

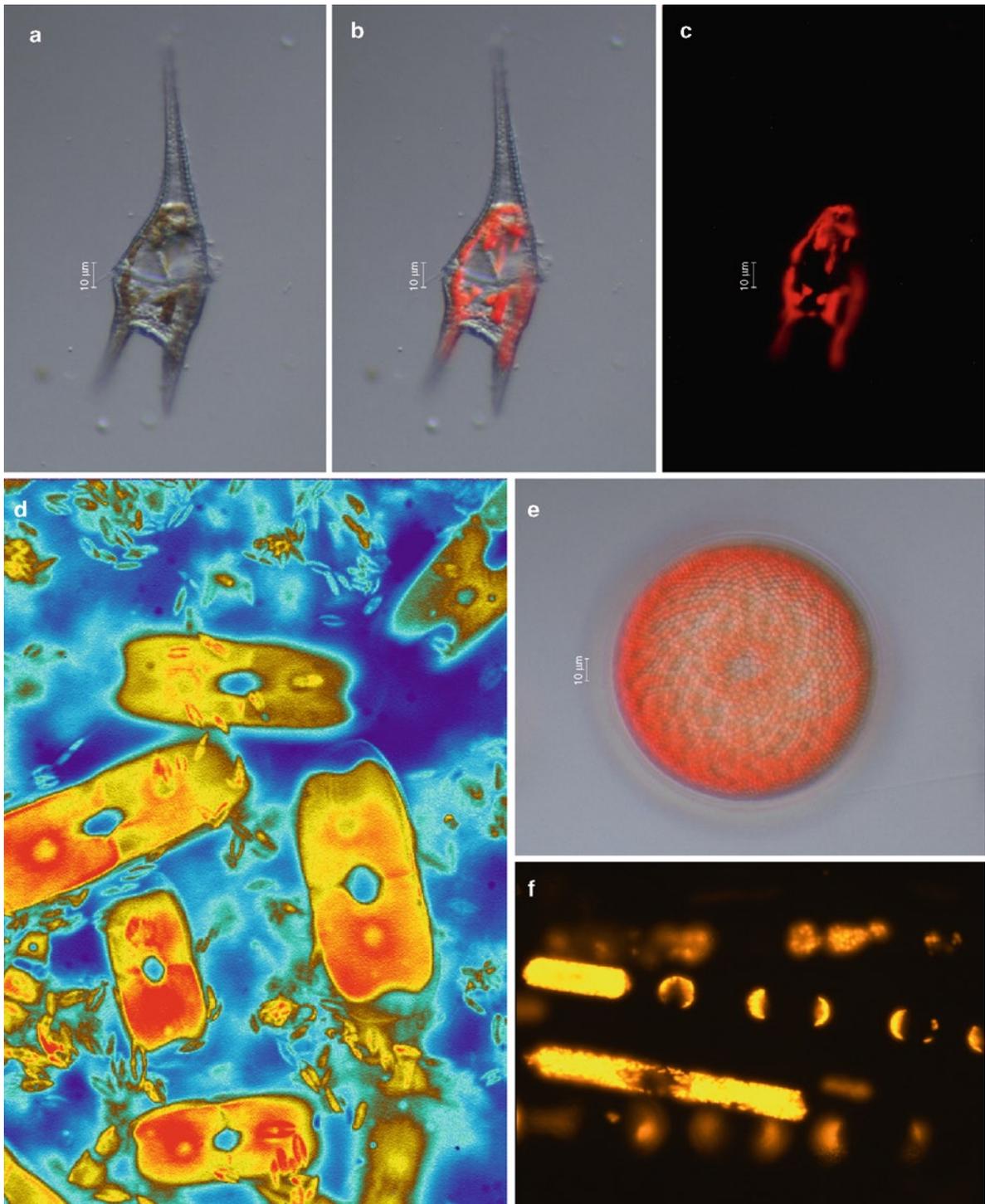
## Preface

It is unquestionable that Chlorophyll *a* fluorescence is quite literally a global phenomenon. Fluorescence merely describes an optical phenomenon where light absorbed at one wavelength is re-emitted at another (longer) wavelength; it exists passively in nature and occurs wherever light exists to be absorbed by Chlorophyll *a* molecules. These molecules are a common property of all photoautotrophic organisms on land and in water; thus Chlorophyll *a* fluorescence is essentially ubiquitous in nature (Fig. 1). It is incredible that such a natural phenomenon has been exploited by such a wide variety of researchers and across the biological and environmental sciences, and perhaps is testament to the importance we place on understanding photoautotrophic activity. We have long known that Chlorophyll *a* fluorescence of photosynthetic organisms varies as a result of changes in the amount (biomass), as well as function (quantum yield), of Chlorophyll *a* present. At operational temperatures that exist in most natural environments, Chlorophyll *a* fluorescence is largely derived from the Chlorophyll *a* associated with photosystem II (PSII), i.e. the oxygen evolving complex; as such, changes in the quantum yield of fluorescence directly relate to changes in photosynthetic (O<sub>2</sub> evolving) capabilities. Thus, by actively inducing changes in Chlorophyll *a* fluorescence using an actinic light source, we can perturb the physiological *status quo* of (PSII) photoautotrophy itself. Packaging of technology to enable induction and measurement of such Chlorophyll *a* fluorescence perturbations has entirely made possible examination of processes associated with plant and algal ecology, physiology and productivity, and at scales from the single cell to the entire planet (van Kooten and Snel 1990). Therefore, it is hard to imagine a future that does not continue to exploit the properties of Chlorophyll *a* fluorescence, not only for research but

also in how we continue to sustainably exploit our ever-changing environment.

The history of using fluorescence to investigate biomass, photosynthetic physiology and primary productivity has been covered in several comprehensive publications, most recently by Papageorgiou and Govindjee (2005) (and chapters therein); however, it is of course important to note the place of aquatic studies in this history, at least for the context of the following chapters. Whilst many major developments in using *variable* Chlorophyll *a* fluorescence have arguably come from studies on terrestrial (vascular) plants, free-living microalgae (chlorophytes in particular in particular) and cyanobacteria have proved to be important laboratory organisms in examining principal photobiological mechanisms. Examining such aquatic organisms under controlled laboratory conditions is a perhaps an obvious step; aside from the relative ease of probing photosynthetic machinery of single celled compared to multi-cellular organisms, microalgae and cyanobacteria dominate photosynthetic activity of much of the Earth's aquatic realm. However, in contrast to working on terrestrial plants, extending such laboratory-based observations to the 'real world' has proven to be the greatest challenge for aquatic scientists and one that has been largely led by technology and engineering. In overcoming the technical challenges, exciting and important discoveries, such as the confirmation of iron limitation of ocean productivity (Behrenfeld et al. 1996) and the discovery of aerobic anoxygenic bacteria (Kolber et al. 2001), have followed.

The earliest application of Chlorophyll *a* fluorescence to aquatic system research (*in situ*) is well recognized as from Carl Lorenzen (1966) who first pumped seawater through a shipboard fluorometer. Such a convenient, rapid approach was quickly adopted by both



**Fig. 1** Fluorescence in action: (a–c) Chloroplast fluorescence in the dinoflagellate *Ceratium* sp. (Photo: L. Novoveska); (d) False colour high resolution fluorescence image of cells of the diatom *Nitzschia dubia*. Fluorescence emanating from the chloroplasts becomes restricted to the

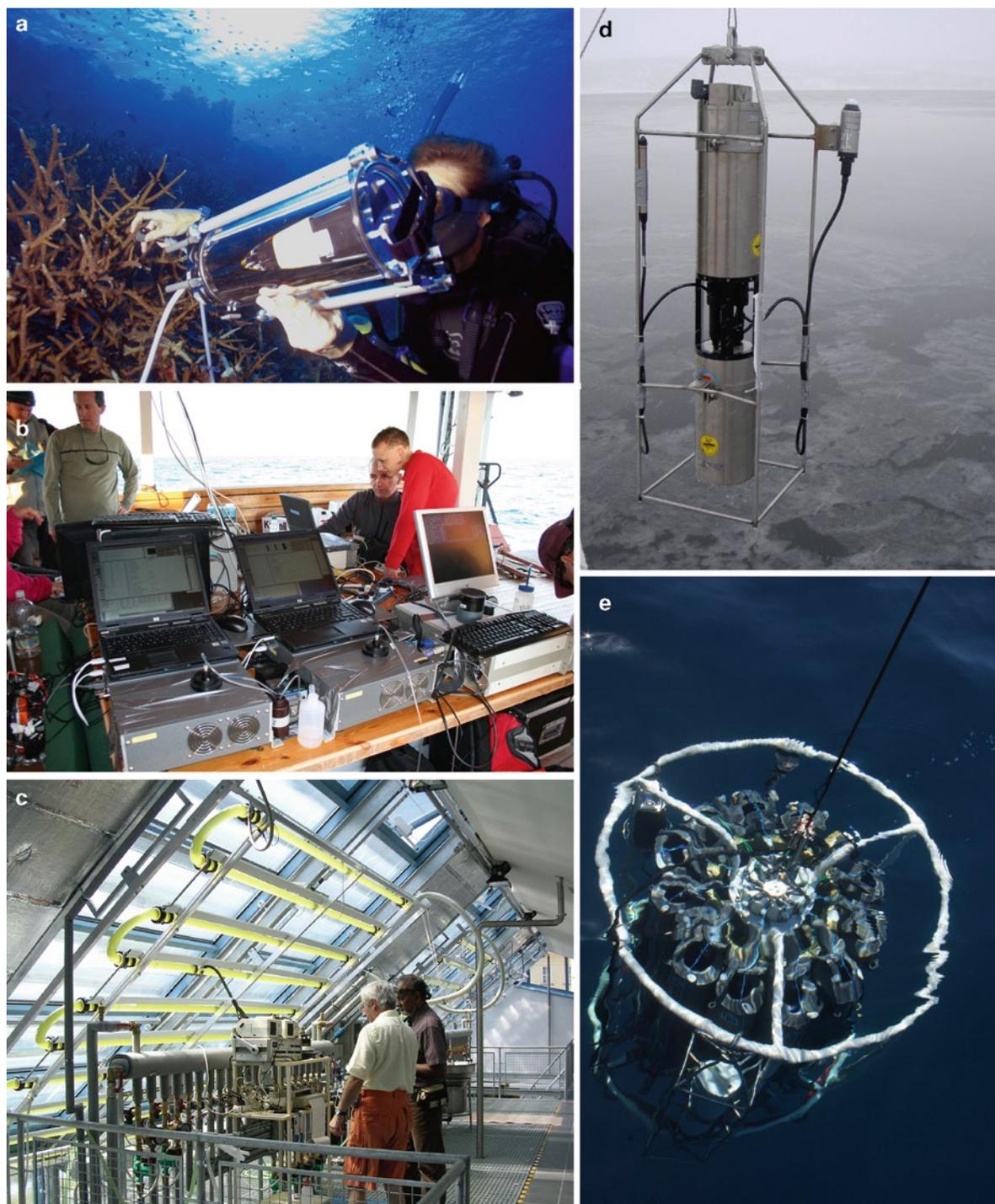
area of the pyrenoid as light intensity increases (Photo: R. Perkins); (e) Chloroplast fluorescence in the centric diatom *Coscinodiscus* sp. (Photo: L. Novoveska); (f) Delayed fluorescence in the colonial diatom *Rhizosolenia* (Photo: M. Berden-Zrimek)

oceanographic and limnological communities; this, not surprisingly quickly led to a wealth of highly novel studies linking physical and biological processes, in particular, the distribution of phytoplankton with ocean turbulence (Platt 1972) and the discovery of the deep chlorophyll maximum of stratified waters (Cullen and Eppley 1981). The major challenge for aquatic scientists to evolve to *in situ* studies was ‘simply’ to package complex and innovative technology into a system that could withstand the constraints of working in water, especially in marine environments where salts and pressure rapidly build. It wasn’t until the 1970s that technology caught up with concept and the first profilable *in situ* fluorometers were truly developed (see Falkowski and Kolber 1995). Ever since, such fluorometers have become smaller and better integrated to sensor arrays, and essentially a routine yet fundamental tool for aquatic scientists. However, despite their rapid adoption by the aquatic community, these fluorometers were still generally restricted to assaying a single chlorophyll fluorescence yield, which was set according to the excitation intensity of the instrument in question, and thus could only ever provide some approximate measure of Chlorophyll *a* biomass *in situ*. An important step to aquatic research was thus in producing fluorometers that induced a variable Chlorophyll *a* excitation (and hence fluorescence emission) protocol (Fig. 2).

Numerous laboratory studies by the 1970s and early 1980s had already demonstrated important concepts linking variable Chlorophyll *a* fluorescence to photosynthetic physiology in aquatic algae (e.g. Mauzerall 1972; Ley and Mauzerall 1982; but note an ISI Web of Science search yields >125 publications in the 1970s alone!), however, modification of these techniques to *in situ* aquatic studies to thus add a physiological component (the variable fluorescence ‘transient’) to measures of fluorescence yield (biomass) was not straightforward. Here, the development of actinic light sources that could deliver the intensity and/or frequency of excitation required to induce variable fluorescence remained an even greater technological challenge to the pre-existing in water operational constraints. Solving this problem essentially had to occur twice since variable fluorescence techniques have already evolved into two parallel but distinct paths (Chapter 3 by Huot and Babin, this volume): Pulse Amplitude Modulation (PAM; Schreiber et al. 1986), where fluorescence is induced by a weak modulated

light source evaluated independently from a relatively long yet moderate intensity light pulse; and Pump and Probe (PP; Mauzerall 1972; Falkowski et al. 1986; but see also Kolber and Falkowski 1993), where variable fluorescence is measured by a weak ‘probe’ actinic flash before and after a saturating ‘pump’ flash. PP later evolved into Fast Repetition Rate (FRR; Kolber et al. 1998), where a complex fluorescence transient could be induced by initially delivering a series of sub-saturating high intensity flashlets followed by a series of more widely spaced ‘probing’ flashlets that examined the subsequent fluorescence decay. All subsequent variable fluorometers have essentially followed one (or a combination) of these paths. Importantly, this new generation of fluorometers not only opened new possibilities for examining photoautotrophic physiology but also a potential revolution in how aquatic scientist would determine primary productivity (Kolber and Falkowski 1993, Kromkamp and Forster 2003; Suggett et al., Chapter 6, this volume).

Evolution of both PAM and FRR (PP) was originally driven from the pioneering laboratory work using microalgae; as such, the first *in situ* variable fluorometers in the 1980s and 1990s were essentially restricted to working on natural phytoplankton suspensions in lakes and oceans. Technical improvements in overall signal resolution since then has enabled researchers to investigate ever more oligotrophic waters of oceans and nutrient impoverished lakes. However, subtle technological changes in sensitivity and the optical configurations within a few years of PAM and FRR fluorometer introduction enabled the photophysiology of benthic autotrophs (corals, microphytobenthic mats, seagrasses and macroalgae) to be examined (see Chapter 9 by Enríquez and Borowitzka, Chapter 10 by Warner et al., and Chapter 11 by Shelly et al., this volume). More recent additional but relatively small optical alterations to the PAM and FRR ‘model’ to examine far red fluorescence (>800 nm) has introduced more new research opportunities, e.g. bacteriochlorophyll *a* (Kolber et al. 2001) and Photosystem I (PSI) variable fluorescence (Dual PAM, e.g. see Sukenik et al. 2009). Modification of the spectral quality of fluorescence excitation and emission detection has also added the potential for variable fluorometers to taxonomically discriminate bulk fluorescence properties (Schreiber 1998; Beutler et al. 2002; Chapter 7 by MacIntyre et al., this volume). All of these advances have unquestionably facilitated the explosion of interest in the use



**Fig. 2** Fluorometers in action: (a) Diving PAM used to measure fluorescence signal in corals, Wakatobi Marine National Park, Indonesia (Photo: D. Smith); (b) Fluorometer comparisons at GAP Workshop, Eilat, Israel, 2008 (Photo: D. Suggett); (c) *In-situ* measurement of fluorescence quenching using the fluorometer PAM 101-103 (H. Walz, Germany) in the

*Haematococcus* culture grown in solar photobioreactor at the Centre of Biological Technologies, University of South Bohemia in Nové Hradky, Czech Republic (Photo: J. Masojidek); (d) FRRF being deployed in winter, Bedford Basin, Canada (Photo: D. Suggett); (e) Fastrack II attached to a CTD frame in water column sampling in Eilat, Israel (Photo: D. Suggett)

of fluorometers for aquatic research in recent years; arguably, compared to 20 years ago, aquatic research investigations are incomplete without some form of fluorescence examination.

Variable fluorometers have clearly provided a platform for aquatic scientists wishing to conveniently assay photosynthetic physiology non-invasively and more accurately scale changes of photosynthesis to the environment. Current acceleration of environmental variability via climate change perhaps provides very real justification for further investing in tools such as fluorometers that have the capacity to link ecosystem processes with environmental regulation. Fluorescence-based technological development (including delayed fluorescence; Chapter 14 by Berden-Zrimec et al., this volume) combined with research has publically produced a tool that can potentially inform stakeholders of the photosynthetic 'viability' (or 'health') of their associated aquatic environment; certainly, a tool that is less labour intensive and costly in the long term than conventional (and destructive) assays that require water or organisms to be removed and analysed in the laboratory. Such applications to those wishing to monitor and subsequently manage ecosystem function was an obvious step in exchanging the knowledge beyond pure research but also necessary for commercial manufacturers to invest further in instrument production. Key examples to date come from the monitoring of lakes and coastal waters for (harmful) algal blooms (Cullen et al. 1997) and coral reefs for pollution and coral bleaching (Jones 1999). PAM Fluorometry has also been demonstrated in action for two BBC documentaries – by Prof Ove Hoegh-Guldberg examining coral bleaching for the BBC documentary *State of the Planet* and by Dr Rupert Perkins investigating stromatolites for *Oceans*) Furthermore, recent developments of algae as biofuels will inevitably require application of fluorometers to optimize and also continually monitor yields (Kromkamp et al. 2009; Sukenik et al. 2009) and thus further move fluorometers from a purely ecological to an industrial monitoring tool.

Despite the potential growth industry that obviously exists for chlorophyll fluorescence, it is clear that the previous growth of fluorometer technological development and the subsequent array of commercially available fluorometers have somewhat superseded our fundamental understanding of the fluorescence signals generated. It is perhaps quite ironic that technological developments have already enabled us to collect vast

fluorescence data sets, however, we are only recently arming ourselves with the key knowledge required to interpret and consequently apply these data into informed opinion. Examining the growth of citations for (variable) fluorescence-based papers over the past decade is perhaps more testament to our confidence in interpreting the data as opposed to reduced constraints in collecting the data itself. Armed with a decades worth of what is arguably 'fluorescence exploration', It is really only now that we are beginning to gain maximum benefit of using fluorescence as a tool to address fundamental research questions in the aquatic sciences.

*Why the need for AQUAFLUO?* — Rapid growth of using active fluorescence across the aquatic science disciplines has inevitably led to divergence in approach and terminology (see Chapter 1 by Cosgrove and Borowitzka, this volume, for recommended terminology). Even though many of us have been attempting to answer similar questions, this divergence has resulted in a lack of consistency required to facilitate information exchange; consequently, the field was not evolving as quickly as originally envisaged. Arguably, the aquatic sciences still communicate fluorescence-based studies in numerous dialects that are often not easily inter-comparable or reconcilable.

Using fluorescence as a non-invasive means for assaying processes, such as (harmful) bloom detection and primary productivity, is still heralded as a key breakthrough for aquatic research and not surprisingly has attracted much funding and research time investment. However, efforts to capitalise on these larger process-scale problems have somewhat overshadowed our need to understand the fundamental nuances of fluorescence measurements using different instrumentation, protocols and for the array of aquatic primary producers that exist. On many occasions, the interpretation of data sets has been confounded by what is real in nature *versus* an artifact of instrument use. Conversations amongst the aquatic sciences community over recent years have increasingly identified the need for conformity in the application and operation of (active) fluorometers, not only to standardise and reconcile existing data sets but also to ensure that fluorometry remained 'accessible' to the ever-growing new user community. Such a step is indeed critical if fluorometry is ever to evolve from a purely academic tool to an everyday, practical and informative management tool. However, despite attempts to call for a common set of approaches (the best example to date for the

aquatic sciences is Kromkamp and Forster 2003), a few researchers have adapted their own approach and/or terms to fit. Perhaps the main limitation in adapting (amongst this rapidly growing and evolving field) has been where on earth should we start?

The concept of AQUAFLUO (AQUATic FLUORescence) was introduced in 2005 following breakout discussions between several of us at a meeting on modeling algal growth in Villefranche, France. It was then apparent that the immense popularity of fluorometry for aquatic studies was not a transient phenomenon. Both researchers and industry were increasingly investing in development of new fluorometers; however, the fundamental operational and conceptual issues that were limiting how confidently trends in variable fluorescence could be scaled to biology had become a bottleneck in supporting (and ultimately rationalising the need for furthering) this industry growth. Therefore, an organizing committee was established from a cross section of the aquatic community and these concerns translated into the ethos for an international meeting to be held at Nové Hradý in the Czech Republic in 2007 (Prášil et al. 2008). Initial priority questions were identified, including:

1. Is it time to step back from trying to focus on using variable fluorescence as a substitute for conventional productivity ( $^{14}\text{C}$ ,  $^{13}\text{C}$ ,  $\text{O}_2$ ) primary productivity techniques? Should we redistribute current research efforts and instead focus on the assessment of algal physiology and the general heterogeneity of algal physiology in nature?
2. How important (for the end users) are nuances in current fluorescence induction methodologies (e.g. single or multiple turnover flashes; single band vs. multi-spectral)? Can we constructively use these differences in experimental techniques to get better insight into algal physiology?
3. Can we expect successful scaling from direct to remotely sensed (LIDARs and satellites) variable fluorescence?

It was immediately clear that addressing such priority questions could only be achieved by bringing together researchers from across the aquatic disciplines (microalgae, macroalgae, submerged vascular plants, corals and aerobic anoxygenic photoheterotrophs (AAPs); lakes, rivers, coasts and oceans) as well as

fluorometer manufacturers and engineers. By bringing this group together, the needs of all interested stakeholders could for the first time identify the common and complimentary needs required to move the field further forward and identify new opportunities; as well, to understand the individual needs of the various aquatic disciplines and develop specific approaches that may help to bridge gaps in consistency in the fluorescence yields that were being measured. A series of talks and workshops at the AQUAFLUO 2007 meeting led by leaders in key aquatic disciplines successfully laid the foundations in exploring these questions. Targeted research activities conducted since the meeting are already beginning to demonstrate that the original ethos of AQUAFLUO and the outcomes of the 2007 meeting are being adopted. However, given the continually evolving nature of using chlorophyll fluorescence in both physiological concept and technological approach, AQUAFLUO is seen as the beginning of a long-term relationship across the aquatic community and an idea with underlying goals and priority questions that will inevitably need to be continually revisited.

The chapters of this book communicate key components of the talks and workshops conducted during the AQUAFLUO 2007. Primarily, they address what measurements can be made and how; the common and aquatic discipline specific pitfalls that may be encountered in both performing measurements and interpreting the fluorescence yields themselves. In essence the book provides a guide to making Chlorophyll *a* fluorescence measurements in the various aquatic sciences and is certainly aimed at experienced and new users alike. *This book is certainly not intended to be a comprehensive review on the subject of Chlorophyll a fluorescence*; several key aspects are not considered in depth, notably remote sensing of variable fluorescence and examination of AAPs.

Each chapter summarises the progress specific to that discipline, the journey that Chlorophyll *a* fluorescence has taken in both approach and application; consequently, the scientific information that can be obtained. Importantly, these chapters also mark the output of that meeting: The first targeted effort to amalgamate the concerted efforts of leaders of the various fields/aquatic disciplines to identify what the next major conceptual (physiological, ecological, biogeochemical) questions that fluorescence measurements can contribute? What are the technological challenges

we need to overcome to realize these contributions? The following chapters highlight fundamental areas for research focusing (a) on a range of organisms from corals and macroalgae to microphytobenthos, and (b) scales, from photosynthetic physiology from the cellular level to mass culture. Importantly, these chapters aim to not only target experienced users but also present best practice, which represents optimisation through past (and often frustrating) research, to those new to the field. Of course, since AQUAFUO 2007 and preparation of this book, our understanding of active fluorescence will have inevitably evolved even further.

Finally, we would like to thank the contributors and the many reviewers of the chapters for their valuable input.

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<http://www.springer.com/978-90-481-9267-0>

Chlorophyll a Fluorescence in Aquatic Sciences:  
Methods and Applications

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2010, XVIII, 326 p., Hardcover

ISBN: 978-90-481-9267-0