

International Society for

Applied Phycology

NEWSLETTER



ISSUE 1-2017

April, 2017

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Message from the President, Dr. Roberto De Philippis

Dear ISAP Members,

I am happy to introduce the first Issue of ISAP Newsletter for 2017, which is also the last Issue of my term as President and of the term of the Editorial Committee composed by Amha Belay (as Chair), Céline Rebours, Sasi Nayar and Stephen O'Leary. I take this occasion for thanking them very much for their strong motivation and dedication in this activity. Their efforts lead to the publication of four Issues in three years, which is a great success considering that before this triennium we published only two Issues from the first one in 2013. We are moving towards the regular publication of two Issues per year. Moreover, thanks to this Committee, and in particular to Sasi Nayar, we have an active presence on Facebook and LinkedIn. We also have an active Website thanks to the dedication of Mike Guiry and Céline Rebours. In particular, I would like to thank very much Mike, who generously helped us since the ISAP Congress held in Galway, hosting and regularly updating the website.

The 6th ISAP Congress (Nantes, 18th to 23rd June; <https://isap2017.sciencesconf.org/>) is approaching and, as you know, my triennium as President will end the last day of the Congress. Thus, even if I hope to meet all of you in France at the General Assembly of ISAP, I would like to anticipate in the Newsletter some considerations on the activity of ISAP in last three years.

First of all, the organization of the 6th ISAP Congress is actively going on thanks to the unremitting activity of the LOC. Up to now, we received more than 380 Abstracts, which were evaluated by the International Scientific Committee constituted by the LOC and the EC of ISAP in order to select oral and poster presentations. I am having frequent and fruitful interactions with the two Co-Chairs of the Congress, Jean Paul Cadoret and Pascal Jaouen, as well as with the Scientific and Administrative Officer Valéria Montalescot. I would like to thank them, as well as the other Members of the LOC, for their dedication to the organization of the Congress. I would also like to thank our two vice Presidents, Céline Rebours and Susan Blackburn, for the fruitful discussions we had several times for taking important decisions regarding the Congress.

I would like to mention the intense activity of the Committee for selecting the Training Courses to be supported by ISAP, constituted by Avigad Vonshak (Chair), David Lewis, Mario Tredici, Emilio Molina Grima, Ioannis Tzovenis. Following the proposal made by Avigad Vonshak, it was established a well-defined procedure for selecting the proposals to be supported. In the three years of my term, our limited balance gave us the possibility to support only three training Courses, but I hope that in the next future we will be able to increase this number. I would like to thank very much the Members of this Committee, and in particular its Chair Avigad Vonshak, for their dedication to this activity.

I signed three Memorandum of Understanding between ISAP and three important International Societies operating the field of Applied Phycology for favouring the interactions between these Societies and the dissemination of information on topics of common interest: (i) the European Aquaculture Society (EAS, <http://easonline.org/>); (ii) the Algae Biomass Organization (ABO, <http://algaebiomass.org/>); (iii) the European Algae Biomass Association (EABA; <http://www.eaba-association.org/en>).

In 2016, it was activated the payment of ISAP dues by using credit card, which was a great improvement in the way of paying the dues. Please, be in good shape with your dues before the Congress, possibly adding a donation for supporting the fellowships for the participation of young scientists in the Congress. We received about 71 applications and at the moment, we are not able to support all of them. In this connection, I also launched a crowdfunding campaign, which until now gave only limited results. Please, continue your efforts for raising additional funds for supporting the fellows currently in the waiting list for the support.

Finally, I would like to remind you some of the activities of the Society that will be carried out during the Congress and in the following few months:

1. election of the next Executive Committee (at the General Assembly if ISAP Members);
2. awards (at the social dinner) to three Distinguished Applied Phycologists, selected by the EC
3. awards (at the closing ceremony of the Congress) for the best posters and the best oral presentations given by young scientists, selected by the International Scientific Committee
4. selection by the EC of the venue for the next Congress (soon there will be a specific call for proposals; the decision will be taken within two months after the end of the Congress);

Looking forward to meeting you soon in Nantes,

with my warm regards

Roberto De Philippis

President, International Society for Applied Phycology

Message from the Editor – Amha Belay

We are happy to present the first issue of the ISAP Newsletter in 2017. ISAP will strive to have the newsletter on regular basis. However, this depends on the contribution of ISAP members and other members of the phycological community. As usual we have two main articles and other news, views and announcements in the current issue. Both articles deal with the effects of anthropogenic factors but from different perspectives, the first describing such event and its consequences on an important lake in Ethiopia while the second deals with proteomics as a method to study the effect of such anthropogenic changes.

The first article deals with the serious subject of global environmental degradation of water bodies due to anthropogenic factors by giving an example of the plight of Lake Abijata, an alkaline lake in the Rift Valley of Ethiopia. This lake which has been designated as a national park has seen its depth dwindling due to diversion of inflows for irrigation and due to the process of extraction of soda ash where the water extracted into the evaporative ponds does not return to the lake. As a mitigation effort, the author suggests a green economy approach for sustainable utilization of the water and the water-shed. As a side note, the editor of this newsletter who happens to be from Ethiopia, had given a warning about the consequences of soda ash extraction from the lake over 30 years ago.

In the second article of this newsletter, Kumar *et al.*, present a stimulating article on the importance of proteomics in the study of adaptive mechanisms of marine macrophytes that are constantly subjected to a range of environmental fluctuations and anthropogenic stressors. While the study of proteomics in macrophytes is at its infancy, the authors see the great role proteomic studies will have in the future in elucidating these adaptive and tolerance strategies on translated proteins. The article guides us through proteomics definitions and methodologies followed by the contribution of proteomics in understanding marine macrophyte stress responses and the future of proteomic research in marine macrophytes.

Greening a National Park as a mechanism to protect and conserve a lake and its resources

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Background

Human interactions with water often involve inland water bodies such as streams, rivers, wetlands, lakes, and ground water. These continental water bodies cover only about 3% of the total water on this planet, of which 0.3% is surface water (Fig. 1). Although tiny, this is the fraction that is easily used, rapidly renewed, essential to life, and a key to viable industrial and domestic supply (Downing 2014). Thus, we rely heavily on a relatively rare commodity (Dodds 2002). However, the quality and quantity of these continental water bodies have been deteriorating primarily due to human population, which is a global phenomenon. There has been widespread decline in biological health of inland waters (UN WWAP 2003). In some regions, more than 50% of native freshwater fish species are at risk of extinction (Vié et al. 2009). Habitat modification and eutrophication are also common problems across the globe. This paper highlights this global problem by taking the plight of an Ethiopian lake as a case.

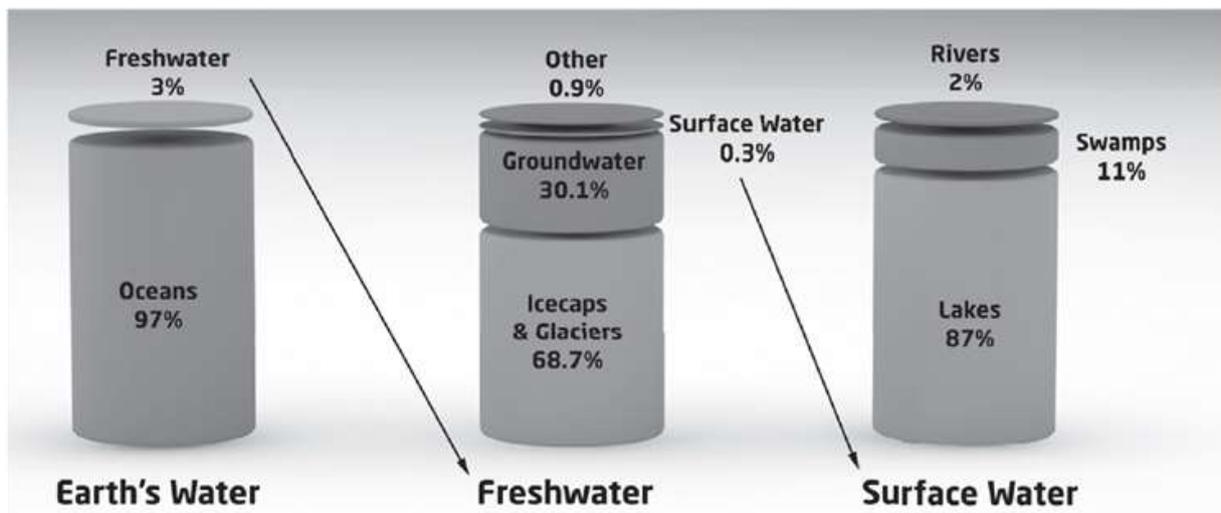


Fig. 1. The distribution of earth's water on the globe (Downing 2014)

Inland water bodies are the sole sources of surface water in Ethiopia. However, the country is frequently referred to as “water tower” of Africa although the water resources face challenges such as multiple water demands and stressors. Water quality degradation, pollution and the worst case scenario, “death” of aquatic ecosystems, are pervasive in the country. Lake Alemaya (now Haromia), for example, has been completely converted to a terrestrial ecosystem as a result of the unbalanced use of freshwater resources.

Lakes Abijata and Shala, which are located in the Rift Valley of Ethiopia, are now in real danger of disappearance though they are officially protected under National Park status as Abijata-Shalla Lakes National Park (ASLNP). The Park was established to protect and conserve the large number of water birds (e.g., Great White Pelicans, Greater and Lesser Flamingos) that use Lake Abijata as feeding grounds and Lake Shala as nesting and breeding grounds (Tefera and Almaw 2002). The islands of Lake Shala are one of the few nesting and breeding sites of pelicans found in Africa (UNESCO 2004). The Park provides a wintering ground and maintenance station for large numbers of terrestrial and aquatic migratory birds including Southern African, Sub-Saharan and Palaearctic species (Legesse et al. 2005). Consequently, ASLNP was recommended for protection under the Ramsar Convention on Wetlands (EWCO 1989).

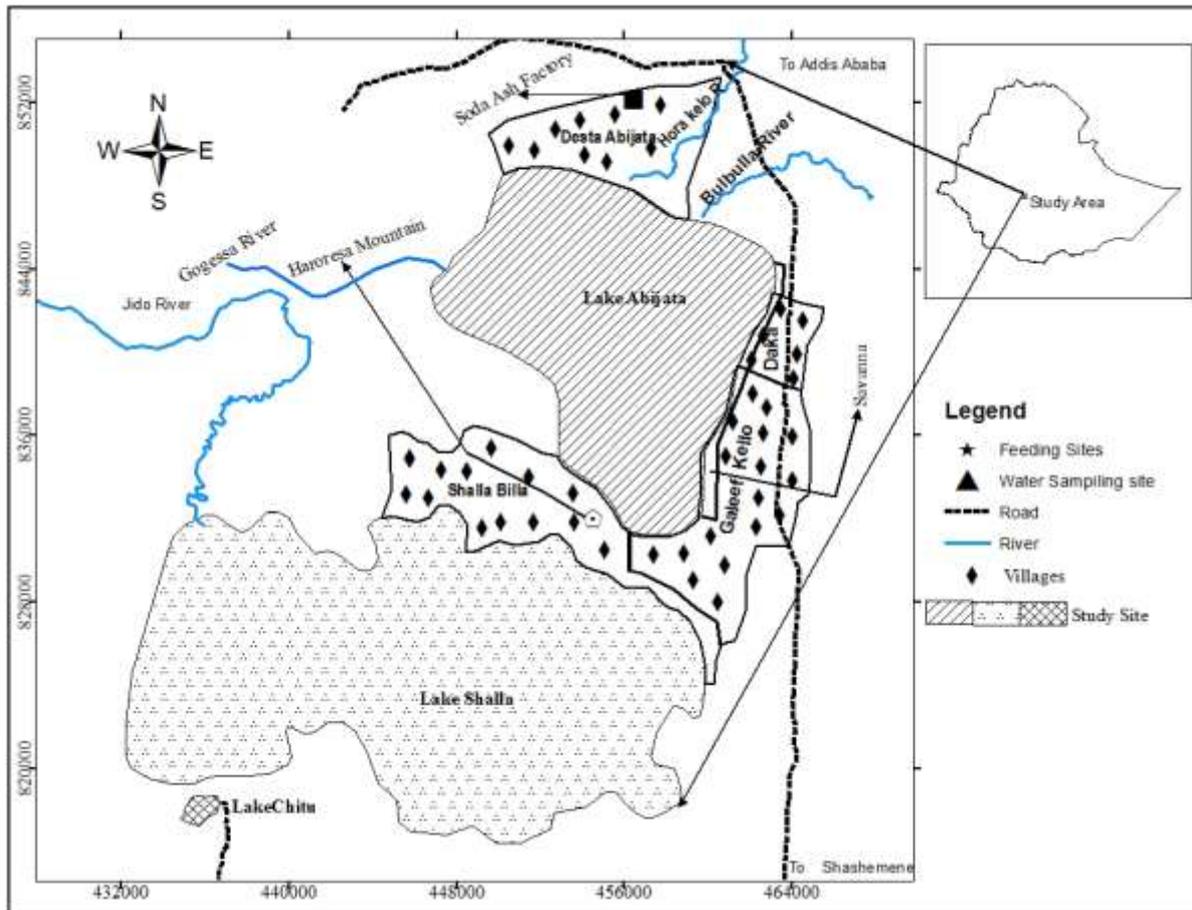


Fig. 2. Map of Lake Abijata with its drainage basin (Kumssa and Bekele 2014).

The natural heritage of this wetland is presently deteriorating due to the presence of large numbers of human settlements in the Park coupled with grazing pressure from domestic animals. The threat of deforestation is omnipresent. The water level of Lake Abijata has significantly diminished from 194 km² in 1973 to 95 km² in 2006 (Fig. 3). This has had adverse impact on the fishery, which has totally disappeared, and also impacted birds such as Lesser Flamingo and Pelican have been migrating (UNESCO 2004; Reaugh-Flower 2011; Ayenew 2002). The water levels in Lake Abijata have dropped some 6.5m between 1985 and 2006, with 70% (~4.5 m) of the loss attributed to human-induced causes (Seyoum, Milewski and Durham 2015). The environmental conditions in the Park are worsening with the lake shrinking, eventually facing an imminent collapse and loss of all its services and benefits unless appropriate measures are immediately taken (Ayenew 2002). In fact, ASLNP is listed as an IBA (Important Bird and Biodiversity Areas) endangered site, identifying it as a priority site for immediate action (Birdlife.org 2016).

This paper documents the status of ASLNP and considers the possible causes for the reduction of water level in the lake. The paper promotes green economy as a way forward for sustainable utilization of natural resources of the Park. The rich biodiversity of the Park is a highway for the Eco-Tourism Industry, *Arthrospira* production and other non-destructive revenue generating activities. The overarching strategy of the paper is to promote the protection and conservation of the common properties, Lakes Abijata and Shala and their resources, through the legally protected ASLNP.

Potential causes for water level reduction of Lake Abijata

The water level of endorheic Lake Abijata has significantly dropped since the mid-1980s (see Fig. 3), which cannot be attributed to natural climatic variability and rainfall record (Temesgen, Nyssen, Zenebe, Haregeweyn, Kindu et al. 2013). A recent study showed that some 70% of the water loss from the lake

can be attributed to human-induced causes (Seyoum, Milewski and Durham 2015). The water budget of Lake Abijata is dependent on seasonal precipitations, river inflows from Hora Kelo (contributing about 8% of the total inflows to the lake) and Bulbula River, which was the largest inflowing river into Lake Abijata from Lake Ziway (Ayenew 2002). Hence, any intervention either on Lake Ziway or Bulbula River affects the water budget of Lake Abijata. An irrigation project on the upper reach of the river is ongoing and as a result the water flow in the Bulbula River has significantly reduced (Ayenew 2002). Irrigation is the largest water user activity in the basin (Ayenew 2002; Vilalta 2010). Water abstraction from Lake Ziway, its tributaries, and the Bulbula River for large-scale irrigation agriculture for the production of fruits, vegetables, and flowers is regarded to have a significant impact (Seyoum, Milewski and Durham 2015).

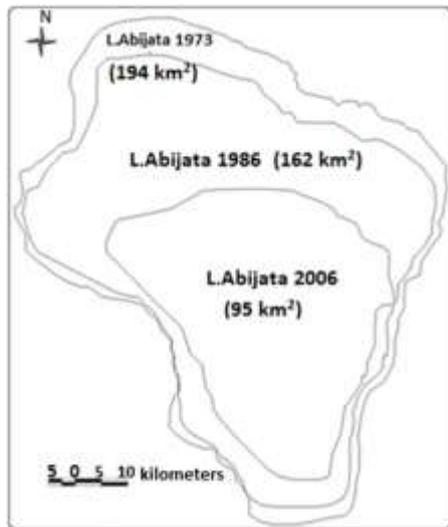


Fig. 3. Surface Area of Lake Abijata as obtained from satellite imagery (1973-2006) (Birdlife.org 2016).

In parallel, Abijata Soda Ash Company could be regarded as the second possible anthropogenic cause for water level reduction (Gebre-Mariam 1998; Kumssa and Bekele 2014). Presently, the factory produces trona by pumping water from the lake into 17 concentration ponds. In doing so, 13, 000,000 m³ of water is removed from the lake each year (Ayenew 2002) possibly increasing to 30,000,000 m³ per year if one were to consider the company's plan to produce 200 thousand tons of soda ash per year. Since the water extracted from the lake into the ponds does not return to the lake, the shore of Lake Abijata has receded over the years. Vilalta (2010) reported that Lake Abijata has receded 3 km from the pumping station and soda ash production has slowed down because of the loss of water in Lake Abijata.

Physico-chemical and biological changes in Lake Abijata

The lake area has shrunk by about 100 km² resulting in conspicuous physical, chemical and biological changes in the lake. Data collected since 1926 show that the lake's salinity has increased by more than 2.6 times (from 8.1 to 26 mg l⁻¹), alkalinity from 80 to 326 mg l⁻¹, and pH from 9.5 to 10.1 (Ayenew 2002; Wood and Talling 1988; Gebre-Mariam 2002).

The lake was dominated by blue-green algae such as *Arthrospira fusiformis*, *Oocystis* and *Anabaenopsis* during the period from 1960 to 1988 (Wood and Talling 1988; Kumssa and Bekele 2014). *Arthrospira* was a dominant species usually found forming dense blooms. However, *Arthrospira* was replaced by *Anabaenopsis* and diatoms subsequent to the establishment of the Soda Ash Company (Kumssa and Bekele 2014; Kebede and Willén 1996). This compositional switch from *Arthrospira* to non-*Arthrospira* phytoplankton species impacted the vast flock of Lesser Flamingo that depends on *Arthrospira* as a primary food source. The numbers of the Lesser Flamingo have been greatly reduced at Lake Abijata as they are now migrating to the nearby Lake Chittu, which is rich in *Arthrospira* (Kebede 1997).

Prior to anthropogenic impacts, about thirteen native fish species inhabited Lake Abijata and a commercial fishery of Tilapia (*Oreochromis niloticus*) prevalent in the 1980s (Reaugh-Flower 2011; Birdlife.org 2016). Currently, there are no fish or fishery, possibly due to a decline in water level, high salinity and associated impacts such as reduced breeding grounds of tilapia and osmotic stress as a result of high salt concentrations. The number of piscivorous birds has also been declining because of the absence of fish at Lake Abijata (Birdlife.org 2016). As an example, pelicans that feed on fish have been migrating to other water bodies (UNESCO 2004).

Green Economy for Abijata-Shala Lakes National Park

A green economy, driven by public and private investments that reduce carbon emissions and pollution, has a potential to boost growth in income and employment, enhance energy and resource efficiency, and

prevent the loss of biodiversity and ecosystem services. ASLNP is worth protecting to promote a green economy as well as biodiversity conservation. The ecosystem services and goods provided by the Park is estimated to range between US\$ 15.9 million to US\$ 308.5 million per year (Reaugh-Flower 2011). In particular, the significance of ecotourism and *Arthrospira* production to the local and national economy in the framework of green economy is discussed below.

Ecotourism Industry

Ecotourism is defined as "responsible travel to natural areas that conserves the environment, sustains the well-being of the local people, and involves interpretation and education". Eco-tourism is in line with Ethiopian government policies, as the country aims to achieve carbon-neutral middle-income status by 2025, whilst developing a green economy, which will enable protection and conservation of tourism destinations such as the ASLNP.

In the Park, a total of 436 bird species (among which 114 species are wetland birds) have been recorded including Lesser Flamingo (*Phoeniconaias minor*), Greater Flamingo (*Phoenicopterus roseus*) and the Great White Pelican (*Pelecanus onocrotalus roseus*) (Estifanos 2008; Birdlife.org 2016). Of the birds, Lesser Flamingo is a major tourist attraction. This species primarily feeds on *Arthrospira* which grow preferentially in the highly alkaline lakes such as Lake Abijata. Hence, Lake Abijata is a source of food (*Arthrospira*) while Lake Shala is a breeding ground for Lesser Flamingo. Lake Shala is also the second-most important of eight regular pelican breeding grounds of the whole of Africa. In light of this, these two lakes deserve protection status.

The magnificent views of the lakes together with the biodiversity they support, such as the abundant number of bird species and mammals, are a strong draw for visitors. More importantly, the proximity of the site to the capital city Addis Ababa, its short drive from the main road, and its position alongside other tourism destinations such as Lake Langano, Hawassa, Arba-Minch (Nechi-Sar Park) are additional underlying factors that attract visitors despite the fact that the Park is not well managed. The Park is in urgent need for rehabilitation and conservation programs, which can be performed by the state together with community groups and professionals. Ecotourism and sustainable tourism have the potential to assist in conserving natural areas, alleviate poverty through revenue generation, empower women, enhance education, and improve the health and wellbeing of local communities.

***Arthrospira* production**

Arthrospira fusiformis is characteristic of soda lakes in tropical Africa forming persistent and almost uni-algal blooms (Kebede 1997). It has been a focus of interest among researchers due to its overall nutritional qualities and commercial significance to humankind as a source of protein (60 to 70 % of dry weight), fatty acids (gamma-linoleic acid-GLA), essential amino acids, and vitamins (high B12 content). The species also has therapeutic effects against hyperlipidemia, nephrotoxicity, diabetes, obesity and hypertension (Gershwin and Belay 2007). As a result of all these benefits, it is regarded as a 'health food' and identified as a food source to combat hunger and malnutrition in developing countries. This alga dominates the phytoplankton community structure in the saline-alkaline lakes of Ethiopia, particularly lakes Abijata, Chittu and Arenguade. *Arthrospira* was also abundant in Lake Abijata (Kumssa and Bekele 2014).

Arthrospira is also the major food source for the large number of Lesser Flamingo and is responsible for non-toxic blooms in the lake. However, Ballot et al. (2005) observed toxic and non-toxic *Arthrospira*, while Krienitz et al. (2005) reported toxic strains of *Arthrospira fusiformis* isolated from Kenyan lakes. Many Lesser Flamingo have perished in East African saline-alkaline lakes, including about 30,000 in Lake Bogoria of Kenya and 43,800 in Lake Manyara in Tanzania. The toxicity of *Arthrospira* was the cause for these deaths, because this is the major food of Lesser Flamingo. To my best knowledge, there have never been reported mass kills of Lesser Flamingo in Ethiopia, potentially suggesting non-toxicity of Ethiopian *Arthrospira* strains.

Mass culture of *Arthrospira*, a green economy initiative, could be undertaken as large commercial scale production or as a social initiative for combating hunger and malnutrition through micro-farms. Mass culture of *Arthrospira* is prevalent in USA, Thailand, India, China, and other countries. For example in China, 19,080 tonnes of *Arthrospira* was first produced in 2003 which rose sharply to 41,570 tonnes (wet weight) in 2004, with a market value of around US\$7.6 million and US\$16.6 million, respectively. In Ethiopia, the cost of nutrient media for biomass production can be significantly reduced by using water from the lakes. In a recent laboratory study, Ogato et al (2014) demonstrated that water from Lake Shala supplemented 25% and 50% with standard *Spirulina* medium could be used to produce *Arthrospira* biomass, thereby reducing the cost of nutrients by 75% and 50%, respectively. Mass cultivation of *Arthrospira* has been ongoing along the shores of Lake Shala (Reaugh-Flower 2011), with the potential of being scaled-up.

Conclusions

The condition of ASLNP is deteriorating largely due to anthropogenic causes such as human encroachment, grazing by cattle, uncontrolled water abstraction and other activities. The water level in Lake Abijata has significantly dropped, resulting in the water spread area receding by about 100 km² reducing the total lake's coverage by about half its original size. Consequently, the physico-chemical and biological variables have changed, adversely impacting many of the organisms inhabiting the lake. The phytoplankton species composition has switched from an *Arthrospira* dominated community to a non-*Arthrospira* community. Tilapia has declined in its overall abundance leading to a collapse of the commercial fishery. The Lesser Flamingos and Pelicans have also migrated from the lake.

Protecting and conserving the Park will not only preserve the biodiversity and habitats, but also improve the economy making it a sustainable and green economy through ecotourism and *Arthrospira* production. The ecological services of ASLNP include provision of services such as food, freshwater, raw materials and medicinal resources, regulating services, supporting services and cultural services (Birdlife.org 2016). The Park's living resources have been valued at US\$ 15.9 million to US\$ 308.5 million per year (Reaugh-Flower 2011). Therefore, rehabilitation and conservation programs should be put in place to restore the biodiversity including *Arthrospira*, fish, Lesser Flamingo and pelicans and to utilize the ecosystems in a sustainable fashion.

Ethiopia has policies, strategies, proclamations and development programs that promote sustainable and equitable utilization of the available water resources (Vilalta 2010). Therefore, the country should practice and implement its novel ideas and policies with an aim to protecting and conserving all water resources for future generations to enjoy.

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Marine macrophytes proteomics: where are we heading?

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Introduction

Marine macrophytes, including seaweeds (macroalgae) and seagrasses (angiosperms), are marine ecological engineers that provide a range of ecological and biological services to marine ecosystems¹. Marine macrophytes inhabit a unique aquatic environment and experience a range of environmental fluctuations as well as numerous anthropogenic stressors. The resilience of these plants to such fluctuations depends on their abilities to reprogram their cellular machinery at the genetic, proteomic and metabolite levels. An increasing amount of transcriptomic-based information is being gathered regarding the acclimation of marine macrophytes to extreme environmental perturbations^{1,2}. However, no coherent explanation has been provided to connect transcriptional responses with functional phenotypic responses in these marine macrophytes when compared to land plants or microalgae.

Taking the above into account, the field of proteomics examines the broader effects of adaptive/tolerance strategies on translated proteins, which ultimately play key roles in adapting to environmental cues. Moreover, proteins act as enzymes or enzyme regulators to mediate changes in metabolite levels. Therefore, changes in a plant's protein profile, or proteome, when under stress can be closely interrelated with phenotypic responses, ultimately determining tolerances. Proteomics has increasingly impacted the study of terrestrial plant responses to biotic and abiotic stresses, however, only a few marine flowering plants have been examined using this novel technology.

Proteomics-definition and methodologies

Proteomic investigation involves a large-scale analysis of all the proteins produced in a biological system under a specific set of environmental conditions. A typical proteomic workflow proceeds with protein extraction followed by protein separation using electrophoresis or chromatography, protein analysis using mass spectrometry (MS), protein identification and quantification and data interpretation. Two complementary approaches, termed "bottom-up" and "top-down," are commonly applied in proteomics. In a bottom-up proteomic approach, proteins are typically separated using two-dimensional gel electrophoresis (2-DE) after which selected protein spots that change in abundance between treatments are enzymatically digested to peptides using trypsin followed by identification using MS³. When a bottom-up (peptide-centric) approach for whole proteome analysis is applied without any prior separation, it is referred to as "shotgun proteomics," or gel-free bottom-up proteomics with chromatographic methods used to separate peptides before being identified in the MS.

Despite the fact that label-free methods exist, most bottom-up approaches rely on stable isotope labelling for peptide quantification of multiple samples simultaneously, greatly reducing variation and instrument time. This labelling is achieved either through the metabolic incorporation of isotopically labelled amino acids into proteins (SILAC approaches) or by post-extraction chemical modifications with other isotopically labelled reagents (ICAT, TMT, iTRAQ approaches)⁴. The technological advancements in shotgun proteomics achieved in recent years have substantially improved the depth of proteome

analysis⁴. Advancements in MS instrumentation provides high mass resolution and accuracy, up to six-orders of dynamic range and high sensitivity, as well as fast scan rates of 100 Hz. However, gel-free bottom-up strategies disconnect peptides derived from sample protein during peptide separation, requiring bioinformatics software to infer the presence of a protein from a small number (>2) of detected peptides. A detected peptide can only be assigned to a protein if the sequence of that protein is known and present in a database, most often derived from genome sequencing projects⁴. Therefore, shotgun approaches in plants are currently applicable only to model species, but could be applied to seaweed and seagrass models for which whole genome sequence is available.

In contrast, the top-down proteomic approach identifies intact proteins without prior enzymatic digestion and characterizes proteins by deducing partial amino acid sequences after gas-phase fragmentation by tandem MS (MS/MS). However, this technique requires proteins to be purified by various chromatographic procedures prior to MS/MS analysis and relies on instruments with extremely high mass resolution to accurately determine the mass of the intact protein⁵. Furthermore, bioinformatics tools for top-down proteomics are underdeveloped, and this proteomic approach is significantly limited due to difficulties with protein fractionation, protein ionization, and fragmentation in the gas phase.

Both of the above-mentioned techniques have expanded the horizons of proteomics and ushered in a new age of promise and challenge for the characterization and identification of proteins. 2-DE based proteomics has remained the preferred strategy for non-model/orphan organisms. This strategy is a protein-based separation and quantification technique in which protein-derived peptide connectivity is retained and only peptides from a single protein are analyzed by MS at one time. This increases protein sequence coverage as poorly ionizing peptides are better detected in a sample of lower complexity and it allows *de novo* sequencing from MS/MS spectra of peptides whose sequences are not previously reported in databases. That said, issues such as gel-to-gel variations, a limited linear dynamic range, and protein co-migration continue to pose challenges in gel-based proteomic approaches. Nevertheless, these issues can, to some extent, be overcome by using 2-DE gel analysis software, though this requires considerable human intervention and visual inspection of gels.

Contribution of proteomics in understanding marine macrophyte stress responses

In marine macrophytes, the application of proteomics-based approaches to increase our understanding of the acclimation and/or tolerance mechanisms to environmental variability is still in its nascency. Possible reasons for this include inefficient protein extraction protocols and a lack of whole genome sequence/transcriptomic information for most macrophyte species. Extraction of proteins from marine macrophytes is particularly difficult due to low protein content and the presence of contaminants that are co-extracted. These contaminants including anionic polysaccharides, polyphenols and salts are highly concentrated in the tissues of marine plants. Due to this limitation, a specific protein extraction protocol need to be optimized and established to enrich the protein profile. Considering this, modified protein extraction protocols have been established to produce well-resolved 1-D SDS-PAGE and 2-DE images with low background speckles from seaweeds such as *Scytosiphon gracilis*, *Ectocarpus siliculosus* and *Ecklonia kurome*^{6,7}. Similar efforts in seagrass proteomics have also been demonstrated in the seagrass *Posidonia oceanica* and other species⁸.

Most proteomic research in seaweeds is 2D-IEF based and has focused on unraveling the mechanisms involved in heavy metal stress tolerance in the brown seaweeds *S. gracilis*, *E. siliculosus* and *Sargassum fusiforme*⁹⁻¹³. These studies have outlined the role of various enzymes in heavy metal stress tolerance, including antioxidant enzymes, such as ascorbate peroxidase and peroxiredoxin; phosphomannomutase, required for cell wall and ascorbate biosynthesis; ABC transporters, involved in the translocations of heavy metals from the cytosol to the vacuole; HSP chaperones; and proteases, among other tolerance pathways. Another environmental condition that influences proteome modifications is desiccation, triggered by daily tidal changes¹⁴. Rapid proteomic modulation when subject to high temperature and desiccation were reported in the seaweeds *Pyropia haitanensis* and *Pyropia orbicularis*, respectively^{14,15}. These modifications principally included proteins involved in energy and biomolecule metabolism, antioxidant and defense functioning, and genetic and environmental information processing. Recently, temperature fluctuations, pathogen interaction, salinity stress, and CO₂ exposure

responses were also investigated using proteomic approaches to unveil oxidative stress tolerance mechanisms as well as strategies for maintaining homeostatic levels in seaweeds.

In seagrasses, the implementation of proteomic approaches recently addressed the negative impacts of light limitation on diverse cellular metabolic pathways. Light limitation has been implicated in the global loss of seagrass due to dredging, eutrophication and other human activities¹⁶. Recently, adaptation and tolerance mechanisms in *Cymodocea nodosa* to salinity were explored using 1D-PAGE and tandem mass spectrometry¹⁷. The results indicated that hypersaline treatments increased the glycolytic protein levels and vacuolar components (e.g., Na⁺/H⁺-antiporter) in seagrass tissues to deal with these conditions.

Future of proteomic research in marine macrophytes

Post-translational modifications of proteins (PTMs)

In a cell, the PTMs of a protein affect its functionality, stability, and localization, as well as protein-protein interactions. Among the various PTMs (including ubiquitination, nitration, S-nitrosylation, sumoylation, glycosylation, carbonylation, methylation, and acetylation), protein phosphorylation (i.e., phosphoproteome) is the most studied in plants¹⁸. PTMs are gaining recognition as signaling molecules that regulate plant cellular processes under a range of biotic and abiotic stresses. Low PTM abundances and/or high PTM instability under normal conditions in a cell are the major challenges in studying these protein alterations. So far, tandem MS to fragment peptides using collision-induced dissociation (CID), electron-transfer dissociation (ETD) or electron-capture dissociation (ECD) have proven promising in studying PTMs due their capacity to identify and/or localize multiple PTMs in the same sample, ultimately determining which amino acid residue carries the PTM. Apart from the detection of phosphoproteins in 2-D gels with phosphoprotein stains, the fractionation and enrichment of digested and labelled (iTRAQ) or non-labelled phosphopeptides in combination with affinity chromatography using cation-exchange (SCX), immobilized metals (IMAC), TiO₂ or ZrO₂ followed by LC-MS/MS is most common procedure for phosphoproteomic studies¹⁹. The study of other PTMs most often relies on the availability of antibodies specific for the modified amino, such as acetylated lysine. These antibodies vary greatly in their quality and efficacy.

Whilst PTM research in land plants has progressed from whole cell/tissue to sub-cellular levels, there is limited progress with work on this area in marine plants. So far, only one report is available on PTMs in marine macrophytes evidencing the enhanced phosphorylation and re-arrangement of the protein subunit of PSII (PsbS) and LHCII proteins in the intertidal seaweed *Ulva* sp. during desiccation stress acclimation²⁰. Recent success of technical advancements in bioinformatics databases (PhosPhAt, Plant Protein Phosphorylation Database (P³DB), Musite, Motif-x, MMFph, Pep2pro, and MASCP) in studying phospho- and other PTMs suggests a certain degree of maturity, and will support their successful implementations in marine plant proteomic workflows.

Organelle proteomics (OP)

Comprehensive whole-tissue proteomics is largely limited by protein abundance and therefore the dynamic range of protein concentration, requiring extensive fractionation to profile the proteome in any depth. In contrast, this issue is less problematic when examining the subcellular/organelle proteome, which can additionally provide vital information on the localization and role of many proteins with currently unknown functions. The fractionation of specific organelles results in the enrichment of proteins that may be relatively rare in whole cell/tissue protein extracts, while decreasing the proportion of the proteome needing to be characterized. Therefore, subcellular proteomics decreases the complexity of proteome discovery and provides information at subcellular level.

Isolation of an intact organelle without non-proteinaceous contaminants while preventing the inadvertent mixing of other cellular compartments is a challenging task in organelle proteomics. Differential/ultra-speed and gradient centrifugation using sucrose and percoll layers have proven effective in isolating intact subcellular fractions from various agriculture crops. While OP is improving our knowledge of the systems biology of land plants under climate change, some studies have started to

focus on studying the PTMs in subcellular compartments²¹. Therefore, OP holds great promise in unveiling the fine-tuned mechanisms that maintain and regulate protein translation, post-translational metabolism, signaling, and trafficking through the cells in response to external perturbations.

Although OP analysis in terrestrial plants has progressed in recent years, there is a lag in the application of this technology to the study of marine macrophytes. However, the scope of OP in marine macrophytes was recently explored through optimization of a method for isolating intact chloroplasts and subsequent proteome analyses in the seagrass *P. oceanica*²². In this work, 72 proteins were identified, of which 43 were exclusively from the chloroplast. Such subcellular proteomics studies are crucial in understating the molecular mechanisms employed by seagrasses when adapting to light limiting conditions in their ecological niche. However, the suitability of these methods needs to be explored in other seagrass and seaweed models, whose whole genome has been sequenced^{1,2}.

Conclusions

The integration of proteomics with transcriptomic and/or metabolomic studies not only serves as a validation tool for studies of gene regulation and their downstream products, including proteins and metabolites (produced by enzymatic activities), but is also useful in revealing central responses of the biological system under study. Although evidence for proteomic modifications is limited, the available information clearly indicates that environmental changes influence the proteome stability of marine macrophytes. In conjunction with other methodologies, the final goal of marine macrophyte proteome research should be the advancement of more productive marine plant cultures and the sustainable management thereof, in addition to furthering the potential ecotoxicological applications of seaweeds and seagrasses in environmental risk assessments. While current interest in this research is restricted both in scope and species under study, we hope this scenario quickly changes, just as the environment is doing today.

Acknowledgement

The first author (Manoj Kumar) gratefully acknowledges the Australian Research Council for awarding him Discovery Early Career Research Award (DECRA Fellowship, DE150100461-2015).

List of abbreviations:

2D-IEF: two dimensional with isoelectric focusing
 CID: collision-induced dissociation
 ECD: electron-capture dissociation
 ETD: electron-transfer dissociation
 ICAT: isotope-coded affinity tags
 iTRAQ: isobaric tag for relative and absolute quantitation
 MS: mass spectrometry
 OP: Organelle proteomics
 PTMs: Post-translational modifications of proteins
 SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis
 SILAC: stable-isotope labelling by amino acids in cell culture
 TMT: tandem mass tag

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News and Views

Conferences



After Kunming - China in 2005, Galway - Ireland in 2008, Halifax - Canada in 2011 and Sydney - Australia in 2014, the International Society of Applied Phycology (<http://www.appliedphycologysoc.org/>) entrusted a scientific team from Western France to organize the 6th congress of the society in Nantes at La Cité Nantes Events Center from June 18th to 23rd 2017. Indeed, with over 300 people working in its universities and research centers and more than 100 businesses, Western France is a European hotspot for the transfer and industrial development of marine biotechnology (Marine Biotech in Western France).

Previous ISAP Congresses have seen the role of applied algal biotechnologies and their potential developed in a commercial, remedial or regulatory context. In 2014, the theme of ISAP was chosen to reflect on the actual successes of algae applications as they already represent a sustainable and relevant field of biotechnology.

In 2017, the scope of the 6th edition of ISAP congress is to appreciate the huge phycological biodiversity and the diversity of its biotechnological applications through the prism of a new and promising industrial sector in full development. The Congress will include speakers and poster presentations, exhibitors and for the first time a BtoB session to meet the right partners.

For details visit <https://isap2017.sciencesconf.org/>



The Wando Seaweed Expo 2017 - Apr. 14 to May 7, 2017. | Wandoo, Korea

Wando Seaweeds Expo 2017 a 'seaweed-themed international expo' will be an opportunity to explore the past, present, and future of seaweeds including laver, kelp, and hijiki at once. As a business-oriented industrial expo with about 150 of seaweed relevant companies and organizations participating from about 20 countries around the world, , this expo will become the platform where diverse information and technology such as ecological and nutritional values of seaweeds as well as its potentials as a future industry are exchanged. In addition, the expo will expand the consumer market of seaweeds both within and outside the country and let us explore opportunities of creating new added values as the power industry of national future development such as advanced materials, new medicines, and bio energy. <http://www.wando.go.kr/expoeng>

The Phycological Society of America Annual Meeting: PSA 2017 - Jun. 4 - 87, 2017. | Monterey Bay, United States

An excellent selection of keynote speakers to address the past, present, and future of algal endeavors, including Mike Guiry and Laura Rogers-Bennett. A bevy of fantastic symposia, including Picophytoplankton in a Changing World (J. Jeffrey Morris), The Ecology of Macroalgal Blooms (the PSA Presidential Symposium, Tim Nelson), and Non-Aquatic (terrestrial, subaerial, etc.) Algae (Nicole Pietrasiak). Excellent education committee is planning several workshops, including, but not necessarily limited to, An Introduction to Metabarcoding Data Analysis (Emily Johnson) and Balancing Science Communication and Science: Achieving Effective Outreach Using Social Media (Susan von Thun). An opportunities to explore the local region, with copious details to follow in the near future. <http://www.psaalgae.org/meetings/2017/6/4/psa-2017-annual-meeting>

The BIO World Congress on Industrial Biotechnology - July 23-26, 2017 | Montréal, Canada

The BIO World Congress on Industrial Biotechnology provides a unique forum for business executives, government officials, academic researchers and industry leaders to share the latest advances in renewable chemicals, synthetic biology, enzymes, food ingredients, biofuels and more! <https://www.bio.org/events/bio-world-congress>



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ABO website - , <http://algaebiomass.org/>

Algae Biomass Summit - <http://algaebiomasssummit.org>



EABA website - <http://www.eaba-association.org/en>

Conference ALGAE Europe, Madrid December 13th-15th 2016 - <http://algaecongress.com/>



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