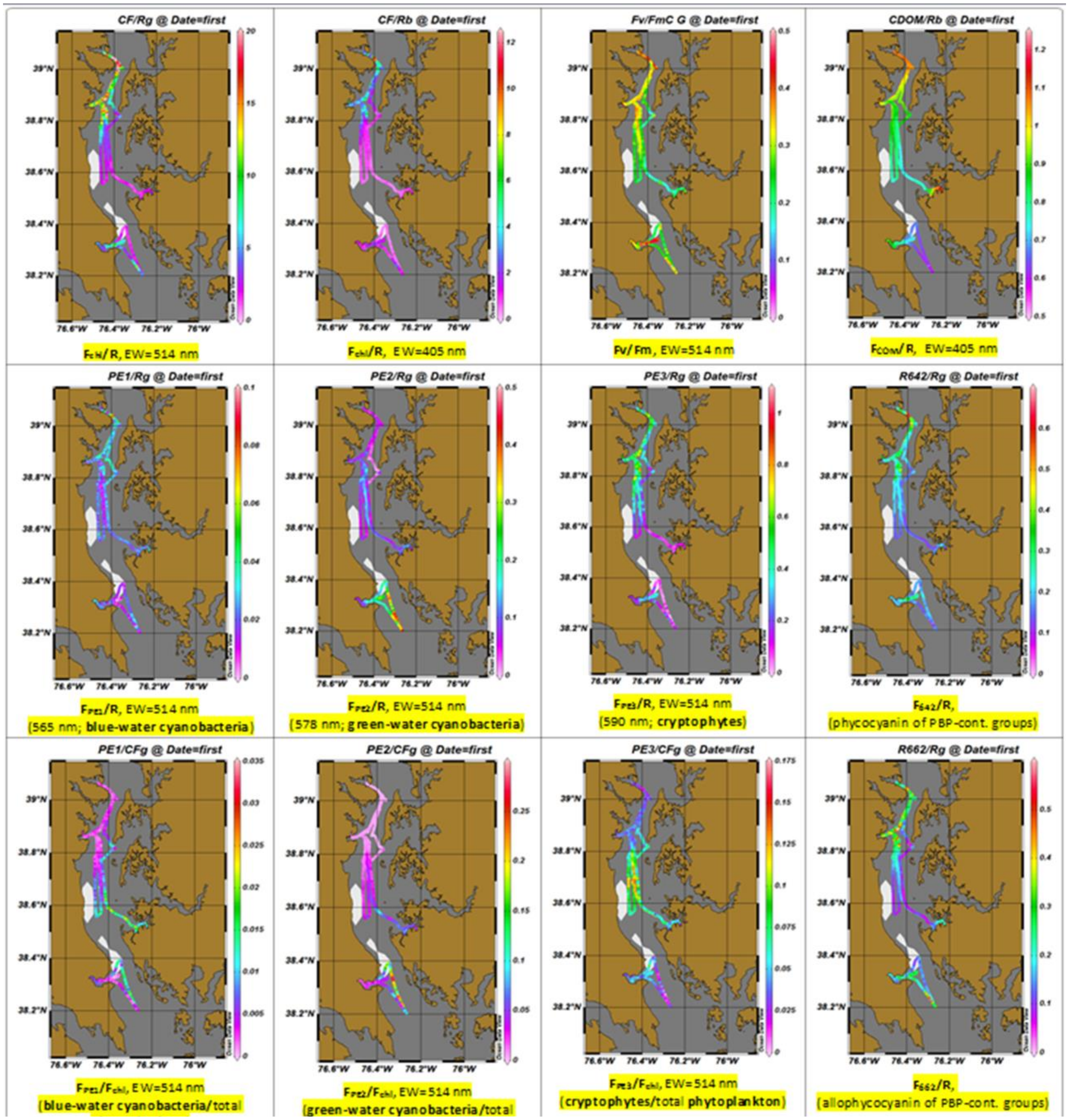

Fig. 14. Underway ALF measurements of the key aquatic characteristics in the upper Chesapeake Bay (Aug. 2013). The rich and informative real-time ALF measurements can provide useful information for detailed bio-environmental characterization of coastal and estuarine waters.

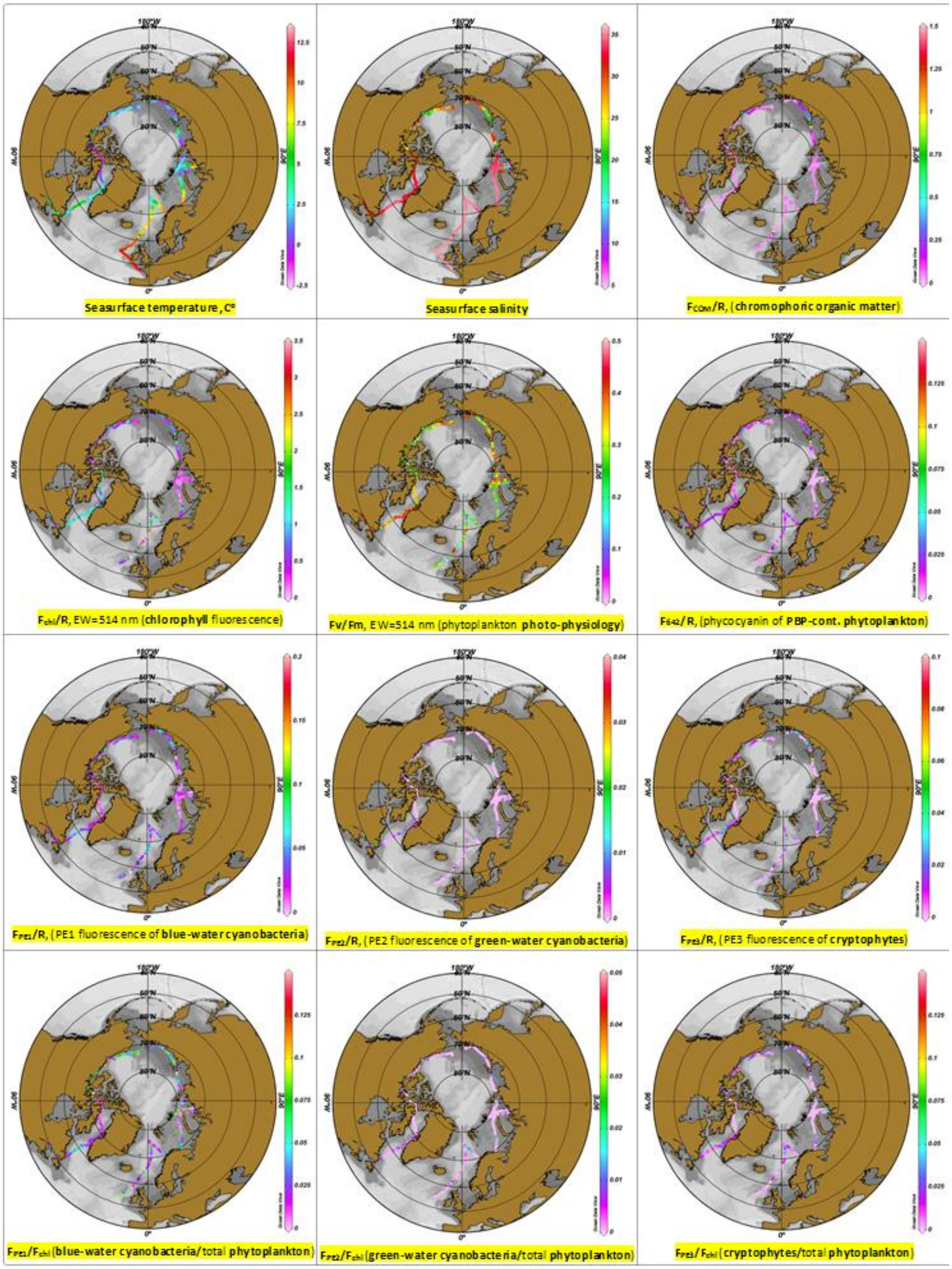

Fig. 15. Integrated data of seven months of the ALFA underway measurements in the Arctic Seas to evaluate the impacts of the climate change. International expedition on the yacht Tara, May-December 2013; research collaboration between the LDEO/WET Labs ALF team and Prof. Emmanuel Boss (U. of Maine).