

**International Society for**  
**Applied Phycology**  
**NEWSLETTER**



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## Message from the President: Dr. Céline Rebours

Dear ISAP Members,

I am happy to introduce this newsletter as the first of my term as president of ISAP and the first edition that is recorded 1-2018.

I would like to thank our outgoing President, **Roberto de Philippis**, for all the actions completed during the triennium 2014-2020 for increasing the influence of ISAP and for his excellent leadership of our Society. I would also like to thank the Members of the outgoing Executive Committee for their contribution to ISAP activities. I am very grateful to Amha Belay, Editor of the Newsletter, for the efforts that he made for the preparation of the issues published since 2013. As **Amha Belay** will now retire, I am delighted to welcome **Sasi Nayar** as our new editor as well as the new members of the ISAP Newsletter Working Group of the Executive Committee for bringing us this new format of the newsletter which has now been credited an ISSN by the National Library of Australia. We are fortunate to have articles from **Amha Belay** and **John Runcie**, without doubt, two important leaders in algal applications research and industry over many years. I anticipate that their articles will make this newsletter a valuable reference for many years to come, as we move forward in a century in which the value of algae as a renewable resource for a wide range of applications will be increasingly realized.

In June 2017, we organized one of the major events for our Society: The 6<sup>th</sup> ISAP Congress took place in Nantes (France), gathering over 560 phycologists and offering a platform to all the stakeholders from the academia to the industry to present, meet and discuss their recent achievements in the field and future collaborations. You will find some information on the Congress on ISAP website and pictures under a [free Flickr webpage](#). I would like also to take the occasion of this message for thanking all the Members of the international Advisory Committee for the selection of the excellent scientific presentations as well as the Members of the Local Organizing Committee, with a particular mention to the Chairs, Valeria Montalescot, Pascal Jaouen and Jean Paul Cadoret, for the excellent work they did in organizing such a successful congress.

During the Congress, the ISAP General Assembly took place (you will find on ISAP website the report of the Assembly and the balance for the triennium 2014-2017), followed by the election of the new Executive Committee. The EC 2017-2020 is composed by the following Members:

Committee Member	Country
<b>Sasi Nayar</b>	Australia
<b>Leila Hayashi</b>	Brazil
<b>Lirong Song</b>	China
<b>Alexandra Busnel</b>	France
<b>Remi Nghiem-Xuan</b>	France
<b>Jean François-Sassi</b>	France
<b>Ioannis Tzovenis</b>	Greece
<b>Fiona Moejes</b>	Ireland
<b>Sammy Boussiba</b>	Israel
<b>Liliana Rodolfi</b>	Italy
<b>Eon-Seon Jin</b>	Korea
<b>Antoinette Kazbar</b>	Lebanon
<b>Rupert Craggs</b>	New Zealand
<b>Vitor Verdelho</b>	Portugal
<b>Leila Ktari</b>	Tunisie
<b>Claire Gachon</b>	UK
<b>Job Schipper</b>	The Netherlands

In addition to the elected Members, the EC also includes the President, **Céline Rebours**, the Outgoing President, **Roberto de Philippis**, the President Elect **Qiang Hu** (elected by the EC Members in September 2017). The President also appointed as Secretary/Treasurer for the triennium 2017-2020, **Valeria Montalescot**, who accepted this role.

One of the first activities achieved by the new EC has been to evaluate the proposal for the next ISAP Congress. It is my pleasure to announce that 7<sup>th</sup> ISAP Congress will be held in Japan in 2020. Please follow the upcoming information through our [ISAP webpage](#).

One of the objectives of ISAP is also to support the organization of workshops and training programs in algal biotechnology for its members. The call for proposal for Training workshops is now opened with deadline March 31<sup>st</sup>, 2018. For further information, please consult our [webpage](#).

We are also wishing to increase the impact of our Society and we are organizing a competition to modernize our logo. We would like to invite all the Members to participate. You will find more information about the logo competition on our webpage or by clicking [here](#).

Furthermore, all Members of ISAP have free access to the electronic version of the Journal of Applied Phycology. You can register yourself to ISAP website and you will find the link to the Journal. I would like to thank Springer very much for this recognition to our Society and for this useful service given to ISAP Members. You are welcome to advertise this opportunity among your colleagues. Payment of ISAP dues by credit cards Membership to ISAP is now available online and with credit card payment. You can become a Member by clicking [here](#).

Finally, all ISAP Members can actively participate in the activities of the Society. We would appreciate your ideas, feedback on ISAP, news and announcements of interest for ISAP Members. We would also be delighted to receive articles that could be published in our Newsletter. For the matters, please contact either the Editor of the Newsletter Sasi Nayar, myself or the ISAP Secretary/Treasurer Valeria Montalescot.

I wish to all the ISAP Members a successful new year in their researches and/or business in applied phycology.

With my warm regards,

Céline Rebours.

President of the International Society for Applied Phycology

## Message from the Editor – Sasi Nayar

As the first issue of the newsletter for 2018, I take this opportunity in wishing you all a very happy, prosperous and productive new year. This is also my first issue as the Editor of the newsletter. I take this opportunity in thanking my predecessor Amha and his Newsletter Team in mentoring me. Of course, one of the preconditions was that Amha would contribute the first article to this edition and he did! You will also notice in this newsletter that we now have an ISSN number issued to us by the National Library of Australia. Going forward, we intend to publish two issues of the newsletter per year.

One of the biggest challenges that the Society faces with the newsletter, is soliciting articles. This is where we need your help. We focus on two articles per issue – one pertaining to microalgae and the other on macroalgae. Besides your contribution, you could also encourage your young graduate students to contribute, making the ISAP newsletter a good way to commence their publication career before they target peer reviewed journals.

We have two very interesting articles in this issue. One by Professor Amha Belay, the immediate Past Editor of our newsletter. Amha is an authority on *Spirulina* (*Arthrospira*) having dedicated many decades of his life researching and later developing the industry on cultivation of *Spirulina* with his efforts at Earthrise. This article focusses on the history of the cultivation of *Arthrospira* sp. With an interesting overview of the more recent efforts made by companies such as Earthrise Nutritionals, Cyanotech and Parry Nutraceuticals.

The second article by Dr. John Runcie relates to the fundamentals of select photophysiological parameters used in applied phycology. John has considerable experience with Pulse Modulated Amplitude Fluorometers (PAM) having undertaken field work in most parts of the world including Antarctica. Most of his work has been conducted with the fluorometers that he designed. PAM fluorometers are powerful tools in understanding physiological responses in microalgae, macroalgae and other macrophytes including seagrasses to various changes made in environmental or anthropogenic. They are gaining considerable attention in applied phycology. However, proper use of these tools and techniques mean having a good understanding of the various photosynthetic parameters. In his article, John explains the fundamentals of photophysiology giving us a good understanding of how these versatile tools can be applied to our research or in cultivation systems.

I also take this opportunity in thanking the current Newsletter Team comprising Remi Nghiem-Xuan, Alexandra Busnel, Sammy Boussiba, Céline Rebours, Qiang Hu and Roberto De Philippis. With 'young blood' as part of this committee, the future of this newsletter and the Society looks bright with a good succession plan in place.

Happy Reading!

Sasi Nayar

Editor of the ISAP Newsletter and Social media administrator

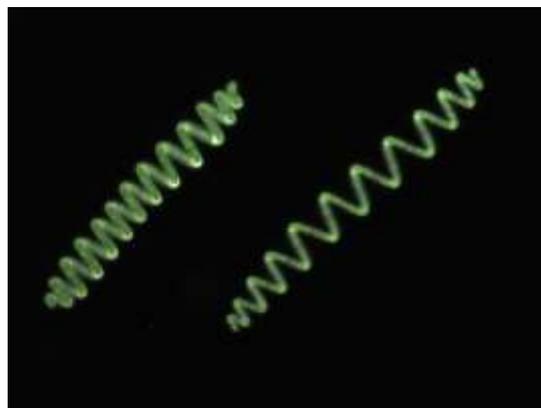
## A Short History of *Spirulina*

**AMHA BELAY, PH. D.**

Senior Vice President & Chief Technology Officer, Earthrise Nutritionals, CA, USA

### Introduction

The history of *Spirulina* is probably as old as the evolution of photosynthesis, some 3.5 billion years ago when blue-green algae or cyanobacteria, as they are now called, may have evolved. Of the thousands of species of these oxygenic prokaryotes only *Arthrospira platensis* and *A. maxima* (possibly the same species), with the common name of *Spirulina*, are cultivated for food, with two or three other cyanobacteria species harvested from the wild for human consumption.



*Arthrospira (Spirulina) platensis*

It is not known when man began to eat microalgae. The recorded history on use of *Spirulina* for food is relatively recent: pre-Spanish Conquest inhabitants of Tenochtitlan (present day Mexico) were reported to eat considerable amounts of a food called ‘*Tecuitlatl*’, now believed to be *Spirulina*, harvested from Lake Texcoco, in which the city was located. Here are a few quotations by historians and travelers that support this:

- Bernal Diaz del Castillo, the first chronicler of the Spanish conquest of Mexico writes describing one of the many strange products that were sold in the market in 1521:  
... and others who sell small cakes made from a sort of ooze which they get out of the great lake, which curdles, and from which they make a bread having a flavor something like cheese”
- Glavigero, a 18<sup>th</sup> century historian writes: “Not contented with feeding on living things, they ate also a certain muddy substance that floats on the water of the lake, which they dried in the sun and preserved, to make use of it as cheese, which it resembled in flavor and taste. ...”
- Sahgun refers to the “clear blue color” of ‘*tecuitlatl*’. This identified it almost certainly as a member of the blue-green algae or cyanobacteria.

Indeed, the first commercial production of *Spirulina* began in the 1970s by harvesting the natural bloom of *Spirulina* from Lake Texcoco, see picture below.

More than 400 years later, in 1964, a Belgian botanist, J. Leonard, taking part in the Belgian Trans-Saharan Expedition, saw dried biscuits “blue-green color” in many village markets of the region of Kanem, in what is now the Republic of Chad in Africa where it is still used as a protein source. The biscuits called ‘*dihe*’ came from several alkaline ponds surrounding Lake Chad. Indeed, *Spirulina* as a

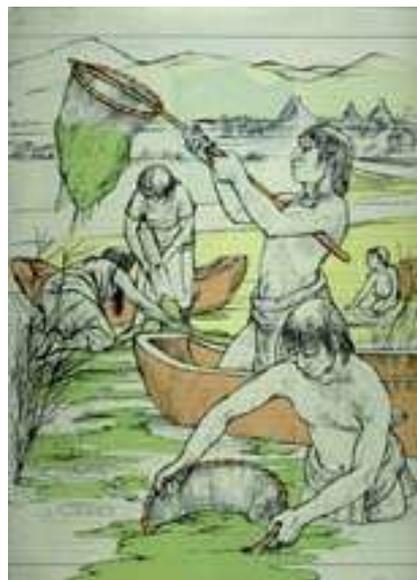
food source could have come to the attention of science even earlier if the first report of the use of “dihe” around Lake Chad, by the French phycologist Dangeard in the 1940s, had not gone unnoticed for 25 years.

Thus, two populations, the Aztecs of Mexico and the Kanembus of Chad, separated by over 10,000 kilometers, had independently discovered the food and nutritional value of *Spirulina*.

The first discussion in the use of *Spirulina* for food was made at the International Conference on Applied Microbiology held in Addis Ababa, Ethiopia (November 10, 1967) where many papers related to the subject were presented including some from Ethiopia. The chairman of the conference said at the time: “*Because of its high protein content Spirulina must be considered as a future food source*”



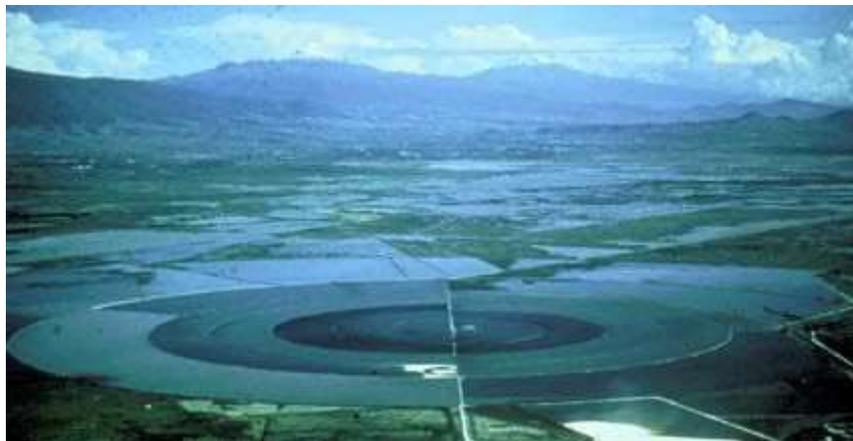
**Collecting *Spirulina* from lakes around Lake Chad. “Dihe” sold in the market.**



**Aztecs harvesting *Spirulina* from lakes in the Valley of Mexico**

## Commercial Production

The first commercial production of *Spirulina* started at Sosa Texcoco in Mexico. Sosa Texcoco was a company that produced sodium carbonate and calcium chloride from a large, several thousand hectares, spiral-shaped solar evaporator ('El Caracol', or the snail) built in the 1940s. The production of *Spirulina* in Sosa Texcoco was a result of the recognition that the nuisance alga clogging the solar evaporators mining operation was the same species as *Spirulina* used for food by the Kanembus. Genevieve Clement of the Institute Francais du Petrole, made this connection while attending an international petroleum congress in Mexico City in the late 1960s. She was engaged at the time in research on systems for the growth of *Spirulina*, following its discovery as a food source, and there met Mr. Hubert Durand-Chastel, the managing director of Sosa Texcoco. Mr. Durand-Chastel recognized the opportunity and by the early 1970s diked off a 20-hectare area, built a harvesting and drying system, and started the first commercial *Spirulina* production facility in the world, soon producing an estimated 300 tons of *Spirulina* powder a year. During the 1970s most of the production was sold to Japan, where it was used to partially substitute for *Chlorella*, which had started to be produced since the 1960s.

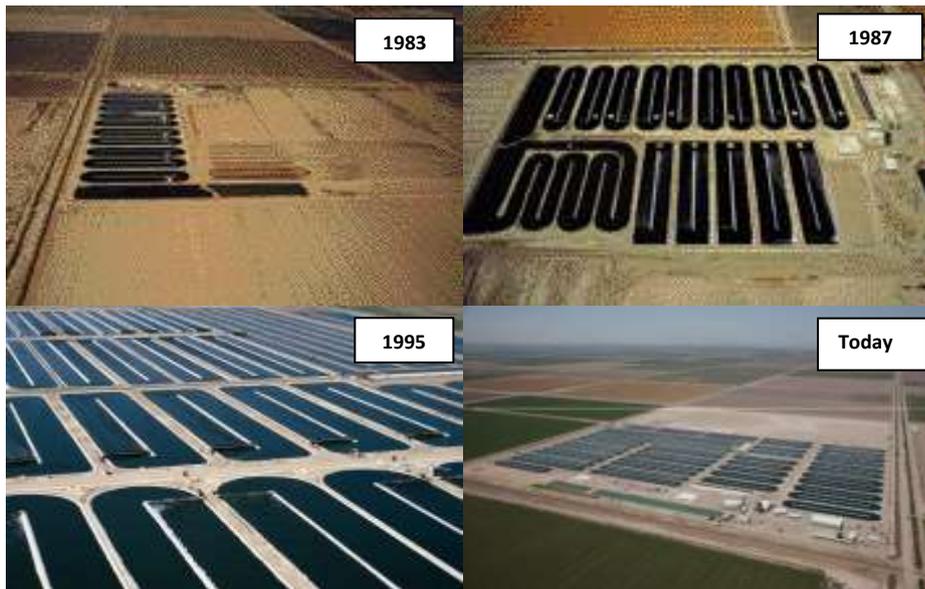


**Sosa Texcoco in Mexico**

The success of the Mexican plant, encouraged the Japanese company, Dainippon Ink and Chemicals (now DIC Corporation) to support research into the potential commercial production of *Spirulina*. They provided funding to Hiroshi Nakamura, at Tokyo University, who had seen a short report on the meeting in Ethiopia under the title "*Micro-organisms à la Carte*" and made him a convert to the cause of feeding the hungry world with *Spirulina*. Indeed, it is Dr. Nakamura's collection of *Spirulina* from Ethiopia in 1969 that was used for most of the initial research and production of *Spirulina*. He describes what he saw during the collection of *Spirulina* from Lake Arenguade, Ethiopia: "...in the local language "*Arenguade*" means green lake and indeed the surface of the water is deep blue-green almost as if the entire surface were covered with a coat of blue-green paint".

DIC's support of the pioneering work of Prof. Nakamura, led to the first commercial production of *Spirulina* ('Siam Algae) in Bangkok, Thailand, using paddle wheel mixed, man-made ponds, the main technology still used today for *Spirulina* production world-wide. A mention must also be made of Dr. Christopher Hills, an early supporter of Prof. Nakamura and enthusiasts of *Spirulina*, who introduced *Spirulina* as a 'health food' into the USA in the late 1970s.

At about the same time, Mr. Larry Switzer, an American entrepreneur, founded Proteus Corporation to produce *Spirulina*. By 1980 Proteus initiated in the Imperial Valley of Southern California, the third commercial *Spirulina* production plant, Earthrise Farms (now Earthrise Nutritionals LLC). Among the initial participants in this enterprise were the late Prof. William Oswald, at the University of California Berkeley, one of his students, Dr. Joseph C. Weissman (now with Exxon Mobil), and Mr. Robert Henrikson (now operating a *Spirulina* 'micro-farm' in N. California).



**Historical progress of Earthrise in California**

Earthrise Farms soon joined with DIC a joint venture, to expand its commercial production plant of *Spirulina* in the Imperial Valley of southern California, with DIC taking over the operation by the mid 1980s. DIC now operates two *Spirulina* production facilities, Earthrise Nutritionals LLC in the USA and Hainan DIC Marketing (HDM) in China. (Siam Algae was closed about 20 years ago). The pioneers of DIC *Spirulina* business were Hidenori Shimamatsu and Yoshimichi Ota.



**DIC group of companies (Clockwise: Siam Algae Company in Thailand, Earthrise Nutritionals in USA and Hainan DIC Marketing in PR China)**

The third major producer of *Spirulina* to initiate commercial production, on the island of Hawaii, was Cyanotech Corporation, founded by Dr. Gerry Cysewski about 1981, who is still leading the company. The vision of *Spirulina* as a potential solution to the food problems of poor people around the world attracted much attention, in particular from the early studies using Sosa Texcoco *Spirulina* in FAO funded studies on malnutrition. In India a consolidated effort was made to study the various applications of algae.



### **Cyanotech Corporation in Hawaii**

To this effect the All-India Coordinated Project on Algae (AICPA) was implemented in 1976 as a multi-institutional program. Besides AICPA several private and regional programs were also begun notable among these being the Central Food Technological Research Institute in Mysore, India and Murugappa Chettiar Research Center (MCRC) in Madras (now Chennai). The pioneers in India of *Spirulina* production to feed the malnourished were L.V. Venkataraman, C.V. Seshadri, and Jeeji Bai. Another promoter of the vision of combating protein malnutrition with *Spirulina* was Mr. Ripley Fox, in France, who initiated several village level projects in Africa and India.



### **Parry Nutraceuticals in India**

In 1995 the Sosa Texococo plant in Mexico, at the time the world's largest producer (with about 300 tons of powder produced annually, was shut down, leading DIC to promptly expand production at Earthrise Farms and build a new plant in Hainan, China, and Cyanotech to do likewise in Hawaii. In India, the Murugappa family, who had supported the Murugappa Chettiar Center, established the first Indian *Spirulina* plant, Parry Nutraceuticals, Ltd, in the State of Tamil Nadu (south of Chennai). Producers started springing up also in China, already since the early 1990s, thanks to the pioneering

efforts of Prof. Hong-Jun Hu considered as the “Father of *Spirulina*” in China. By the late 1990s *Spirulina* production already exceeded the level before the closure of the Sosa Texcoco plant, and production has greatly increased since, particularly in China with an estimated 80% of world production, reaching about 8,000 tons of dry powder in 2014, from 65 registered *Spirulina* producers.



**A *Spirulina* facility in Inner Mongolia, China**

Other than China, USA and India, the major producers, there is active *Spirulina* production now ongoing world-wide, albeit in mostly small scales. Noteworthy is the harvest of *Spirulina* directly from lakes in Myanmar since the late 90's, pioneered by Min Thein, with assistance from Dr. Otto Pulz in Germany. But perhaps the most noteworthy development is the growth of *Spirulina* microfarms all over the world. In France over 100 microfarms, typically less than 0.1 hectare in size, with a few paddle wheel ponds, are currently operating and selling air dried *Spirulina* to local consumers. Many similar microfarms are also operating in Africa. Microfarms now are also appearing in many other countries.

This short history of commercial *Spirulina* production has neglected the extensive basic and applied research carried out for the past fifty years on *Spirulina* physiology, biochemistry, and genetics. Two centers of research deserve special mention, in Israel, led by Amos Richmond, Avigad Vonshak and Sammy Boussiba, and in Italy, where Prof. R. Materasi and Mario Tredici, both groups carrying out pioneering work on *Spirulina* mass culture since the 1980s. This short list of course neglects many others who contributed to this field. Perhaps a future more complete and expanded review will be able to mention many others.

### **Current State and Future Prospects of *Spirulina***

The most important aspect of *Spirulina* has not yet been mentioned: its nutritional importance and health benefits. Although it has not yet fulfilled its promise of ending protein malnutrition around the world, it certainly is contributing to the health and nutrition of its many consumers, estimated at ten million world-wide. The benefits of *Spirulina* in supporting nutrition and health are supported by many hundreds of studies over the years. The increasing focus on non-animal proteins is increasing the interest in *Spirulina* as a human food, in particular as a non-allergenic plant protein. Most recently, the use of a safe blue food coloring, phycocyanin, extracted from

*Spirulina*, was approved by regulatory agencies in the USA and Europe, and is sold under its brand name Linablue® by DIC/Earthrise, and to some extent by others. What will the future bring? Clearly, lower cost of production processes would greatly increase the markets for *Spirulina*. Larger-scale production, higher productivities and cheaper cultivation medium (for example growing *Spirulina* on seawater) would all help to reduce costs. Much research is still required to achieve the goal of the early *Spirulina* pioneers, to feed the world with this amazing, highly nutritional and health promoting food source.

#### About the author



Until recently, Dr. Amha Belay was Sr. Vice President and CTO of Earthrise Nutritionals, a company that has been producing *Spirulina* for over 30 years. He oversaw all scientific, technical and regulatory aspects of *Spirulina* production and external research collaborations. Prior to Earthrise, he was Associate Professor at Addis Ababa University, Ethiopia and a Senior Fulbright Fellow at UC Santa Barbara to mention some.

Dr. Belay is the Director of the Algae Biomass Organization and a technical advisor of several organizations. He is a member of several scientific associations and is a recipient of the “Distinguished Applied Phycologist Award” from the International Society for Applied Phycology.

He is currently a Visiting Scholar at UC San Diego and works as an independent consultant in the area microalgae biomass production, product development, quality assurance and regulatory affairs.

## Selected photophysiological parameters in applied phycology

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### Introduction

Chlorophyll fluorescence techniques are widely used in photosynthesis research. In particular, modulated chlorophyll fluorescence measurements (where the excitation light is modulated at a very high frequency) offer the advantages of effective use in the presence of sunlight, and the ability to provide information describing light utilisation efficiency. There is a multitude of publications describing how the physiology of photosynthesis and other biological processes of phototrophs can be investigated using modulated chlorophyll fluorescence techniques (*e.g.* Suggett et al. 2011). In this article, I aim to discuss a few commonly used photophysiological parameters with a focus on how these parameters can be used in applied phycology. While I refer to algae from here onwards, most of the principles can equally well be applied to plants.

### Quantum yield: $\Phi_{II}$ and Fv/Fm

One of the most fundamental measurements obtained from modulated chlorophyll fluorometers (including PAM, OJIP and FRR fluorometers), is the quantum yield of photochemistry of PSII. This is widely termed  $\Phi_{II}$  when measured in sunlight and Fv/Fm when measured on dark-acclimated samples. This parameter aims to describe the proportion of light received that is converted to electrons and directed into PSII. As it's common for biological measurements, there is a multitude of assumptions and caveats that dilute the purity of this description, but generally this is accepted to be a true description of this parameter.



**Shutter Fluorometer measuring *Posidonia oceanica* in the Mediterranean.**

The advantage of this parameter is that it provides information describing an essential component of the most fundamental physiological process of any photosynthetic organisms, that is the conversion of energy from photons to energy in chemical bonds within the organism. The efficiency of this energy conversion process is a direct reflection on the capacity of the alga to do biological work, including growth, reproduction, pigments production and secondary metabolites production.

However, the quantum yield of photochemistry in PSII of a dark-acclimated alga ( $F_v/F_m$ ) can be very different from that of the same alga after exposure to light ( $\Phi_{II}$ ). If we remove that light source and place the alga back in the dark, will  $\Phi_{II}$  revert back to its previous value of  $F_v/F_m$ , and how fast?

In most circumstances,  $F_v/F_m$  will eventually be re-attained (if light exposure was extreme,  $F_v/F_m$  may not be attained back and the alga has suffered irreparable damage). In most situations, the rate of recovery is a function of a multitude of factors including the intensity of light the sample have been exposed, the duration of this exposure, and the capacity of the alga to tolerate exposure to high light. For example, light exposure may have caused  $F_v/F_m$  to drop by half (e.g.  $F_v/F_m=0.70$ ,  $\Phi_{II} = 0.35$ ) but as there is no long-term damage, recovery back to  $F_v/F_m$  may only take from minutes to less than an hour. In contrast, the same decline in  $F_v/F_m$  may have been attributable to longer term damage and the recovery phase could take several hours. These two situations describe one alga which is able to do biological work, and another which is much less capable do do it, as it devotes more resources to recovery. There is no way of distinguishing these two possibilities just by measuring  $\Phi_{II}$ . So, if  $\Phi_{II}$  has to be most useful, it needs to be taken in context.

### **Diel measurements**

Perhaps the simplest approach to a meaningful interpretation of  $\Phi_{II}$  is repeated measurements over time. Concurrent measurements of incident light will help in interpretation, where a rapid decline in  $\Phi_{II}$  can be linked with intense sunlight (or not). For macroalgae, regular measurements of the same sample will allow one to compare values of  $\Phi_{II}$  with  $F_v/F_m$  (usually measured before dawn). One would expect a daily (diel) change in  $\Phi_{II}$  with the lowest value coincident with the most intense sunlight. With all other environmental parameters being the same, one could then compare the diel fluctuation in  $\Phi_{II}$  between algae exposed to different treatments (e.g. fertilizers, micro-nutrients, salinity, temperature) to determine which treatment caused the greatest decline in  $\Phi_{II}$ . A similar approach would work with microalgae and the FRR fluorometry approach, where the response of a subset of the entire algal population is assumed to be representative of the physiological state of bulk population. However, we can go one step further than just comparing diel patterns in  $\Phi_{II}$ . We can calculate the rate of electron transport through PSII.

### **Electron transport rate**

Photosynthetic rates have long been used to characterise the photosynthetic capacity of plants and algae. Oxygen consumption (respiration) and production (photosynthesis), and radioactively labelled carbon uptake (photosynthesis) provide robust estimates of the rates of photosynthesis and respiration. However, these techniques are difficult to properly execute and are time consuming, so not necessarily the technique of choice for the impatient phycologist. Electron transport rate (ETR) is another way of describing photosynthetic rate (although it is fundamentally different from oxygen- and labelled carbon-derived photosynthetic rates) and can be determined from just four values including  $\Phi_{II}$ .

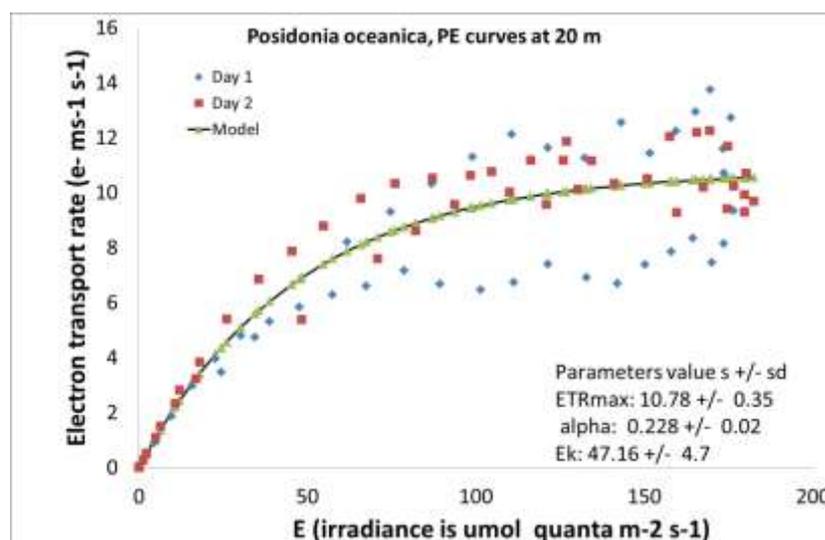
Recall that  $\Phi_{II}$  describes the quantum yield of photochemistry of PSII, where the term quantum yield defines the proportion of quanta used in a process as a proportion of the quanta received. If we measure the number of quanta received with a light sensor (in units of photosynthetically active radiation, or PAR (400-700 nm), and we also measure  $\Phi_{II}$ , then we can easily determine the number of electrons derived from the light received that could theoretically be directed into PSII. For example, if  $\Phi_{II}$  was 0.50 and PAR was measured to be  $400 \mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , then we would calculate  $200 \mu\text{mol electrons m}^{-2} \text{ s}^{-1}$  to be generated and directed into PSII.

Of course, the situation is not that simple. Now, there are two other major aspects of the photosystem we must account for. Firstly, not all the light reaching the alga is necessarily absorbed by chlorophyll. Some of this light is absorbed by non-photosynthetic structures. So, we need to measure “absorptance”, that is the proportion of light received that enters the photosystem. This value varies widely depending on species-specific attributes and sometimes physiological variable, such as cytoplasmic streaming where chloroplasts may clump in various parts of the organism. Nevertheless, many researchers adopt an average value of 0.84 as has been reported in the literature. Measurements of absorptance are easier now that miniaturised devices are now available such as small integrating spheres and portable absorptance meters.

Secondly, of the light that does reach the alga and is absorbed by the photosynthetic pigments, what proportion of this light is absorbed by PSII, and what proportion is absorbed by PSI? As the typical modulated fluorometer measures the photochemical efficiency of PSII, our final estimate of electrons transported into PSII should necessarily account for those entering PSII only. Again, many researchers use a commonly assumed value of 0.5, where half the photons reaching an alga are absorbed by PSII and the other half are absorbed by PSI. This can also be measured using a variety of optical and biochemical techniques (*e.g.* Sharon *et al.* 2011).

Our estimate of ETR is therefore the product of  $\Phi_{II}$ , PAR, absorptance and PSII/(PSII+PSI). This relation was derived by Genty in the late 1980s (Genty *et al.* 1989) and is widely used. A simpler approximation is relative ETR, calculated as the product of  $\Phi_{II}$  and PAR but with no values for absorptance or PSII/(PSII+PSI). Values of rETR are less useful as they cannot be compared amongst algae that may have differing values of absorptance and/or PSII (PSII+PSI) but can provide an indication of differences in ETR between samples of the same species collected from a similar environment. True ETR is preferred with units in  $\mu\text{mol electrons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

How would we use ETR to tell us something about our algae production? Again, a simple way to use ETR to assess algal performance is to measure it regularly over time and examine the extent of ETR declining between treatments (assuming all else is equal). One would focus on the absolute values of ETR, the extent of decline of ETR at midday, and the rate of recovery of ETR over the course of the afternoon period.



**Diel PE curve of *Posidonia oceanica* at 20 m depth**

Another approach that can provide additional information is a plot of ETR versus PAR obtained from regular measurements over the course of the day using ambient light intensity as PAR. This takes the form of a classic light-response curve, where photosynthetic rate on the y axis increases as light intensity (on the abscissa) increases. From this curve one can obtain values for several useful parameters. Note these parameters are presented in units that can be compared against values reported in the literature.

1.  $\alpha$ , or the slope of the linear part of the curve describes the efficiency of light conversion under low light conditions. When true ETR is used, the slope is equivalent to  $\Phi_{II}$  as a measure of photon conversion efficiency in PSII as it is described in units of electrons per quanta.
2. **ETRmax** is the maximum ETR attained and represents the maximum photosynthetic rate achieved. This value is in units of  $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ .
3. **Ek** represents the irradiance where ETR would reach its maximum value if there was no inhibitory effect on ETR under higher light conditions. This value is in units of  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ .

The ETR vs PAR plot can be used to compare the capacity of different algal treatments to cope with natural growing conditions. A reduction in values of any of these three parameters could indicate an impaired capacity to cope with high light.

We have addressed the fundamental measure of photosynthetic conversion efficiency ( $\Phi_{II}$  and Fv/Fm), and many ways, the difference between these parameters can show us whether or not an alga is performing suboptimally. While this approach is useful and relatively easy to implement in an algae culturing facility, can we extract more information from the measured samples? In particular, can we obtain more information about the physiological processes that cause quantum yield to decline from Fv/Fm to some value of  $\Phi_{II}$ ?

### **Non-photochemical quenching**

An alga, or population of algal cells, fully acclimated to the dark and subjected to a PAM, OJIP or FRR measurement will provide a value of Fv/Fm. Once exposed to light, maximum fluorescence declines and the quantum yield of photochemistry in PSII declines and is now termed  $\Phi_{II}$ . The difference between Fv/Fm and  $\Phi_{II}$  can be attributed to non-photochemical quenching, and various aspects of non-photochemical quenching indicate the physiological state of the sample being measured.

#### *Induced non-photochemical quenching*

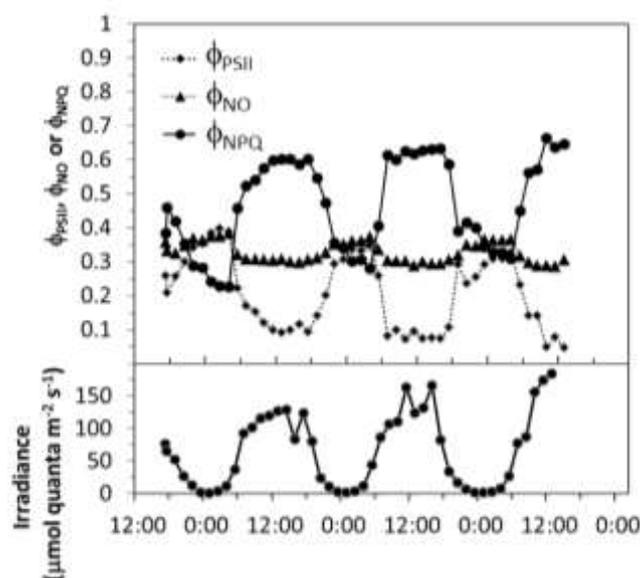
Non-photochemical quenching is induced with exposure to light, and then becomes less after the alga is placed in darkness. The rate of relaxation, or recovery, indicates the type of stress the alga has experienced, where rapid recovery indicates the activation of a photoprotective regulatory mechanism, and longer-term slow recovery indicates potential damage to the photosystem. One way to address non-photochemical quenching is to subject the sample to a light treatment, then place in the dark and measure the recovery of that sample over time. Most fluorometers provide an ability to perform this sequence of measurements automatically, where one provides either an actinic light for a predetermined interval and at a set intensity, or a light response curve which is equally well characterised. Immediately after the light treatment (either actinic light interval, or light response curve), one removes ambient light placing the sample in darkness, and subsequent measurements are made for an extended period. Typically, measurements are first made at a higher frequency in order to best characterise the recovery rates of the rapidly recovering regulatory processes. This is then followed by less frequent measurements that represent more slowly recovering processes that often represent photodamage. The two processes can then be separated out using models such as a two-compartment exponential model. The half-life of the short- and long-term recovery processes can be calculated, as can the proportional contribution to non-photochemical quenching. A detailed description can be found in Runcie and Riddle (2011) and references therein.

In an applied phycology context, the approach would be to collect samples perhaps early in the day, at noon and in the evening and conduct these experiments on collected samples. Comparison of the rates of recovery would indicate quite clearly the extent of photoinhibition that is regulatory, and that which is more likely associated with photodamage. One would want to see any photodamage to be largely restricted to midday measurements, with a minimal contribution of this component of non-photochemical quenching to be found in the evening, and preferably zero in the early morning. By changing culture conditions, one could potentially increase light, nutrients and/or temperature until a threshold was reached where the slowly recovering component began to dominate.

#### *Diel variation in non-photochemical quenching*

An alternative approach is to use a continuously monitoring fluorometer to take regular measurements throughout the day, enabling one to build up a picture of the diel fluctuation in non-photochemical quenching. To use this approach, one needs to be able to provide a far-red light to the alga, and to eliminate ambient light at various times during the measurement. This can be done manually, or with a fluorometer with an automated dark-acclimation function.

The diel technique has the distinct advantage of enabling one to distinguish both regulatory non-photochemical quenching processes (downregulation), and other non-regulated processes for each time point. As the sample is not exposed to a light treatment for any appreciable length of time one can assume some degree of independence between samples taken, that is the sample can be assumed to have completely recovered from measurements previously taken when the next measurement is to be made. The distinction between the two types of non-photochemical quenching can be calculated according to the analysis described by Kramer *et al* (2004), where  $F_o'$  is measured on a briefly darkened sample exposure to far red light. This measurement can be made any time of the day and is independent of previous light exposure and the dark acclimated state of the pre-dawn sample. In contrast, the value of calculated  $F_o'$  provided by some fluorometers and based on equations described in Klughammer and Schreiber (2008) is based on the assumption that the sample has not experienced photoinhibitory light exposure between dawn and the time of measurement. In many cases this is not true, and under these conditions one would incorrectly interpret an increase in regulatory non-photochemical quenching.



**Regulated ( $\Phi$  NPQ) and non-regulated ( $\Phi$ NO) non-photochemical quenching, and the effective quantum yield of PSII photochemistry ( $\Phi$ PSII) of an Antarctic macroalga.**

In summary, regulated non-photochemical quenching can be distinguished from non-regulated or constitutive non-photochemical quenching. Optimal algal conditions would be indicated by a high value of  $\Phi_{II}$  indicating high photosynthetic efficiency, and a high proportion of regulated non-photochemical quenching relative to non-regulated non-photochemical quenching.

### Summary and conclusions

Modulated chlorophyll fluorometers can be very useful in optimising algal culturing conditions by helping identify environmental conditions that lead to a reduction in fluorescence-based values of algal photosynthetic performance. Depending on the equipment available, or the budget at hand, one can devise relatively simple measurement protocols that may be repeated three times in a day (*e.g.* light response curves and recovery) or repeated continually over 24+ hour periods. Careful analysis of these measurements will enable the operator to identify poor, good and better conditions for growth, reproduction and/or production of pigments and secondary metabolites. Constant tweaking of the algal culturing system can then be guided with the fluorescence-based measurements of algal condition mentioned here.



***In situ* diel measurements of *Ulva* at 100m depth.**

**Shirley Island, abundant macroalgae in an ice-free exposed location.**



**The author about to start work under the sea ice**

## References

- Klughammer C and Schreiber U (2008) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method, *PAM Application Notes* 1: 27 -35.
- Kramer DM, Johnson G, Kiirats O, and Edwards GE (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. *Photosynth Res* 79: 209–218.
- Runcie JW and Riddle MJ (2011) Distinguishing downregulation from other non-photochemical quenching of an Antarctic benthic macroalga using in situ fluorometry. *European Journal of Phycology*, 46: 171–180.
- Sharon Y, Levitan O, Spungin D, Berman-Frank I and Beer S (2011) Photoacclimation of the seagrass *Halophila stipulacea* to the dim irradiance at its 48-meter depth limit. *Limnol. Oceanogr.*, 56(1), 357–362.
- Suggett DJ, Borowitzka MA and Prášil O (2010) Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications Vol 4 *Developments in Applied Phycology*, Springer Netherlands 326pp.

## About the author

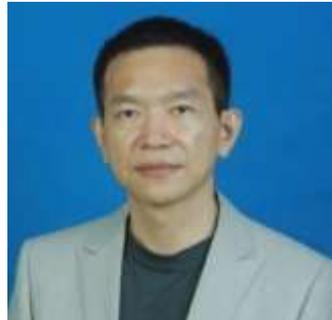


Dr John Runcie was awarded a PhD from the University of Sydney where he examined nutrient uptake and assimilation in marine macroalgae in the context of environmental pollution. Since then John has focused on the use of PAM fluorometers to address environmental issues including eutrophication using macroalgae and seagrasses as bioindicators. He is CEO of Aquation Pty Ltd ([www.aquation.com.au](http://www.aquation.com.au)), an Australian company that designs and manufactures innovative marine science instrumentation including specialised chlorophyll fluorometers. John also maintains an active research program in an adjunct capacity with the University of Sydney and the Sydney Institute of Marine Science.

## News and views

### ISAP President - Elect (2017 - 2020)

We congratulate Professor Hu Qiang on being elected to serve as President-Elect (2017-2020) and the President (2020-2023) of the International Society for Applied Phycology (ISAP).



#### Professor Hu's vision for the society

“Reflecting on the tremendous successes that ISAP has made for the past 20 years, I am very excited to work with all of you to create a new chapter in the history of the Society. I commit myself to be a facilitator for the Society to serve the members of applied phycology and algal biotechnology communities at large. The algal biotechnology field is at a crossroad that requires ISAP's leadership to capture the momentum of successes for the past ten years. Since the demand for expanding the existing and creating new commercial market spaces for algal products and algae-based services is real and urgent, our Society can play a significant role in building bridges between scientists/technologists and industry leaders towards transforming knowledge into the marketplace”.

#### Biography

Professor Hu is a Distinguished Professor and Director of the Center for Microalgal Biotechnology and Biofuels, Institute of Hydrobiology, Chinese Academy of Sciences. He also serves as the Director of Microalgal Biotechnology Center of the State Development & Investment Corporation (SDIC), which is one of the largest algal research centers in the world. Professor Hu is one of the leading authorities in fundamental and applied research on algae and has published over 100 peer-reviewed articles on photosynthesis, biosynthesis of lipids and carotenoids, growth physiology of high-density algal culture, photobioreactor system design, and applications of algal mass culture technology for food, feed and fine chemicals, and for environmental bioremediation. With Dr. Amos Richmond, he co-edited the ‘Handbook of Microalgal Culture: Applied Phycology and Biotechnology’ (Wiley Blackwell, 2013), which is considered as one of the most comprehensive textbooks on algal biotechnology. He currently serves as an associate editor of the journals ‘Algae’ (since 2010) and ‘Phycologia’ (2011-2014), and as an editorial board member of the journals ‘Algal Research’ (since 2010) and ‘Journal of Applied Phycology’ (since 2012). Professor Hu also has considerable experience of working for professional organizations. As a Board of Director of Algae Biomass Organization, Professor Hu was involved in organizing the first and following ‘Algae Biomass Summit’ until his return to China in 2013. As the Chair of the Organizing Committee, Professor Hu organized the ‘4th Asia and Oceania Algae Innovation Summit – AOAIS 2016’ in Wuhan, China. Throughout his career, Professor Hu established a broad network of colleagues and maintained deep personal friendships with a large number of global leaders in algal biotechnology. Additionally, he has been successful in renewing his connections with both academic and industry leaders in China since his recent return in 2013 from the USA following his distinguished career there. Professor Hu Qiang now joins the illustrious list of the past presidents as the first Asian President-Elect and President in the history of ISAP.

## ISAP Logo Competition

The Society would like to renew the ISAP logo to represent a modern image of the innovative and growing field of Applied Phycology. All the members can propose their concept to the Executive Board by the **31st of March 2018** and be eligible to win a free registration for the next ISAP congress!

To participate:

1. Conceive a new concept for the logo.
2. Write a short description (200 words max) of your concept and draw a draft of the logo.
3. Send to [secretary@appliedphycologysoc.org](mailto:secretary@appliedphycologysoc.org) as a single pdf file (please name pdf file as follow: LASTNAME\_logoISAP2018).

All new concepts will be submitted to the Executive Committee of the society and 3 finalists will be chosen. A vote will be organized among all the members of the society and the winning design redesigned by a professional.

The society would like to thank the European Algal Biomass Association (EABA) for their financial support for the redesigning of the logo by a professional.

See more details visit [our webpage](#).

**Deadline for submission: 31st March 2018.**

## ISAP Training Workshops: Call for Proposals

One of the objectives of ISAP is to support the organization of workshops and training programs in algal biotechnology for its members.

Funding for Trainings workshops are offered after evaluation of the proposal by the ISAP working Group, in consultation with the ISAP Executive Committee. For further information, consult

- 2018 call PDF document:  
[https://docs.wixstatic.com/ugd/e9f50b\\_17af9de093744a7b9d7af0a17137c4ce.pdf](https://docs.wixstatic.com/ugd/e9f50b_17af9de093744a7b9d7af0a17137c4ce.pdf),
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Please note that the deadline for the 2018 call is the **31st of March 2018!**

For further information, please contact Professor Roberto De Philippis ([roberto.dephilippis@unifi.it](mailto:roberto.dephilippis@unifi.it)), chair of the ISAP Working Group for Sponsored Courses and Workshops, or Dr. Valeria Montalescot ([secretary@appliedphycologysoc.org](mailto:secretary@appliedphycologysoc.org)), Secretary of ISAP.

## 7th ISAP Congress: Tokyo Japan

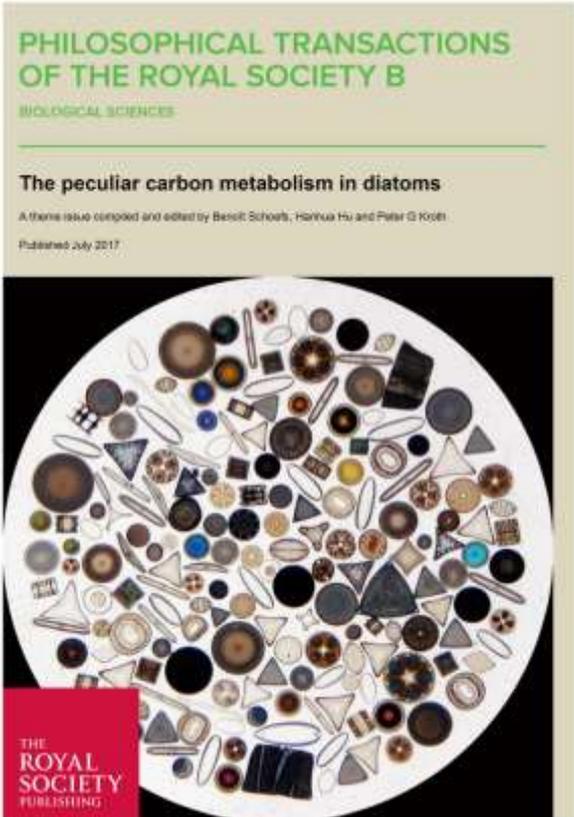
The 7th ISAP Congress will be held in Tokyo, Japan. Additional information will be posted on ISAP webpage, Facebook page (<https://www.facebook.com/AppliedPhycology1/>) and the congress webpage in the coming days.

## 6th ISAP Congress: Nantes France

The 6th ISAP Congress in 2017 was held in Nantes, France. Visit <https://www.flickr.com/photos/153196206@N08/sets/72157686795223655/> to see photographs taken during the congress.

### New publication on diatoms

Royal Society Publishing has recently published a special issue of Philosophical Transactions B entitled 'The peculiar carbon metabolism in diatoms', compiled and edited by Benoît Schoefs, Hanhua Hu and Peter G Kroth. This content can be accessed at <http://bit.ly/PTB1728> and the articles can be accessed directly at <http://rstb.royalsocietypublishing.org/content/372/1728>



**PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B**  
BIOLOGICAL SCIENCES

**The peculiar carbon metabolism in diatoms**

A theme issue compiled and edited by Benoît Schoefs, Hanhua Hu and Peter G Kroth  
Published July 2017

**THE ROYAL SOCIETY PUBLISHING**

**About this issue**

As diatoms, microalgae convert sunlight energy into chemical energy in the unbalanced, long CO<sub>2</sub> from their environment and releasing oxygen. Ignored for a long time, it is now recognised that the diatom contribution to carbon fixation and the biogeochemical system is significant. Up to 60% of the total carbon fixation in oceans and a substantial being plant higher marine trophic food web. In addition, the potential of microalgae for isolated ecological application is huge. Despite the biomimetic progress that have occurred in synthetic biology engineering, the improvement of bioengineering in terms of production and economic value remains fragile, possibly due to a lack of understanding of the metabolism and physiology of microalgae.

In this theme issue, we present a multidisciplinary understanding of carbon metabolism in microalgae, involving physiological and ecological aspects. We highlight the importance of carbon metabolism for biotechnological applications and economics.

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