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NEWS REPORT

Aquafluo 2007: chlorophyll fluorescence in aquatic sciences, an international conference held in Nové Hrady

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"The topic of this conference was somewhat quirky and possibly unique in that most of us who study chlorophyll fluorescence in aquatic systems don't really care about what we measure so carefully and study so thoroughly. It's not the fluoresced photons that matter; it's what fluorescence can tell us about biological and ecological properties and processes" is how one of us (J.C.) characterized the first international meeting for chlorophyll fluorescence in aquatic sciences—"Aquafluo 2007" held in Nové Hrady, Czech Republic, from May 28 to June 1, 2007, attracting more than 120 participants from 24 countries. (For a pdf file of the book of abstracts and many photographs, see: http://www.aquafluo.cz). Figure 1 shows a group photograph of many of the participants.

Methods for assaying chlorophyll fluorescence were introduced into aquatic science several decades ago, once the main principles had been developed and tested in mainstream photosynthesis research (see e.g., Chlorophyll a Fluorescence: A Signature of Photosynthesis, edited by G.C. Papageorgiou and Govindjee, Advances in Photosynthesis and Respiration, Volume 19, Springer, Dordrecht, 2004). A central goal for studies using these methods within aquatic systems was originally (and still is) to examine and explain environmental controls on the photosynthesis, growth and community structure of phytoplankton, and other chlorophyll-containing organisms in aquatic systems. Therefore, it is perhaps not surprising that chlorophyll fluorescence has become established as an independent method for assessing algal physiology in the aquatic environment, although only recently. Chlorophyll fluorescence techniques are now proven as indicators of environmental stresses, such as iron limitation in the ocean, and have led to the discovery that significant numbers of photoheterotrophic bacteria inhabit the surface ocean and deep sea vents. Fluorescence applications in flow cytometry and microscopy have contributed to a fuller understanding of the heterogeneity of phytoplankton photosynthesis on the cellular level. A key advantage of fluorescence as a tool in aquatic science is that fluorescence measurements facilitate observations of biological and ecological processes on scales that matter, whether they be temporal scales—of the primary processes of photosynthesis, diel migration of benthic diatoms, or seasonal succession of microalgal communities—or spatial scales ranging from differentiated locations on a single cyanobacterial filament to chlorophyll-containing subsurface maximum layers in lakes and ultimately to synoptic views

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Fig. 1 A group photograph of the participants at the Aquafluo 2007 meeting in Nové Hrady

Fig. 2 Selected photographs of individual participants from the Aquafluo 2007 meeting in Nové Hrady. **Top Row (Left)**: (Left to right): Ondrej Prasil (organizer), Paul Falkowski (plenary speaker) and Tom Bibby. **(Right)**: (Left to right): Mariella Ragni (recipient of the first poster prize) and Govindjee (plenary speaker). **Middle Row (Left)**: (Left to right): John Cullen (organizer), Tammy Richardson, Evelyn Lawrenz and David Suggett (organizer). **(Right)**: (Left to right): Zbigniew Kolber and Ivan Setlik. **Bottom Row (Left)**: (Left to right): John Cullen and Marcel Babin. **(Right)**:(Left to right): Jun Minagawa, Michal Koblizek and Wim Vredenberg. All other photographs may be found at: http://www.aquafluo.cz

of sun-induced phytoplankton fluorescence across ocean basins.

The understanding of marine ecosystem dynamics is based on element fluxes through various compartments such as phytoplankton, bacteria, zooplankton and sediment fauna. The major elements of interest are those that form a large fraction of biomass and/or are possibly limiting. The most commonly used are C, N, and P; however, C is especially important as a 'currency' given the growing surge of research and legislation tied to increasing atmospheric CO_2 and its effect on climate. Carbon is also the closest of those elements to the fluorescence process: electrons produced by Photosystem II (PS II) reduce CO_2 to organic carbon, competing with fluorescence for the energy in absorbed photons. (For full information on PSII, see "Photosystem II: Water Plastoquinone Oxido-reductase," edited by T.J. Wydrzynski and K. Satoh, Advances in Photosynthesis and Respiration, Volume 22, Springer, Dordrecht, 2006.) A huge interest rests on using chlorophyll fluorescence to estimate primary productivity; however, multiple publications have either supported or discounted fluorescence measurements as an alternative for direct but laborious conventional measurements of aquatic primary production. As concluded by Paul Falkowski, pioneer of active fluorescence techniques for use in aquatic environment: "whilst the link between electrons and carbon fluxes will always be a key aspect of the use of fluorescence in aquatic sciences, the use of fluorescence will never be a substitute to carbon flux measurements but rather a tool among others." (See top row, left, Fig. 2 for a photograph of one of the two plenary speakers, Paul Falkowski, with one of us (O.P.) and Tom Bibby.)

The conference, that was called Aquafluo 2007, took place in Nové Hrady at a former chateau, which dates from the early 19th century, and was recently renovated and converted into modern research center with conference facilities. Such a setting allowed Aquafluo 2007 to combine presentations with practical workshops in well-equipped laboratories, as convened and organized by the Czech Academy of Sciences and the University of South Bohemia.

Topics covered throughout the meeting bridged a wide range of uses of chlorophyll fluorescence methods in aquatic sciences: from the satellite-borne determination of fluorescence yields and phytoplankton physiology on large spatial scales down to techniques to study the heterogeneity of photosynthesis with subcellular spatial resolution (for a detailed program, see www.aquafluo.cz). Other presentations dealt with methodological overviews of available techniques, current understanding of the photobiology of aquatic photoautotrophic groups, such as corals and aerobic anoxygenic phototrophic (AAP) bacteria, fluorescence quenching (better said: decrease in fluorescence intensity) mechanisms of key microalgae and global change effects on nitrogen fixation in marine cyanobacteria. Participants also received intensive overviews of the theoretical backgrounds to chlorophyll fluorescence.

During the afternoons, participants were split into small groups to attend workshops that allowed direct "hands-on" experience with several experimental or analytical techniques that have become highly popular amongst aquatic scientists. Individual workshops dealt with questions of how to calculate primary productivity from fluorescence data, what complications to anticipate when measuring benthic photosynthetic communities, how to interpret differences between data obtained using single and multiple-turnover excitation protocols, how to use multispectral excitation of fluorescence in order to determine the taxonomical composition of natural phytoplankton populations, how can algal biotechnology benefit from chlorophyll fluorescence, what information can be obtained when using microscopes equipped for measuring fluorescence kinetics and finally, how to operate automated growth chambers with on-line fluorescence detection.

Many students presented posters to compete for prizes initiated by Govindjee, organized by Aquafluo 2007, and sponsored by Springer. The first prize was a book, "Chlorophyll a Fluorescence: A Signature of Photosynthesis," mentioned above; it was won by Mariella Ragni from the University of Essex, who presented a poster entitled "Light dependence of PSII photoinhibition, recovery and the size of the light harvesting antenna in *Emiliania huxleyi* under high light and UV stress." The second prize was a book "Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications," edited by B. Grimm et al., Volume 25, Advances in Photosynthesis and Respiration, Springer, Dordrecht, 2007; it was awarded to Katerina Rottnerova from the University of South Bohemia in Ceske Budejovice for her poster entitled "Circadian rhythms of photosynthesis in the marine diatom *Thalassiosira weisflogii* under nitrogen replete and deplete conditions." Figure 2, top row, right, shows a photograph of Mariella Ragni receiving an autographed copy of her Prize from Govindjee, one of the two plenary speakers at the symposium.

Following the intensive week of presentations and workshops, participants concluded that fluorescence does provide information about [phytoplankton] photosynthesis, but it is difficult to interpret (and can even become confusing) when trying to understand the observations in terms of ecophysiology or ecosystem dynamics when used alone or at best with few ancillary measurements. The photosynthesis literature is rich enough to provide solid bases for several different interpretations of a single observation; as one of us (Govindjee) commented after one talk during the meeting, "*you have to prove it.*" So, fluorescence must be one tool among others.

The use of chlorophyll fluorescence in aquatic sciences over the last decades has allowed us to identify key processes related to phytoplankton photoacclimation and nutrient stress. The diversity of phytoplankton is such, however, that we could expect an infinite number of peculiarities among the myriad of existing species. To make a major step forward, it is now time for (1) generalization as much as possible, and (2) interpretation of fine processes in terms of ecology. This requires screening over many different representative species, and studies conducted as much as possible in the natural environment, using methods that are conducted and calibrated in such a way as to be comparable between studies. Efforts toward that direction have been initiated in the recent past and must be pursued.

Consideration of the capabilities of fluorescence measurements and their relevance to the ultimate goals of aquatic research may help us to better decide which approaches currently in use are most worthy of pursuing and which new fluorescence-based methods should be developed for applications in aquatic systems over the coming years. Some of the most important scientific questions will no doubt be the same ones we have asked for decades; however, the answers may be addressed more effectively only now because the appropriate observation technologies are finally available. Some questions will, of course, be new—in particular, those that are raised with the advent of new technology. Regardless, the development and evaluation of fluorescence-based tools should be driven by the scientific questions on which the research is based:

how can fluorescence reveal that which could not be resolved previously? We must look to the rich history of research within both photosynthesis and aquatic systems, for insight and practical guidance. Meanwhile, we should keep in mind the fundamental questions that we are really trying to pursue, so we can distinguish critically important controversies from marginally relevant quibbles! Fluorescence is much more than emitted photons; it is clear that we can and should continue to make such a phenomenon central to answering many of the fundamental questions that fuel the aquatic sciences.

We end this report with photographs of some of the authors and other participants when they were relaxing outside the conference room. The left photograph in the middle row of Fig. 2 shows two of us (J.C. and D.S.) with Tammi Richardson and Evelyn Lawrenz, enjoying a meal on the steps of the former chateau; further, the right photograph of the middle row, in Fig. 2, shows Zbigniew Kolber with Ivan Setlik, a pioneer of photosynthesis and fluorescence research in The Czech Republic. The left photograph of the bottom row, in Fig. 2, shows two of us (J.C. and M.B.) announcing the winners of the poster competition. Finally, the right photograph of the bottom row, in Fig. 2, shows three other speakers: Jun Minagawa, Michal Koblizek, and Wim Vredenberg, standing near the registration desk. All other photographs may be found at: http://www.aquafluo.cz.

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