Newsletter of the Community of Duckweed Research and Applications, edited by the ISCDRA

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 7 (1), issue 24, pages 1-40 (2019)

5th International Conference on Duckweed Research and Applications to be held at Weizmann Institute of Science, Rehovot, Israel from September 9 - 12, 2019

Cover page

5th International Conference on Duckweed Research and Applications to be held at Weizmann Institute of Science, Israel from September 9 - 12, 2019

Topics at this Conference in September 2019 will include advances in duckweed genomics, physiology, microbiomes, ecosystems, ecotoxicology, nutrients, natural products, biomass production and other commercial applications.

The venue is the conference center at the Weizmann Institute of Science, a world-class research institution set in a lushly landscaped campus in the university town of Rehovot, 25 min. from Tel Aviv and 55 min. from Jerusalem. The institute is host to 240 experimental and theoretical research groups across five faculties—Biology, Biochemistry, Chemistry, Mathematics/Computer Science, and Physics, and to 1400 advanced degree students and postdoctoral fellows.

Israel, the start-up nation, is a relatively small country with a long history. Archeological findings from neolithic to biblical to crusader periods are everywhere. Although small, the country contains several climatic zones, ranging from coastal Mediterranean to the arid Dead Sea, from the Hermon mountain to the Negev desert. Major cities include Jerusalem, a spiritual center for Jews, Christians and Muslims and Tel Aviv, a modern nonstop city.

We invite you to enjoy the science of duckweed together with the heritage of Israel at the Weizmann Institute of Science.

In this issue

Letter from the Editor....1

5th ICDRA: 3rd Circular....2

Research Topic in Frontiers in Chemistry....3

Historical account: William Griffith, Esq., F.L.S. (1810-1845)....8

Student Spotlight: Kenneth Acosta....13

From the database....15

Instructions to Contributors for the Duckweed Forum....38

Links for further reading....40

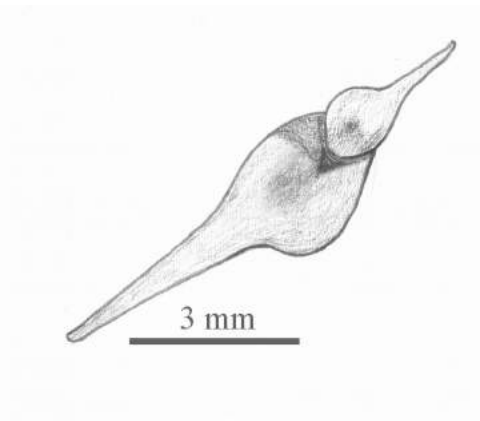
The 3rd International Steering Committee on Duckweed Research and Applications Members

- **Chair: Prof. Eric Lam**, Rutgers, The state University of NJ, New Brunswick, USA; ericL89@hotmail.com
- **PD Dr. Klaus-J. Appenroth**, Friedrich Schiller University of Jena, Germany; Klaus.Appenroth@uni-jena.de
- **Prof. Marvin Edelman**, Weizmann Institute of Science, Rehovot, Israel; marvin.edelman@weizmann.ac.il
- **Dr. K. Sowjanya Sree**, Central University of Kerala, Periye, India; ksowsree9@cukerala.ac.in
- **Dr. Yubin Ma**, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China; mayb@qibebt.ac.cn
- **Dr. Tsipi Shoham**, GreenOnyx Ltd., Tel Aviv, Israel; tsipi@greenonyx.biz

- **External Advisor: Tamra Fakhoorian**, International Lemna Association, Mayfield, KY, USA; tamraf9@gmail.com

All prior Duckweed Forum issues: http://www.ruduckweed.org/

Science meets art: *Wolffiella caudata* Landolt



Like many of the species in the genus Wolffiella, *Wolffiella caudata* also has an attractive and almost exotic morphology. The tail-like appendix (the Latin word for tail is “cauda”) helps in identification of this species and gave it the name. Prof. Elias Landolt (1926 – 2013) described this species when he inspected a Lemnaceae collection from Bolivia in 1992, in “Berichte des Geobotanischen Instituts der ETH Zurich”. The vegetation zone is the humid Savanna in southwestern part of the Amazon Basin. Physiological and biochemical properties were described recently by Pagliuso et al. in Frontiers in Chemistry 6: article 291 (2018). Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India

Letter from the Editor

Dear Duckweed Community,

A warm Happy New Year of 2019 to you all! On behalf of all the members of our Steering Committee, I have the pleasure to wish everyone a most prosperous and healthy new year. As you may have noticed, our Cover Photo for this issue of the Duckweed Forum (DF) features the Weizmann Institute in Rehovot, Israel, the site for our upcoming 5th International Conference in September of this year. In the third circular for this biennial event of our community, you can find a list of confirmed speakers as well as updated information on the venue. I hope many of you will be able to attend this conference to share in the exciting new development that are happening in the duckweed arena, both in terms of basic and applied research.

As many of us in the field are preparing for the upcoming conference, the Research Topic with 13 articles in *Frontiers in Chemistry* that was dedicated to duckweed research and applications has been completed in 2018. This research topic was to commemorate our 4th International Conference at Kerala, India, and I like to thank Marvin Edelman, Klaus Appenroth and Sowjanya Sree for their hard work in serving as guest editors to process all the submitted papers during their peer review. The success and impact of this research topic, and a reflection of the growing interest in duckweed research and applications worldwide, can be gleamed from the metrics such as over 15,000 views online and thousands of download by interested readers in just the half year or so after its completion last year. If this trend is an accurate gauge for the rising recognition of duckweed, I believe we will see an accelerated rate of improvement in our knowledge of duckweed biology and breakthroughs in its applications in the near future. The 5th International Conference may very well be the place to see these advances reported for the first time. I thus encourage everyone to consider attending this event so that you can share in the renaissance of this truly remarkable model plant.

In this DF issue, we have an interesting Historical Account on the life of William Griffith, the discoverer of the species *Wolffia microscopica*. It is an inspiring account by our committee member Sowjanya and Prof. Maheshwari (Jaipur, India) on the remarkable career of an exceptional botanist from the 19th Century. In spite of a life cut short at a young age of 35 by disease, he was able to make lasting contributions to duckweed research through his remarkable observational powers in plant anatomy and systematics. His tale of discovery is a reminder of all the wonders in Nature that are likely still awaiting discovery by the discerning eyes of a careful and knowledgeable observer.

For the Student Spotlight section, Kenneth Acosta, a talented young student in my group, contributed a personal description of what draws him to Science and specifically Duckweed Research. Together with the Database of new publications assembled by Prof. Klaus Appenroth, I hope these sections will provide both interesting and informative readings for everyone interested in duckweed research and applications. Finally I would like to remind everyone to consider sending in an interesting duckweed-related photo to be considered for the next DF's Cover Picture. We are looking forward to sharing what you think is exciting about duckweed with the community.

A big thanks to all the contributors for this issue of DF and I wish you a great year again.

Eric Lam, Chair of the ISCDRA

5th ICDRA: 3rd Circular

Lectures & Posters

Advances in duckweed genomics, physiology, microbiomes, ecosystems, ecotoxicology, nutrients, natural products, biomass production and other commercial applications. Registration Fee includes admission to all sessions, conference kit, coffee breaks, lunch and dinner on conference days, half day trip, transport from and back to airport

Conference Co-chairs
Marvin Edelman WIS, Israel
Asaph Aharoni WIS, Israel
Avi Levy WIS, Israel

International Committee
Eric Lam Rutgers U, USA
Klaus Appenroth FSU, Germany
Marcel Jansen UCC, Ireland
Sowjanya Sree CUK, India
Yubin Ma CAS, China
Tsipi Shoham GreenOnyx, Israel
Autar Mattoo USDA, USA

Local Committee
Ron Vunsh WIS, Israel
Uwe Heinig WIS, Israel
Sagit Meir WIS, Israel
Barak Cohen WIS, Israel
Miri Lapidot Hinoman, Israel

Coordinator
Talia Suissa
talias@Weizmann.ac.il

Sponsors
THE CHORAFAS INSTITUTE FOR SCIENTIFIC EXCHANGE
FACULTY OF BIOCHEMISTRY WIS, Israel
DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES WIS, Israel

Confirmed Speakers (so far)

* Keynotes

Asaph Aharoni, Israel*
Klaus Appenroth, Germany
Nikolai Borisjuk, China
Jay Cheng, USA*
Uwe Heinig, Israel
Marcel Jansen, Ireland*
Eric Lam, USA
Yubin Ma, China
Rob Martienssen, USA
Todd Michael, USA*
Tokitaka Oyama, Japan*
Ingo Schubert, Germany
Iris Shai, Israel
Sowjanya Sree, India*
Shuqing Xu, Germany

Registration Deadline
July 9th 2019

CONFERENCE WEBSITE http://www.weizmann.ac.il/conferences/DRA2019/

REGISTRATION http://www.weizmann.ac.il/conferences/DRA2019/registration

Research Topic in Frontiers in Chemistry

"Duckweed: Biological Chemistry and Applications"

Guest Editors:

Marvin Edelman, Rehovot, Israel; Klaus-J. Appenroth, Jena, Germany; K. Sowjanya Sree, Periye, Kerala, India.

The duckweeds (*Lemnaceae*) are a family of simple, higher plants at the far end of the monocotyledon subdivision. All of the 5 genera and 37 species are aquatic, mostly sweet water, mostly floaters or slightly submerged, and all have extremely reduced anatomies. The leaf-like frond of the largest is only about 1-1.5 cm in size, while the smallest possesses a simple thallus measuring about 0.5 mm. Duckweeds can flower, but normally propagate vegetatively, both in nature and in the laboratory, by budding from one or two meristematic zones on the frond or thallus. Some genera have adventitious roots with root caps which may be more for stability in the water than nutrient uptake; the latter task is managed by the entire underside of the frond. Some genera are devoid of roots altogether.

As its name implies, duckweeds are a favorite food source for fowl and several fish and other animals as well. Under optimal conditions in nature or in the laboratory, several species can double their biomass almost daily, with protein content reaching 40% or more of the dry weight. In controlled environments, they can be grown axenically under either autotrophic, mixotrophic, or heterotrophic conditions with defined inorganic or organic liquid medium, or on agar. In addition, the genomes of some duckweeds are among the smallest for a higher plant. Coupled with the increasing abilities of several groups to genetically transform various species of this aquatic family, think "upcoming model system" or "biotech applications."

Indeed, R&D and applications involving duckweeds are in bloom. Advances across the board in duckweed biochemistry, molecular biology/genomics and physiology, ecotoxicology and phytoremediation, interactions with different environments, ecosystems and climatic conditions, nutrients and natural products, biofuels and biomass production, are among the subject matters addressed in this Research Topic.

Link: https://www.frontiersin.org/research-topics/6799

Research Topic Articles:

Nutritional Value of the Duckweed Species of the Genus *Wolffia* (Lemnaceae) as Human Food

Klaus-J. Appenroth[1]*, K. Sowjanya Sree[2], Manuela Bog[3], Josef Ecker[4], Claudine Seeliger[4], Volker Böhm[5,6], Stefan Lorkowski[5,6], Katrin Sommer[7], Walter Vetter[7], Karla Tolzin-Banasch[8], Rita Kirmse[8], Matthias Leiterer[8], Christine Dawczynski[5,6], Gerhard Liebisch[9] and Gerhard Jahreis[5,6]

[1]Matthias Schleiden Institute, Plant Physiology, Friedrich Schiller University Jena, Jena, Germany; [2]Department of Environmental Science, Central University of Kerala, Kasaragod, India; [3]Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany; [4]Chair of Nutrition Physiology, Technical University Munich, Freising, Germany; [5]Institute of Nutritional Sciences, Friedrich Schiller University Jena, Jena, Germany; [6]Competence Cluster for Nutrition and Cardiovascular Health (nutriCARD) Halle-Jena-Leipzig, Jena, Germany; [7]Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany; [8]Thuringian State Institute of Agriculture, Jena, Germany; [9]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00483/full; doi.org/10.3389/fchem.2018.00483

Mass Production of *Lemna minor* and Its Amino Acid and Fatty Acid Profiles

Rina Chakrabarti[1]*, William D. Clark[2], Jai Gopal Sharma[3], Ravi Kumar Goswami[1], Avanish Kumar Shrivastav[3] and Douglas R. Tocher[2]

[1]Aqua Research Lab, Department of Zoology, University of Delhi, New Delhi, India; [2]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, Scotland; [3]Department of Biotechnology, Delhi Technological University, New Delhi, India

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00479/full; https://doi.org/10.3389/fchem.2018.00479

Structural and Biochemical Properties of Duckweed Surface Cuticle

Nikolai Borisjuk[1]*, Anton A. Peterson[1], Jiyang Lv[2], Guorun Qu[2], Qian Luo[2], Lei Shi[2], Guimin Chen[1], Olena Kishchenko[1], Yuzhen Zhou[1] and Jianxin Shi[2]*

[1]Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Science, Huaiyin Normal University, Huaian, China; [2]Joint International Research Laboratory of Metabolic and Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00317/full; https://doi.org/10.3389/fchem.2018.00317

Development of *Wolffia arrhiza* as a Producer for Recombinant Human Granulocyte Colony-Stimulating Factor

Pavel Khvatkov[1,2]*, Alexsey Firsov[1,3], Anastasiya Shvedova[1], Lyubov Shaloiko[3], Oleg Kozlov[3], Mariya Chernobrovkina[1], Alexander Pushin[1,2,3], Irina Tarasenko[3], Inna Chaban[1] and Sergey Dolgov[1,2,3]

[1]Laboratory of Plant Gene Engineering, All-Russia Research Institute of Agricultural Biotechnology, Russian Academy of Sciences, Moscow, Russia; [2]Sector of Plant Bioengineering, Nikita Botanical Gardens – National Scientific Centre, Russian Academy of Sciences, Yalta; [3]Laboratory of Expression Systems and Modification of the Plant Genome "BIOTRON", Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Puschino, Russia

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00304/full; https://doi.org/10.3389/fchem.2018.00304

Correlation of Apiose Levels and Growth Rates in Duckweeds

Débora Pagliuso[1], Adriana Grandis[1], Eglee S. Igarashi[1], Eric Lam[2]* and Marcos S. Buckeridge[1]*

[1]Laboratory of Plant Physiological Ecology, Department of Botany, Systems and Synthetic Biology Center, Institute of Biosciences, University of São Paulo, São Paulo, Brazil; [2]Department of Plant Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00291/full; https://doi.org/10.3389/fchem.2018.00291

Bacterial Production of Indole Related Compounds Reveals Their Role in Association Between Duckweeds and Endophytes

Sarah Gilbert[1], Jenny Xu[1], Kenneth Acosta[1], Alexander Poulev[1], Sarah Lebeis[2] and Eric Lam[1]*

[1]Department of Plant Biology, Rutgers University, New Brunswick, NJ, United States; [2]Department of Microbiology, University of Tennessee, Knoxville, TN, United States

Links: https://www.frontiersin.org/articles/10.3389/fchem.2018.00265/full; https://doi.org/10.3389/fchem.2018.00265

Effect of Exogenous General Plant Growth Regulators on the Growth of the Duckweed *Lemna minor*

Desi Utami, Ami Kawahata, Masayuki Sugawara, Rahul N. Jog, Kyoko Miwa and Masaaki Morikawa*

Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00251/full; https://doi.org/10.3389/fchem.2018.00251

Development of Efficient Protocols for Stable and Transient Gene Transformation for *Wolffia globosa* Using Agrobacterium

P. P. M. Heenatigala[1,2], Jingjing Yang[1], Anthony Bishopp[3], Zuoliang Sun[1], Gaojie Li[1], Sunjeet Kumar[1], Shiqi Hu[1], Zhigang Wu[1], Wei Lin[1], Lunguang Yao[4], Pengfei Duan[4] and Hongwei Hou[1]*

[1]The State Key Laboratory of Freshwater Ecology and Biotechnology, The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Wuhan, China; [2]Inland Aquatic Resources and Aquaculture Division, National Aquatic Resources Research and Development Agency, Colombo, Sri Lanka; [3]Centre for Plant Integrative Biology, University of Nottingham, Nottingham, UK; [4]Collaborative Innovation Center of Water Security for Water Source Region of Mid-Line of South-to-North Diversion Project, College of Agricultural Engineering, Nanyang Normal University, Nanyang, China

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00227/full; https://doi.org/10.3389/fchem.2018.00227

Genomes and Transcriptomes of Duckweeds

Dong An[1], Changsheng Li[2], Yong Zhou[2], Yongrui Wu[2] and Wenqin Wang1*

[1]Department of Plant Sciences, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China; [2]National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00230/full; https://doi.org/10.3389/fchem.2018.00230

Competition Between *Lemna minuta*, *Lemna minor*, and *Azolla filiculoides*. Growing Fast or Being Steadfast?

Simona Paolacci[1,2]*, Marcel A. K. Jansen[1,2] and Simon Harrison[1,2]

[1]Enterprise Center Distillery Field, School of Biological, Earth and Environmental Sciences, University College of Cork, Cork, Ireland; [2]Environmental Research Institute, University College of Cork, Cork, Ireland

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00207/full; https://doi.org/10.3389/fchem.2018.00207

Metabolic Patterns in *Spirodela polyrhiza* Revealed by ^{15}N Stable Isotope Labeling of Amino Acids in Photoautotrophic, Heterotrophic, and Mixotrophic Growth Conditions

Erin M. Evans[1,2], Dana M. Freund[1,2], Veronica M. Sondervan[1], Jerry D. Cohen[1,2] and Adrian D. Hegeman[1,2,3]*

[1]Department of Horticultural Science, University of Minnesota, Twin Cities, Saint Paul, MN, United States; [2]Plant and Microbial Genomics Institute, University of Minnesota, Twin Cities, Saint Paul, MN, United States; [3]Department of Plant and Microbial Biology, University of Minnesota, Twin Cities, Saint Paul, MN, United States

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00191/full; https://doi.org/10.3389/fchem.2018.00191

A Comparison of Growth on Mercuric Chloride for Three Lemnaceae Species Reveals Differences in Growth Dynamics That Effect Their Suitability for Use in Either Monitoring or Remediating Ecosystems Contaminated With Mercury

Jingjing Yang[1], Gaojie Li[1], Anthony Bishopp[2], P. P. M. Heenatigala[1], Shiqi Hu[1], Yan Chen[1], Zhigang Wu[1], Sunjeet Kumar[1], Pengfei Duan[3], Lunguang Yao[3] and Hongwei Hou[1]*

[1]The State Key Laboratory of Freshwater Ecology and Biotechnology, The Key Laboratory of Aquatic Biodiversity and Conservation of Chinese Academy of Sciences, Institute of Hydrobiology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Wuhan, China; [2]Centre for Plant Integrative Biology, University of Nottingham, Nottingham, United Kingdom; [3]Collaborative Innovation Center of Water Security for Water Source Region of Mid-line of South-to-North Diversion Project, College of Agricultural Engineering, Nanyang Normal University, Henan, China

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00112/full; https://doi.org/10.3389/fchem.2018.00112

Expression and Immunogenicity of M2e Peptide of Avian Influenza Virus H5N1 Fused to Ricin Toxin B Chain Produced in Duckweed Plants

Aleksey Firsov[1]*, Irina Tarasenko[1], Tatiana Mitiouchkina[1], Lyubov Shaloiko[1], Oleg Kozlov[1], Leonid Vinokurov[1], Ekaterina Rasskazova[1], Arkadii Murashev[1], Alexander Vainstein[2] and Sergey Dolgov[1]

[1]Institute of Bioorganic Chemistry (RAS), Moscow, Russia; [2]Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot, Israel

Links:https://www.frontiersin.org/articles/10.3389/fchem.2018.00022/full; https://doi.org/10.3389/fchem.2018.00022

Historical account: William Griffith, Esq., F.L.S. (1810-1845)

the discoverer of *Wolffia microscopica*, a unique duckweed species endemic to India

K. Sowjanya Sree[1] and Satish C. Maheshwari[2]

[1]Dept. Of Environmental Science, Central University of Kerala, Periye, India (ksowsree9@cukerala.ac.in; ksowsree@gmail.com)

[2]Centre for Converging Technologies, University of Rajasthan, Jaipur, India (profsatishcmaheshwari@gmail.com)

The Indian subcontinent through its long history has witnessed the rise and fall of many kingdoms and empires. The British ruled India for many decades. Although their rule ended in 1947 after a long freedom struggle, in many ways we also benefitted from their presence in India. We were fortunate enough to have many explorers, scholars and among them several botanists. One of the earliest among them was William Griffith during the rule of East India Company (it is this trading company that later paved way for the establishment of the Government of India which functioned from 1858 until 1947 under British rule). William Griffith was born on 4th March, 1810 in Ham, Surrey, England. Although he had studied medicine, he had a great admiration towards plants. This led him to learn botany privately at the garden of the apothecaries at Chelsea. His excellence in the field had earned him the Linnean Gold Medal from the society of apothecaries in 1830. Shortly thereafter Griffith was appointed as the Assistant Surgeon in the Madras Presidency of East India Company. Fortunately, coinciding with his interests, he served for a short while as the superintendent of the Company's botanical garden at Calcutta (presently called Kolkata), India (Lang, 1913). A memorial stands till date in the Indian Botanical Garden in Kolkata in memory of William Griffith.

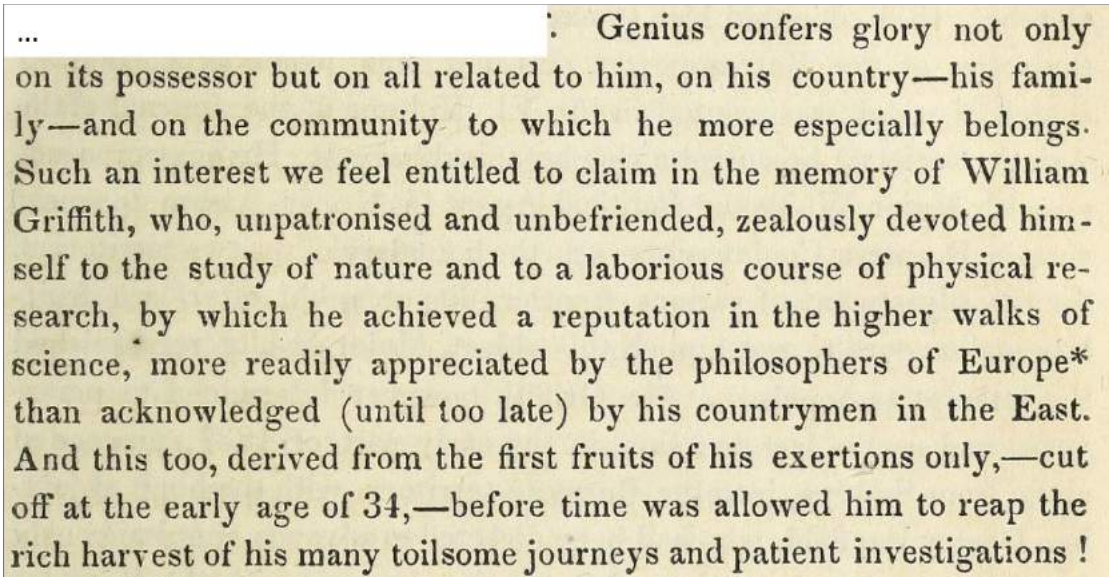
William Griffith (1843), reproduced from Lang (1913)

Memorial in honour of William Griffith at the Indian Botanical Garden, Kolkata

William Griffith was a great morphologist of his time who made keen observations of the live samples, in most cases collected by himself. He was especially interested in flowering samples and often investigated the ovaries and the ovules. Griffith worked for more than one third of his life in India,

investigating the flora of Southern Asia. Major contributions of his service came from his botanical expeditions to treacherous places in India, Burma, Afghanistan among many others (Griffith, 1847; Lang, 1913). Apart from this, Griffith was also interested in birds and he made use of these several excursions to add to his ornithological collection as well (M' Clelland, 1845).

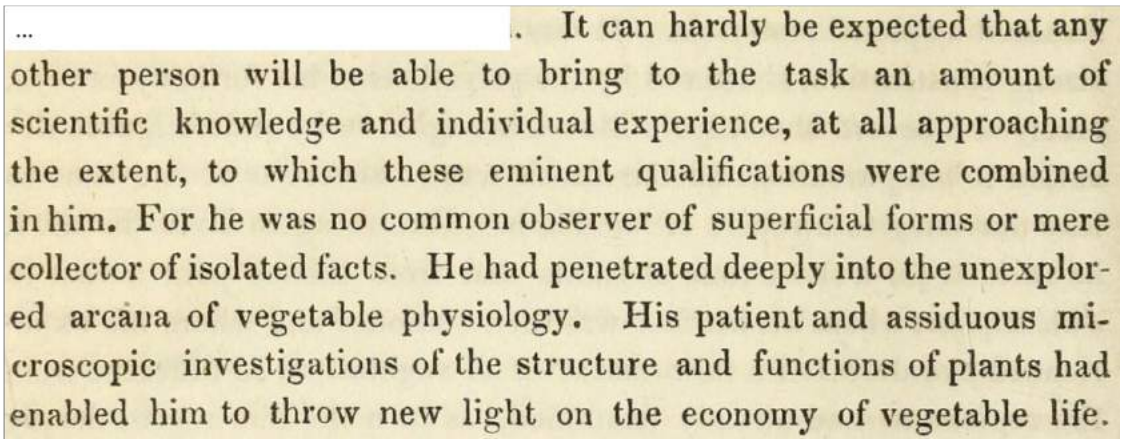
An excerpt from biographical memoir of the late William Griffith (Committee of the Madras Literary Society, 1847):



... . Genius confers glory not only on its possessor but on all related to him, on his country—his family—and on the community to which he more especially belongs. Such an interest we feel entitled to claim in the memory of William Griffith, who, unpatronised and unbefriended, zealously devoted himself to the study of nature and to a laborious course of physical research, by which he achieved a reputation in the higher walks of science, more readily appreciated by the philosophers of Europe* than acknowledged (until too late) by his countrymen in the East. And this too, derived from the first fruits of his exertions only,—cut off at the early age of 34,—before time was allowed him to reap the rich harvest of his many toilsome journeys and patient investigations !

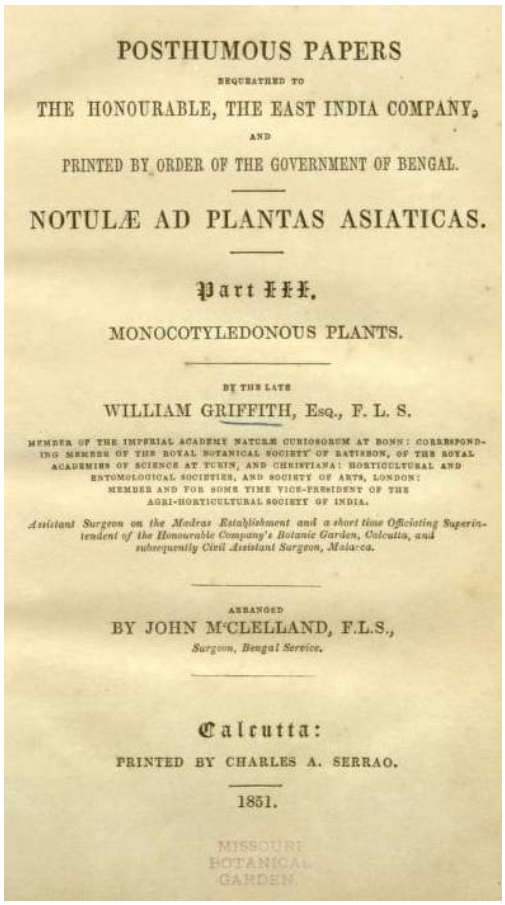
In 1835, Griffith, in a team together with Dr. Nathaniel Wallich (Botanist) and Dr. John M'Clelland (Geologist and a good friend of Griffith), was deputed to examine the tea forests of Assam (Griffith, 1847). Dr. Wallich, in fact, knew Griffith as an extraordinarily talented young colleague even before his arrival to India. Griffith, who was a pupil of Professor Lindley and Mr. Robert Brown at the London University, had contributed a beautiful drawing of the plant, *Phytocrene gigantea* to Dr. Wallich's Plantae Asiaticae Rariores (Wallich, 1832; Committee of the Madras Literary Society, 1847). Griffith's detailed scientific investigations of the tea plants in Assam played an important role in the East India Company's decision to develop tea estates in Assam and other similar geographical locations in India. Thanks to Griffith that the world today enjoys the refreshing Assam tea (Lang, 1913).

An excerpt from biographical memoir of the late William Griffith (Committee of the Madras Literary Society, 1847):



... . It can hardly be expected that any other person will be able to bring to the task an amount of scientific knowledge and individual experience, at all approaching the extent, to which these eminent qualifications were combined in him. For he was no common observer of superficial forms or mere collector of isolated facts. He had penetrated deeply into the unexplored arcana of vegetable physiology. His patient and assiduous microscopic investigations of the structure and functions of plants had enabled him to throw new light on the economy of vegetable life.

In this article we would like to draw attention to his discovery of the presently known *Wolffia microscopica*, a unique species of duckweed from Calcutta and Serampore, Bengal in 1838 that is endemic to the Indian sub-continent, possibly on one of his several explorations in India. Griffith had originally named it as *Grantia microscopica* sp. novo and genus novo in honour of James William Grant, Esq. for his success as "first-rate microscopic observer" (Voigt, 1845; Griffith 1851a, b). Voigt's manuscript, however, contemplated on its inclusion in the genus *Wolffia* Horkel ex Schleid., belonging to the family Lemnaceae (Voigt, 1845).



POSTHUMOUS PAPERS

BEQUEATHED TO

THE HONOURABLE, THE EAST INDIA COMPANY,

AND

PRINTED BY ORDER OF THE GOVERNMENT OF BENGAL.

NOTULÆ AD PLANTAS ASIATICAS.

Part III.

MONOCOTYLEDONOUS PLANTS.

BY THE LATE

WILLIAM GRIFFITH, Esq., F. L. S.

MEMBER OF THE IMPERIAL ACADEMY NATURÆ CURIOSORUM AT BONN: CORRESPONDING MEMBER OF THE ROYAL BOTANICAL SOCIETY OF RATISBON, OF THE ROYAL ACADEMIES OF SCIENCE AT TURIN, AND CHRISTIANA: HORTICULTURAL AND ENTOMOLOGICAL SOCIETIES, AND SOCIETY OF ARTS, LONDON: MEMBER AND FOR SOME TIME VICE-PRESIDENT OF THE AGRI-HORTICULTURAL SOCIETY OF INDIA.

Assistant Surgeon on the Madras Establishment and a short time Officiating Superintendent of the Honourable Company's Botanic Garden, Calcutta, and subsequently Civil Assistant Surgeon, Malacca.

ARRANGED

BY JOHN M'CLELLAND, F.L.S.,

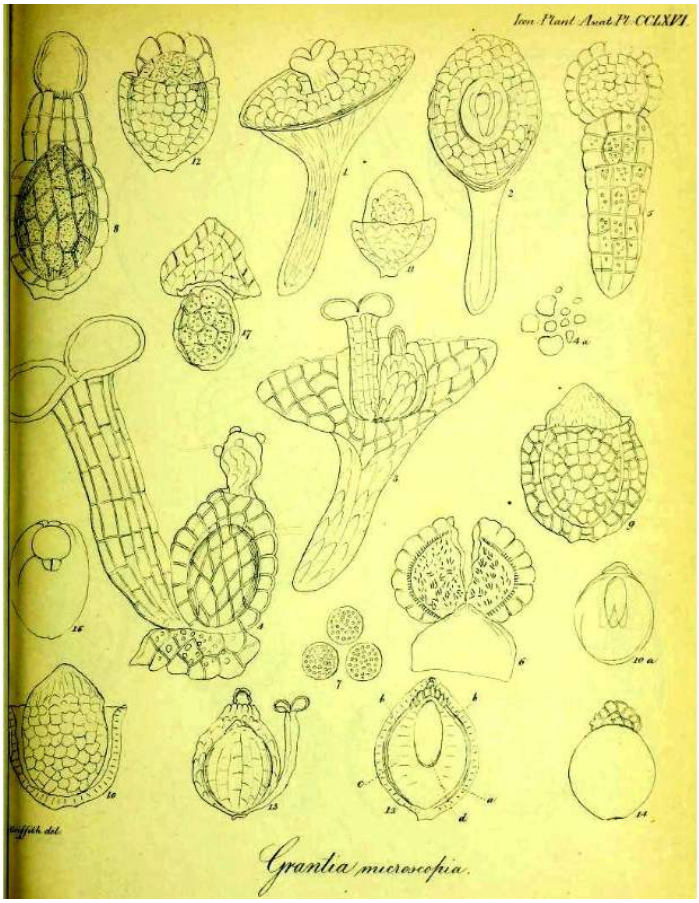
Surgeon, Bengal Service.

Calcutta:

PRINTED BY CHARLES A. SERRAO.

1851.

MISSOURI BOTANICAL GARDEN



Left: Posthumous papers of William Griffith (1851a). Right: Drawings of *Wolffia microscopica* (then named as *Grantia microscopica*) by William Griffith (1851b); The figure available from the online resource was delicate to be reproduced and hence, the contrast of the figure had to be increased.

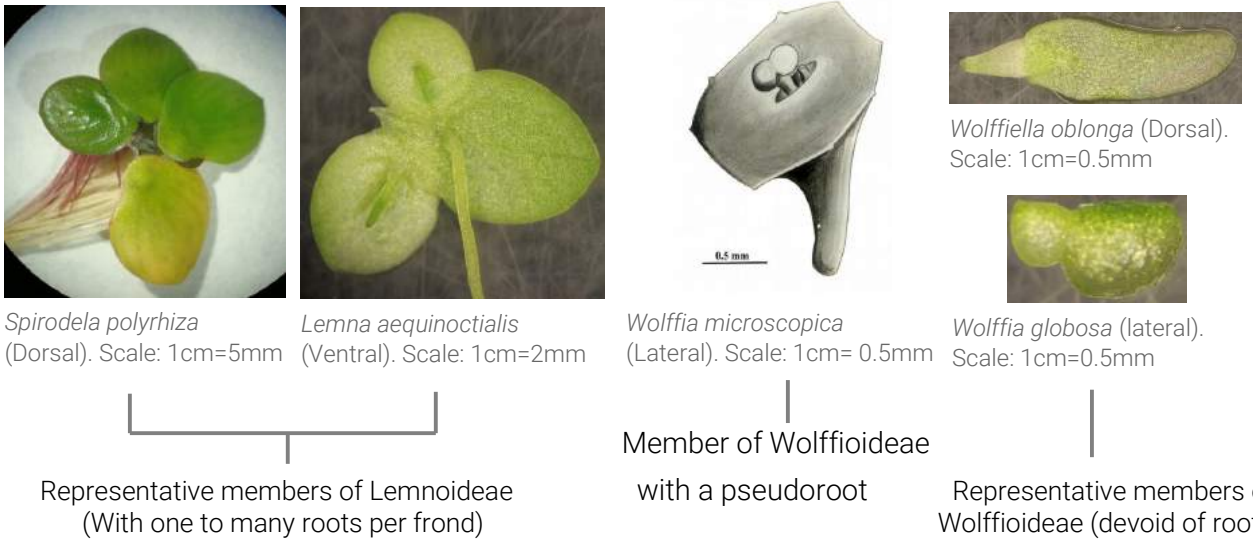
Describing the genus Grantia, Griffith had an interesting remark about this family of plants:

"To those indeed who estimate the interest of a plant merely from the size of its flower and the gorgeousness of its colouring, this family is not likely to be attractive. These minute plants are however interesting as exhibiting indications of wonderful design in the adaption of the most simple structure to the highest functions of vegetable life." (Griffith 1851a).

With his first hand microscopic observations of the collected samples, Griffith presented detailed descriptions of *W. microscopica* in both vegetative as well as reproductive phases of its life. Already in these first descriptions of this plant by Griffith, the unique ventral protrusion present in this plant species was discussed. Griffith has described it as an included part and had discussed on whether

this included part be considered as a root (Griffith 1851a). In a very recent study on this unique duckweed species, Sree et al. (2015) have described this ventral projection as a pseudoroot.

The presence of this included part as Griffith called it, imparts a unique evolutionary significance to the duckweed species, *W. microscopica*. The two sub-families of Lemnaceae can be clearly demarcated based on the presence or absence of roots with the Lemnoideae having one to many roots and the Wolffioideae without any roots. Interestingly, out of all the members of the sub family Wolffioideae including the genera Wolffia and Wolffiella, *W. microscopica* is one of the species with a ventral projection at the node, which suggests certain links towards this included part being a remnant of the roots from its relatives belonging to Lemnoideae. On an evolutionary scale, *W. microscopica* could act as a connecting link between the root-bearing Lemnoideae and the rootless Wolffioideae. The very thought of whether this included part can be considered as a root and the comparison of this species with the already described species of Lemna, shows the immense scholarly potential of Griffith on working with these tiny plants.



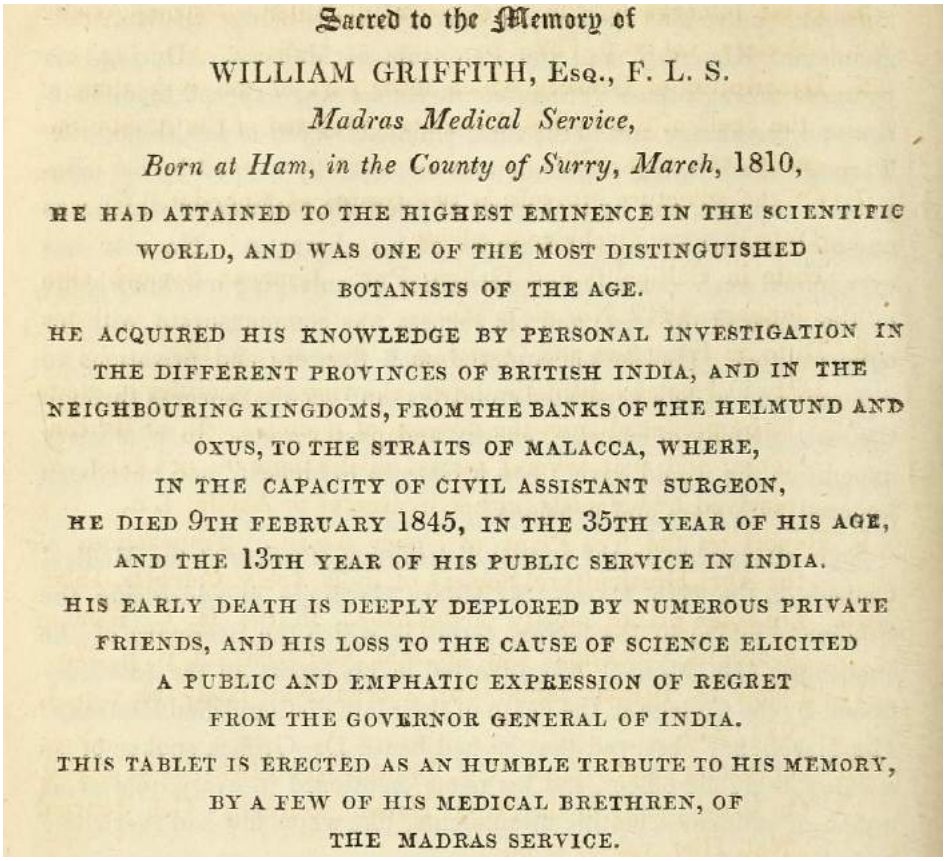
Wolffia microscopica: a possible connecting link between the two subfamilies Lemnoideae and Wolffioideae

The detailed descriptions of different stages of this unique plant species by Griffith already in the 19th Century had contributed immensely to its later inclusion and reclassification into the already existing genus, Wolffia Horkel ex Schleid.

Genus Wolffia could have been Genus Grantia: In the late 20th century, it was realised that the type specimen used for typification of Wolffia Horkel ex Schleid. i.e., *Wolffia delilii* was later identified as belonging to Wolffiella, *Wolffiella hyalina*. This movement of the type specimen to another genus, led to a discussion on the nomenclature of the genera: Wolffia and Wolffiella. One of the options was to rename the genus Wolffia with the available generic name, Grantia Griff. ex Voigt. It was, however, decided to forego this option as this renaming would have directed the replacement of the nomenclature of 13 of the then known 15 species under the two genera (Hartog, 1969). As a consequence, the contribution of William Griffith to Lemnaceae could not find the place it deserves.

Unfortunately, the works of Griffith were published only posthumously because of his sudden death in Malacca at a very young age of 35 years on 9th February, 1845, caused by the attack of a deadly

pathogen. He was married to Ms. Henderson, who was the sister of his brother's wife, only a few months before on 21st September, 1844 (M'Clelland, 1845).


Sacred to the Memory of

WILLIAM GRIFFITH, Esq., F. L. S.

Madras Medical Service,

Born at Ham, in the County of Surry, March, 1810,

HE HAD ATTAINED TO THE HIGHEST EMINENCE IN THE SCIENTIFIC WORLD, AND WAS ONE OF THE MOST DISTINGUISHED BOTANISTS OF THE AGE.

HE ACQUIRED HIS KNOWLEDGE BY PERSONAL INVESTIGATION IN THE DIFFERENT PROVINCES OF BRITISH INDIA, AND IN THE NEIGHBOURING KINGDOMS, FROM THE BANKS OF THE HELMUND AND OXUS, TO THE STRAITS OF MALACCA, WHERE, IN THE CAPACITY OF CIVIL ASSISTANT SURGEON, HE DIED 9TH FEBRUARY 1845, IN THE 35TH YEAR OF HIS AGE, AND THE 13TH YEAR OF HIS PUBLIC SERVICE IN INDIA.

HIS EARLY DEATH IS DEEPLY DEPLORED BY NUMEROUS PRIVATE FRIENDS, AND HIS LOSS TO THE CAUSE OF SCIENCE ELICITED A PUBLIC AND EMPHATIC EXPRESSION OF REGRET FROM THE GOVERNOR GENERAL OF INDIA.

THIS TABLET IS ERECTED AS AN HUMBLE TRIBUTE TO HIS MEMORY, BY A FEW OF HIS MEDICAL BRETHREN, OF THE MADRAS SERVICE.

A mural tablet in the Cathedral Church of St. George, Madras (now called Chennai), India as a tribute to William Griffith

References:

- Committee of the Madras Literary Society, Biographical memoir of the late William Griffith. The Madras Journal of Literature and Science, 1847, 14: 187-197.
- den Hartog, C., Nomina Conservanda Proposita. Taxon, 1969, 18: 591-592.
- Griffith, W., 1847. Journals of travel in Assam, Burma, Bootan, Affghanistan and the neighbouring countries. Arranged by M'Clelland, J. Bishop College Press, Calcutta, India.
- Griffith, W., Notulae ad plantas asiaticas. Bishop College Press, Calcutta, India, 1851a, 3, 223-229.
- Griffith, W., Icones plantarum asiaticarum. Bishop College Press, Calcutta, India, 1851b, 3, plates CCLXVI-CCLXVIII.
- Lang, W. H., William Griffith 1810—1845. In *Makers of British Botany* (ed. Oliver, F. W.), Cambridge University Press, Cambridge, UK, 1913, 178-191.
- M'Clelland, J., Memoir of the late William Griffith, Esq. F.L.S. Assistant Surgeon in the honourable East India Company's Service on the Madras Establishment. Journal of the Agricultural and Horticultural Society of India, 1845, 4: 1-39.
- Sree, K. S., Maheshwari, S. C., Boka, K., Khurana, J. P., Keresztes, A., Appenroth, K-J., The duckweed *Wolffia microscopica*: A unique aquatic monocot. Flora, 2015, 210: 31-39.
- Voigt, J. O., Hortus Suburbanus Calcuttensis. Bishop College Press, Calcutta, India, 1845, pp. 692.
- Wallich, N., 1832. Plantae Asiaticae Rariores. Treuttel and Wuertz and Richter, London, UK.

Student Spotlight: Kenneth Acosta

Rutgers, the State University of New Jersey, New Brunswick, NJ, USA (ikennethacosta@gmail.com)

My first childhood encounters with science include watching the "Bill Nye The Science Guy" TV show and a failed attempt to observe leaf cells under a microscope. I retained this scientific inclination throughout my education. Eventually, my curiosity of how living things worked and a need for a principle-based understanding of biology led me to graduate with my BS in Biochemistry from Rutgers University.

Top: In the Lam laboratory.
Bottom: *S. polyrhiza* 9501 fronds (left) and turions (right).

For my undergraduate research I was fortunate enough to be given the opportunity to join Dr. Eric Lam's laboratory. I spent a lot of time in the laboratory my last two years volunteering during winter and summer breaks. During this time I was introduced to duckweed and began working on a project looking at Methionine levels in different duckweed species.

Conducting research was something new and completely different than what I expected. It involved a different way of thinking and the process of experimental design was completely new to me. As I finished my undergraduate education I knew I still had a lot to learn. At the time a company collaborated with the laboratory to perform a large-scale duckweed screen and by then I had gained a sufficient amount of expertise in handling duckweed. I saw this as an opportunity to keep doing research and learn how to properly conduct it.

Since then, I was able to complete the large-scale screen, assist in several different duckweed projects, train undergraduate students to work with duckweed, manage a laboratory and the Rutgers Duckweed Stock Cooperative (RDSC), the largest collection of living duckweed strains in the world. Duckweed has given me the opportunity to go on many duckweed-hunting trips with the most notable one being to Argentina. Perhaps, the biggest thing duckweed has given me, along with proper guidance from my mentor Dr. Eric Lam, is the ability to conduct biological research. By trying

Duckweed hunting and wastewater remediation in Salta, Argentina. Left: Eduardo Mercovich; Right: Kenneth Acosta.

to understand duckweed biology I have come to learn how to turn a biological question/observation into a well-designed experiment that produces objective data. I have come to appreciate the creativity and freedom that scientific research offers and that one of the biggest rewards of research, if not the biggest, is discovery. While I have learned a great deal I know I still have a lot more to learn about doing research and the scientific process. In the end I would like to run my own laboratory while teaching others how to perform scientific research and making some interesting discoveries along the way.

I started my PhD studies during the 2018 fall semester in Dr. Eric Lam's laboratory at Rutgers University. My research involves studying the duckweed microbiome. Most of plant microbiome research has focused on terrestrial plants. These studies have revealed a conservation of plant microbiota at higher taxonomic levels and their ability to re-establish themselves on their plant host. Duckweed has a less complex architecture than other plants, small size, fast growth rate, and an aquatic habitat. As the field moves to a more mechanistic understanding of plant microbiota assembly I believe duckweed can be a facile model system to study the mechanisms involved in the establishment of plant microbial communities. The knowledge gained from this type of investigation should help to create more environmentally-friendly and sustainable crop management approaches.

When I am able to find some time outside the lab, I enjoy working on cars, spending time with loved ones, exercising, and more recently trying to teach myself programming.

From the database

Highlights

Several of the published papers in a Research Topic section of Frontiers in Chemistry “Duckweed: Biological Chemistry and Applications” are “Highlights”. We have presented them in a separate chapter of this issue of “Duckweed Forum”.

Biotechnology

Utilization of nutrient rich duckweed to create N, P Co-doped porous carbons for high performance supercapacitors

Wang, T; Zhang, JZ; Hou, QH; Wang, S (2018) JOURNAL OF ALLOYS AND COMPOUNDS

Fast-growing duckweeds are a potential source for useful biomass because of enrichment of N and P. Herein, we demonstrate a facile procedure for converting the duckweed biomass into N, P co-doped porous carbon material, a promising high-performance electrode material for use in supercapacitors. Under an optimum KOH activation temperature (750 degrees C), the carbonized duckweed sample with enriched N, P contents of 3.39 at.% and 0.25 at.%, respectively, exhibits a hierarchical mesoporous structure with a high specific surface area of similar to 1636m^2g^{-1}. The duckweed electrode displays a specific capacitance of 315.2 Fg^{-1} in the three-electrode electrochemical configuration at a current density of 1Ag^{-1}. In addition, the symmetric duckweed electrode offers a maximum capacitance of 225.0 Fg^{-1} and energy density of 25.3 Wh kg^{-1} as well as a high stability (about 95% of capacitance retention after 10 000 cycles at 5 A g^{-1}). This proof of concept provides a promising route for mitigating "biohazardous" duckweed blooms and for generating high capacity, low-cost electrodes for supercapacitors as a "treasure" from environmentally friendly raw materials.

Turion, an innovative duckweed-based starch production system for economical biofuel manufacture

Xu, YL; Fang, Y; Li, Q; Yang, GL; Guo, L; Chen, GK; Tan, L; He, KZ; Jin, YL; Zhao, H (2018) Industrial Crops and Products 124: 108-114

Turion is a kind of dormant tissue from *Spirodela polyrhiza* (L) Schleid and represents another duckweed-based starch production system, which owns several potential technical merits than the conventional one. This paper systematically investigated the physiological, biochemical and production characteristics of turion and preliminarily evaluate its feasibility for bioetbanol production. Turion productivity of 3.78 g/m^2/d and starch productivity of 2.90 g/m^2/d was achieved in strain 0196. Full-component analysis revealed that turion is high quality substrate as it contained high starch content (65.63%) and low lignocellulose content (12.82%). Besides, turion can be continuously produced by strain 0196 up to 6 weeks, indicating a sustainable manufacture of them is possible. Finally, harvested turion was used for ethanol fermentation for the first time with an

ethanol yield of 0.34 g g^{-1}(turion), resulted in an annual yield of 4,69 t/ha. This research elucidated that turion from duckweed is a novel biomass for biofuel production.

Catalytic conversion of duckweed to methyl levulinate in the presence of acidic ionic liquids

Chen, ZJ; Ma, XY; Xu, L; Wang, Y; Long, JX (2018) Bioresource Technology 268: 488-495

In this study, an efficient strategy is proposed for selective methyl levulinate production from duckweed, a typical fast-growing aquatic microalgae in warm and humid regions, in the presence of acidic ionic liquids (ILs). The results show that IL structure has a significant effect on its acidic strength, which finally determines the process efficiency for levulinate methyl generation. With the optimized catalyst of $[C_3H_6SO_3HPy]HSO_4$, 88.0% duckweed is consumed, resulting in a comparable methyl levulinate yield of 73.7% and a process efficiency of 81.8% at 170 °C particularly, it is significantly temperature-dependent. In addition, solvent has a remarkable intensified effect on the process efficiency, which dramatically decreases from 81.8 to 53.7% when methanol is replaced by water.

Remark of "Duckweed Forum": duckweed are not microalgae but angiosperms (flowering plants)!

Duckweed (*Lemna minor*) is a novel natural inducer of cellulase production in *Trichoderma reesei*

Li, Chen; Li, Demao; Feng, Jun; Fan, Xiang; Chen, Shulin; Zhang, Dongyuan; He, Ronglin (2018) Journal of Bioscience and Bioengineering DOI:10.1016/j.jbiosc.2018.09.017

An inducer is crucial for cellulase production. In this study, duckweed was used as an inducer of cellulase production by *Trichoderma reesei* RUT C30. In a reaction induced by 50g/L duckweed in shake flasks, the filter-paper activity (FPA) reached 6.5FPU/mL, a value comparable to that induced by avicel. The enzyme-hydrolysis rate induced by steam-exploded corn stalk was 54.2%, representing a 28% improvement over that induced by avicel. The duckweed starch was hydrolyzed to glucose, which was subsequently used for biomass accumulation during the fermentation process. Furthermore, to optimize the control of the fermentation process, a combined substrate of avicel and duckweed was used to induce cellulase production by *T. reesei* RUT C30. The cellulase production and hydrolysis rates of the combined substrate, compared with avicel alone, were 39.6% and 36.7% higher, respectively. The results of this study suggest that duckweed is a good inducer of cellulase production in *T. reesei*, and it might aid in decreasing the cost of lignocellulosic materials hydrolysis.

High performance duckweed-derived carbon support to anchor NiFe electrocatalysts for efficient solar energy driven water splitting

Kumar, A; Chaudhary, DK; Parvin, S; Bhattacharyya, S (2018) Journal of Material Chemistry A 6: 18948-18959

Solar-energy-driven overall water splitting using sustainable energy resources is extremely desirable for high purity hydrogen fuel production, and one of the ways is to couple cost-effective solar cells in series with earth-abundant electrocatalysts for oxygen and hydrogen evolution reactions, OER and HER, respectively. Developing highly efficient and earth-abundant electrocatalysts however remains

one of the grand challenges. Herein, we developed biomass (duckweed, DW) derived N,S-doped mesoporous carbon matrix supported NiFe-alloy nanoparticles (NPs) as efficient electrocatalysts for overall water splitting. While the annealed catalyst required 267 mV overpotential at 10 mA cm^{-2} for the OER, the best HER performance was demonstrated by the unannealed electrocatalyst requiring 106 mV at -10 mA cm^{-2} in 1 M KOH. For overall water splitting, this couple required only 1.61 V cell voltage to deliver 10 mA cm^{-2}, with continuous release of O_2 and H_2 gas bubbles for more than 200 h. On integrating with perovskite solar cells, the homologous DW electrolyzer exhibited unassisted solar-energy-driven overall water splitting with a solar-to-hydrogen (STH) conversion efficiency of 9.7%.

Effect of pH and temperature on microbial community structure and carboxylic acid yield during the acidogenic digestion of duckweed

Calicioglu, O; Shreve, MJ; Richard, TL; Brennan, RA (2018) Biotechnology for Biofuels 11, Article Number: 275

Duckweeds (Lemnaceae) are efficient aquatic plants for wastewater treatment due to their high nutrient-uptake capabilities and resilience to severe environmental conditions. Combined with their rapid growth rates, high starch, and low lignin contents, duckweeds have also gained popularity as a biofuel feedstock for thermochemical conversion and alcohol fermentation. However, studies on the acidogenic anaerobic digestion of duckweed into carboxylic acids, another group of chemicals, which are precursors of higher-value chemicals and biofuels, are lacking. In this study, a series of laboratory batch experiments were performed to determine the favorable operating conditions (i.e., temperature and pH) to maximize carboxylic acid production from wastewater-derived duckweed during acidogenic digestion. Batch reactors with 25g/l solid loading were operated anaerobically for 21 days under mesophilic (35°C) or thermophilic (55°C) conditions at an acidic (5.3) or basic (9.2) pH. At the conclusion of the experiment, the dominant microbial communities under various operating conditions were assessed using high-throughput sequencing. The highest duckweed-carboxylic acid conversion of 388±28 mg acetic acid equivalent per gram volatile solids was observed under mesophilic and basic conditions, with an average production rate of 0.59 g/l/day. This result is comparable to those reported for acidogenic digestion of other organics such as food waste. The superior performance observed under these conditions was attributed to both chemical treatment and microbial bioconversion. Hydrogen recovery was only observed under acidic thermophilic conditions, as 23.5 ± 0.5 ml/g of duckweed volatile solids added. More than temperature, pH controlled the overall structure of the microbial communities. For instance, differentially abundant enrichments of *Veillonellaceae acidaminococcus* were observed in acidic samples, whereas enrichments of *Clostridiaceae alkaliphilus* were found in the basic samples. Acidic mesophilic conditions were found to enrich acetoclastic methanogenic populations over processing times longer than 10 days. Operating conditions have a significant effect on the yield and composition of the end products resulting from acidogenic digestion of duckweed. Wastewater-derived duckweed is a technically feasible alternative feedstock for the production of advanced biofuel precursors; however, techno-economic analysis is needed to determine integrated full-scale system feasibility and economic viability.

Hydrotreatment of bio-oil distillates produced from pyrolysis and hydrothermal liquefaction of duckweed: A comparison study

Wang, F; Tian, Y; Zhang, CC; Xu, YP; Duan, PG (2018) Science of the Total Environment 636: 953-962

A comprehensive comparison of hydrothermal liquefaction (HTL) to the pyrolysis of duckweed was conducted to determine the yields and components of the crude bio-oils and their distillates. The upgrading behaviors of the distillates were thoroughly investigated with the use of used engine oil as a solvent. With all other variables fixed, HTL produced crude bio-oil with a lower H/C ratio (1.28 ± 0.03) than pyrolysis did (1.45 ± 0.04). However, its distillates had a higher H/C ratio (1.60 ± 0.05) and total yield (66.1 ± 2.0 wt %) than pyrolysis (1.46 ± 0.04 and 47.2 ±.4 wt %, respectively). Phenolics and nitrogenous heterocycles constituted relatively major proportions of the two crude bio-oils and most of their distillates. Obvious differences in molecular composition between the two crude bio-oils and their distillates were ascribed to the distinct impacts of HTL and pyrolysis and were affected by the distillate temperature. Co-hydro treating with used engine oil (UEO) provided the upgraded bio-oils much higher H/C ratios (similar to 1.78 ± 0.05) and higher heating values (similar to 45.5 ± 1.4 MJ . kg^{-1}), as well as much lower contents of N, O and S compared to their initial distillates. Aromatics and alkanes constituted a large proportion in most of upgraded bio-oils. N removal from the pyrolysis distillates was easier than from the HTL distillates. Distinct differences in yields and molecular compositions for the upgraded bio-oils were also attributed to the different influences associated with the two conversion routes.

Enhancing bioconversion potential of duckweed by acid and hydrogen peroxide pretreatment methods to improve biofuel productivity

Gonen, C (2018) Sugar Tech 20: 474-481

One of the main triggers of the climate change is the consumption of fossil derivative fuel to satisfy the energy need, and also it is clearly known that the future of the fossil fuel supply is limited. That is why, finding and using alternative, sustainable, renewable, and eco-friendly energy sources are inevitable to fight climate change and to reduce the global warming. Biomass is a well-known renewable material for energy production and is called as biofuels. There are some limitations to utilize the biomass effectively, because of the structure of their molecular forms. For this very reason, the pretreatment pathways to increase the biomass sugar concentration potential to increase the bioconversion potential are attempted in this study. Two different pretreatment methods, i.e., hydrogen peroxide and acid, were applied to biomass, which in this case is duckweed, taken from artificial pond at Nigde, Turkey. In order to determine important factors of the processes, Plackett-Burman design was used. Chemicals dosages, timing, temperature, solid ratio, and mesh size are identified using this methodology to obtain the interested results. Box-Behnken statistical design method was applied to make the optimization of the factors chosen from the factorial design. Consequently, Box-Behnken test indicated that acid pretreatment method showed slightly better results than the hydrogen peroxide application per total sugar concentration, which are 0.60 and 0.48 g/L, respectively.

Ecology

Are alien species necessarily stress sensitive? A case study on *Lemna minuta* and *Lemna minor*

Paolacci, S; Harrison, S; Jansen, MAK (2018) FLORA 249: 31-39

It is widely assumed that environmental stressors contribute to the protection of habitats from invasion by alien species, and that native species are better stress-tolerators. This assumption was tested by comparing the performance of the invasive alien *Lemna minuta* Kunth with that of the co-generic native *L. minor* Linnaeus, under several environmental stressors. The effects of temperature and drought, important determinants of the distribution of Lemnaceae, on growth and photosynthesis were explored. Also, tolerance to, and accumulation of aluminium and copper were studied. Finally, tolerance to Reactive Oxygen Species (ROS) was compared by growing the plants at different concentrations of the ROS generator paraquat (methyl-viologen). The present study shows that specific stressors (such as low temperature in this study) disproportionally affect growth of alien *L. minuta*. Yet, in the case of three other stressors (aluminium, copper, drought), effects on biomass growth are similar for the two species, or they are even less severe on *L. minuta*. Remarkably, *L. minuta* dries out faster, and accumulates more metals than *L. minor*, suggesting that the former species has a greater physiological tolerance, whilst the latter species has an avoidance strategy. Thus, the current study on the role of environmental stressors in facilitating alien invasions does not support the notion that the presence of stressors impedes alien invasions, but rather shows that differences between an alien and a native species are multi-faceted, and stressor-specific.

Skimming the surface: duckweed as a model system in ecology and evolution

Laird, Robert A; Barks, Patrick M (2018) American Journal of Botany 105: 1962-1966

We highlight the potential of one particular group—duckweeds—as a model system for research in the disciplines of ecology and evolution. Duckweeds are floating or submergent aquatic monocots that comprise the “simplest and smallest of flowering plants” (Hillman, 1961). Their small stature and morphological simplicity are just two of the many traits that make duckweeds well suited for addressing a variety of questions in ecology and evolution. Furthermore, because of a long history of use in fields such as ecotoxicology (e.g., Wang, 1990) and plant development (e.g., Hillman, 1976), and ongoing interest in using duckweed for industrial applications such as feed and biofuel production (e.g., Cheng and Stomp, 2009) and bioremediation (e.g., Ziegler et al., 2016), researchers studying duckweed today benefit from a mature literature, established research procedures, repositories with hundreds of live strains, and ample molecular resources including genome sequences for *Spirodela polyrhiza* (Wang et al., 2014) and *Lemna minor* (Van Hoeck et al., 2015).

Riding invasion waves: Spatial and temporal patterns of the invasive *Lemna minuta* from its arrival to its spread across Europe

Ceschin, S; Abati, S; Ellwood, NTW; Zuccarello, V (2018) Aquatic Botany 150: 1-8

In Europe, the duckweed *Lemna minuta* is an invasive alien that can cause severe abiotic-biotic alterations of lentic aquatic ecosystems. Its invasion history across Europe was spatio-temporally reconstructed from its various introductions to its present distribution by analyzing georeferenced historical records obtained from different sources (bibliography, national-international digital databases). Fuzzy clustering, a form of classification in which an element belongs to different clusters according to a degree of belonging, was used to identify the most important temporal invasion events (pulsations). Geographical representation of these pulsations in invasion maps simplified the detection of the main dynamics of *L. minuta* movements across Europe. Based on the analyses of the historical data, five main invasion pulsations were identified (1965, 1982, 1991, 2000, 2010). Invasions in Europe began along the Atlantic coasts around the 1950s-1960s. Around the 1980s, *L. minuta* spread extensively across the United Kingdom and Central Europe. After this, it started to invade Southern and also Eastern Europe, mainly stabilizing around the 2000s. In the last decade, *L. minuta* consolidated its occurrence in Western and Central Europe, while it continues to colonize new Mediterranean and Eastern European regions. This analysis type is effective in determining spatial-temporal dynamics of invasive alien plants using georeferenced historical data and it also offers insight into the behavior of a biological invader by identifying the main invasion routes and areas most susceptible to future invasion. This latter information can be useful for development of management strategies for preventing invasions and conserving aquatic ecosystems potentially under threat.

Toxic oligopeptides in the cyanobacterium *Planktothrix agardhii*-dominated blooms and their effects on duckweed (Lemnaceae) development

Pawlik-Skowronska, B; Toporowska, M; Mazur-Marzec, H (2018) Knowledge and Management of Aquatic Ecosystems 419, Article Number: 41

Cyanobacterial toxins are a global threat to aquatic organisms; however, they represent only one group of bioactive cyanobacterial metabolites. Very little is known about the effects of other cyanobacterial products (e.g., non-ribosomal oligopeptides) on freshwater macrophytes. Our experimental study revealed that the development of young duckweed *Spirodela polyrhiza* was inhibited by two aquatic extracts of cyanobacterial bloom samples predominated by *Planktothrix agardhii* and pure microcystin-LR (MC-LR). The extracts differed considerably in the content of MCs and other oligopeptides; they contained three or four MC variants and several other oligopeptides such as anabaenopeptins, aeruginosins, and planktocyclin. Their toxic effects on young plants (first frond area, root number, fresh biomass, and chlorophyll a content) were different. The more phytotoxic extract obtained from a higher cyanobacterial biomass contained a lower total MC concentration and different anabaenopeptin variants (e.g., anabaenopeptin H, G, HU892, and E/F) as well as planktocyclin, which were not present in another extract with a higher MC concentration. The obtained results suggested that *P. agardhii* oligopeptides other than MCs are harmful to young duckweeds and may exert even stronger toxic effects than MC-LR. The production of various variants of MCs together with other oligopeptides, and their toxicity to aquatic plants varied over seasons depending on the taxonomic composition of the cyanobacterial bloom. Germinated turions of *S. polyrhiza* can be used as a sensitive bioindicator of the cyanobacterial threat in aquatic ecosystems.

Dynamic simulation of a duckweed-dominated wetland in north China based on a system dynamics model

Zhao, YW; Dong, BQ; Li, ZM; Cheng, GH; Zhou, LQ (2018) Ecological Indicators 92: 268-277

Duckweed is a common category of floating plant which floats on or beneath the surface of still or slow-moving bodies of wetlands. Its growth has much effect on the hydro-ecological process of wetlands. However, in the existing wetland-ecosystem simulation models which contained floating- or emergent-plant modules, the shading effect of macrophytes and the relationship between macrophytes and phytoplankton in the wetland ecosystem were not considered sufficiently. When these models were directly used in the modeling of duckweed-dominated wetlands, the simulation effects of main state variables such as phytoplankton and dissolved inorganic phosphorus in water were poor and the models could not completely respond to the changes of the external variables. Through a field experiment and parameter calibrations, the shading effect of duckweed and the relationship between duckweed and phytoplankton were considered originally in this study. A duckweed-dominated wetland structurally dynamic (DWSD) model was developed for the Hanshiqiao wetland in Beijing. The development of this model included three procedures. (1) The relationship between the shading effect of duckweed and the biomass of duckweed was investigated based on a field experiment. (2) The equations of transmitted light intensity in water and of the growth of phytoplankton were revised according to the relationship obtained. (3) A module of duckweed was developed, and was incorporated into the structurally dynamic model. The DWSD model was calibrated and verified using the ecological survey data of the Hanshiqiao wetland in 2014. The decisive coefficients (R^2) and Nash-Sutcliffe coefficients (E_{NS}) for model calibration were not less than 0.9 in reproducing A and FP, and meanwhile R^2 and E_{NS} of A and FP for model validation were all above 0.8. They showed that this model had acceptable simulation efficiencies both in calibration and verification. The DWSD model was used to predict changes of main state variables in 2015. As the simulation results showed, the module of duckweed posed significant impacts on the modeling of results, which performed in various aspects. For instance, the precision of this model was increased. The simulated values of phytoplankton and detritus in water for the DWSD model were all closer to the observed values than that without the module of duckweed. The transmitted light intensity in water from July to October was decreased. The growth of phytoplankton was inhibited and the simulated values of the content of detritus and the concentration of phosphorus in water were increased.

Feed & Food

Nutrients and bioactive compounds of the *Lemna gibba* and *Ulva lactuca* as possible ingredients to functional foods

Aguilera-Morales, ME; Canales-Martinez, MM; Avila-Gonzalez, E; Flores-Ortiz, CM (2018) Latin American Journal of Aquatic Research 46: 709-716

Lemna gibba freshwater macrophyte and seaweeds *Ulva lactuca* of the middle basin Papaloapan River, southeast of Mexico were chemically characterized in their nutrients and bioactive compounds for possible use in the formulation of functional foods. The proximate chemical analysis showed that ashes contents (g 100 g^{-1}) sample) of *L. gibba* and *U. lactuca* were 20.10 and 33.07, crude protein 21.5 and 17.2, lipids 4.45 and 1.7, nitrogen-free extract 32.4 and 38.34, respectively.

Significant differences ($P < 0.05$) were found in the chemical composition between the two species of aquatic plants. *L. gibba* resulted in a protein source, and *U. lactuca* resulted in an energy source. They had eight essential amino acids for fish and other aquatic species and were abundant in lysine and methionine. Both aquatic plants had an essential quantity of inulin (functional fiber) this data not been reported. Also, they had xanthophyll and variety of antioxidant (beta-carotenes, lutein, lycopene and neoxanthin). *L gibba* had only had one polyunsaturated fatty acid (PUFA; alpha-linolenic (ALA) 30.31 mg g^{-1}. *U. lactuca* had a variety of essential PUFA's (ALA, LA, AA; 3.93, 6.73 and 0.41 mg g^{-1}) of fatty acids, respectively). Based on these results, both of the aquatic plants of the middle basin Papaloapan River studied are susceptible to take advantage in the formulation of functional food, since according to the literature the compounds identified have shown beneficial effects as immunonutrients, immunostimulants, antioxidants or modulators of intestinal flora. In aquaculture production, it is suggested to prove the combined use of these two plants as functional ingredients or some particular component in the diets as prevention strategy of diseases as well as to promote aquaculture sustainable through the use of these plants in the productions.

Duckweed as human food. The influence of meal context and information on duckweed acceptability of Dutch consumers

de Beukelaar, MFA; Zeinstra, GG; Mes, JJ; Fischer, ARH (2018) Food Quality and Preference 71: 76-86

Duckweed is considered a promising source of protein for human food products due to its high protein content and environmentally friendly production properties. In order to achieve successful inclusion in the diet, duckweed should be presented to consumers in an acceptable way. This paper explores Western consumers' perceptions towards duckweed as human food and investigates in what contexts duckweed could be acceptable to consumers who are not used to eating it. In a first interview study (N = 10), consumers generally responded positively towards duckweed as human food, although associations with turbid ponds also did come up. According to the respondents, duckweed belonged to the food category vegetables. So, duckweed was considered to fit best in meals where vegetables and greens are expected. In a larger online survey (N = 669), it was confirmed that consumers had a more positive deliberate evaluation of duckweed and were more likely to accept a meal with duckweed if duckweed was applied in a fitting meal. It was also shown that providing information about nutritional and sustainability benefits increased deliberate evaluation and acceptability for fitting meals, but decreased it for non-fitting meals. Automatic evaluations positively influenced deliberate evaluation and acceptability, supporting the 'yuck' effect, but they did not differ between the meal applications. The current paper shows that if applied in a meal context that fits with consumer expectations, under the assumption that sensory properties like taste are satisfactory, there appear no major objections from consumers against the introduction of duckweed as human food at a larger scale.

Duckweed in irrigation water as a replacement of soybean meal in the laying hens' diet

Zakaria, HA; Shammout, MW (2018) Brazilian Journal of Poultry Science 20: 573-582

Water lentils (Duckweed [DW])(*Lemna gibba*), in irrigation ponds, was evaluated by replacing two levels of soybean meal (SBM) on performance and egg quality of laying hens of 54 weeks of age. A total of 72 white Lohmann laying hens were randomly allocated into 3 treatments with 6

replicates/treatment, 4 hens/replicate in a randomized complete block design. Treatments were: control group (DW0%) with (SBM) as the main source of protein, T1 (DW10%) and T2 (DW20%), where duckweed replaced 10% and 20% of SBM for 9 weeks. No significant differences were observed among the dietary treatments in body weight change, feed conversion ratio, egg weight and mortality rate. Replacement with (DW20%) decreased ($p<0.05$) feed intake, egg laying rate and egg mass. The dry albuminin (DW10%) decreased ($p<0.05$) from 7 to 9 weeks and in the total period. Yolk pigmentation was highly ($p<0.001$) improved by the replacement. Blood spots were increased ($p<0.05$) with (DW20%). Duckweed grown in good quality irrigation water can replace up to 10% of the SBM as a source of protein without adverse effects on hen performance and egg quality in addition to profitability.

Interaction with microorganisms

Duckweed (*Lemna minor*) and Alfalfa (*Medicago sativa*) as bacterial infection model systems

Kamal, Fatima; Radziwon, Alina; Davis, Carly M; Dennis, Jonathan J (2018) Methods in Molecular Biology 1898:191-198

Alternative animal host models of bacterial infection have been developed which reproduce some of the disease conditions observed in higher animals. Analogously, plants are useful for modeling bacterial pathogenesis, in some cases revealing broadly conserved infection mechanisms. Similar to animals, plants have been shown to possess innate immune systems that respond to invading viruses, bacteria, and fungi. Plant infection models often yield results faster, are more convenient, and less expensive than many animal infection Molecular Biology discovery of virulence genes and factors involved in bacterial pathogenesis.

"Duckweed-Microbe Co-Cultivation Method" for isolating a wide variety of microbes including taxonomically novel microbes

Tanaka, Yasuhiro; Tamaki, Hideyuki; Tanaka, Kazuya; Tozawa, Erina; Matsuzawa, Hiroaki; Toyama, Tadashi; Kamagata, Yoichi; Mori, Kazuhiro (2018) Microbes and Environments DOI:10.1264/jsme2.ME18067

We herein described a new microbial isolation method using the interaction between the floating aquatic plant, duckweed, and microbes. We harvested microbial cells from Japanese loosestrife roots and co-cultivated these cells with aseptic duckweed using artificial inorganic medium for the plant for four weeks. During the co-cultivation, some duckweeds were collected every week, and the roots were used for microbial isolation using a low-nutrient plate medium. As a result, diverse microbial isolates, the compositions of which differed from those of the original source (Japanese loosestrife root), were obtained when the roots of duckweed were collected after 2 weeks of cultivation. We also successfully isolated a wide variety of novel microbes, including two strains within the rarely cultivated phylum, *Armatimonadetes*. The present study shows that a duckweed-microbe co-cultivation approach together with a conventional technique (direct isolation from a microbial source) effectively obtains more diverse microbes from a sole environmental sample.

Effects of co-inoculation of two different plant growth-promoting bacteria on duckweed

Yamakawa, Y; Jog, R; Morikawa, M (2018) Plant Growth Regulation 86: 287-296

Aseptic *Lemna minor* was soaked for 4 h in pond water where wild *L. minor* was naturally flourishing. Seven of the eight surface-colonizing bacterial strains were found capable of promoting the growth of *L. minor*. This high appearance of plant growth-promoting bacteria (PGPB) suggests that association of environmental bacteria is generally beneficial rather than harmful for host plants. One of the PGPB, *Pseudomonas* sp. Ps6, enhanced the growth of *L. minor* by 2-2.5-fold in 10 days. This activity was higher than that previously reported for *Acinetobacter calcoaceticus* P23, which enhanced growth of *L. minor* by 1.5-2-fold. Ps6 mostly adhered to and colonized the root rather than the frond, a leaf-like structure of duckweed where P23 preferentially adheres. It was expected that these two strains can share niches, coexist, and enhance the growth of duckweed additively upon co-inoculation. However, contrary to expectation, the growth of *L. minor* was enhanced by only 2.3-fold by co-inoculation of these two bacteria. P23 lowered the initial adhesion of Ps6 cells by 98.2% on the fronds and by 79.5% on the roots. However, initial adhesion of P23 cells to the roots increased dramatically, by 47.2-fold, following co-inoculation with Ps6. However, the number of P23 cells decreased dramatically to 0.7% on the root and to 3.6% on the frond after 10 days, whereas Ps6 cells increased by 12.5-fold on the frond and kept 69% on the root, thereby eventually restoring the population on the plant surfaces. Because duckweed is the fastest growing vascular plant and it is easy to grow an aseptic and axenic plant, the duckweed/bacteria co-culture system will be a model platform for studying multiple interactions among host plants and the associated bacteria.

Complete genome sequences of two plant growth-inhibiting bacteria, *Acinetobacter ursingii* M3 and *Asticcacaulis excentricus* M6, isolated from duckweed (*Lemna minor*)

Ishizawa, H; Kuroda, M; Inoue, D; Ike, M (2018) Microbiology Resource Announcements 7: Article Number: UNSP e01092-18

Acinetobacter ursingii M3 and *Asticcacaulis excentricus* M6 are plant growth-inhibiting bacteria that reduce the yield of the duckweed *Lemna minor*. We report here the complete genome sequences of *A. ursingii* M3 and *A. excentricus* M6, sequenced using the PacBio RS II platform.

Duckweed diversity decreases heavy metal toxicity by altering the metabolic function of associated microbial communities

Zhao, Z; Shi, HJ; Liu, CQ; Kang, XJ; Chen, LC; Liang, XF; Jin, L (2018) Chemosphere 203: 76-82

Mono-cultured and mix-cultured duckweed species were investigated with respect to the function of their associated microbial communities in heavy metal contaminated wastewater. Results show that the carbon source utilization patterns of the *L aequinoctialis*- and *S. polyrhiza*-associated microbial communities were different. The relationships between microbial activity, antioxidant enzyme activity (CAT, GSH, and SOD) and growth was positive and significant. The microbial activity of *L. aequinoctialis* and *S. polyrhiza* in mixture was higher than in monoculture in low and high heavy metal, respectively, thereby altering the utilization of specific carbon source types and increasing duckweed growth and antioxidant enzyme activity, when compared to the monocultured duckweed.

Furthermore, results indicate that duckweed species in mixture are protected from damage through regulation of the associated bacterial communities.

Molecular Biology

Frond transformation system mediated by *Agrobacterium tumefaciens* for *Lemna minor*

Yang, GL; Fang, Y; Xu, YL; Tan, L; Li, Q; Liu, Y; Lai, F; Jin, YL; Du, AP; He, KZ; Ma, XR; Zhao, H (2018) Plant Molecular Biology 98: 319-331

The Lemnaceae, known as duckweed, the smallest flowering aquatic plant, shows promise as a plant bioreactor. For applying this potential plant bioreactor, establishing a stable and efficient genetic transformation system is necessary. The currently favored callus-based method for duckweed transformation is time consuming and genotype limited, as it requires callus culture and regeneration, which is inapplicable to many elite duckweed strains suitable for bioreactor exploitation. In this study, we attempted to establish a simple frond transformation system mediated by *Agrobacterium tumefaciens* for *Lemna minor*, one of the most widespread duckweed species in the world. To evaluate the feasibility of the new transformation system, the gene CYP710A11 was overexpressed to improve the yield of stigmasterol, which has multiple medicinal purposes. Three *L. minor* strains, ZH0055, D0158 and M0165, were transformed by both a conventional callus transformation system (CTS) and the simple frond transformation system (FTS). GUS staining, PCR, quantitative PCR and stigmasterol content detection showed that FTS can produce stable transgenic lines as well as CTS. Moreover, compared to CTS, FTS can avoid the genotype constraints of callus induction, thus saving at least half of the required processing time (CTS took 8-9 months while FTS took approximately 3 months in this study). Therefore, this transformation system is feasible in producing stable transgenic lines for a wide range of *L. minor* genotypes.

Callus induction and plant regeneration of *Spirodela polyrhiza*

Huang, MX; Ma, XY; Zhong, YS; Hu, QX; Fu, MH; Han, YL (2018) Plant Cell Tissue and Organ Culture 135: 445-453

This study reports efficient protocols of tissue culture of *Spirodela polyrhiza*, the only species whose whole genome (including nuclear, mitochondrial and chloroplast genome) has been sequenced in Lemnaceae. The callus induction, callus maintenance and plant regeneration have been established. Sixteen kinds of combinations of phytohormones from the orthogonal combinations of four auxins and four cytokinins, and 64 kinds of orthogonal combinations of concentration for each combination of phytohormones were tested for callus induction. Callus were induced with a high efficiency from the root apical meristem on the MS medium with the phytohormone combination of 2,4-D and thidiazuron by a method called Rhizoids-hovering. Unlike other reported species in Lemnaceae whose callus has been induced from frond, frond of *S. polyrhiza* cannot be induced into callus under all tested conditions in this study.

A protocol for efficient callus induction and stable transformation of *Spirodela polyrhiza* (L.) Schleiden using *Agrobacterium tumefaciens*

Yang, JJ; Lia, GJ; Hua, SQ; Bishopp, A; Heenatigala, PPM; Kumar, S; Duan, PF; Yao, LG; Hou, HW (2018) Aquatic Botany 151: 80-86

Spirodela polyrhiza represents the largest specie with the smallest genome of all the members of the Lemnoideae. Its genome features have been delineated, revealing its fewest predicted genes of any known plant genome. It is also ideal system for basic biological researches and various practical applications including toxicity testing, bioreactor, biomonitoring and biofuel. In this study, we reported the successful induction of *S. polyrhiza* callus coupled with the efficient stable transformation using the *Agrobacterium tumefaciens* strain LBA4404 by optimizing each step of the process. We found that the highest callus induction efficiency was achieved with 22.62 μM 2,4-D and 8.88 μM 6-BA, with above 90% of fronds forming calli. We also determined that 100 M acetosyringone in the co-cultivation medium and the maintenance of pH value at 5.2 were crucial for high transformation efficiency (up to 13 ± 1.5%). As proof of concept, we transformed *S. polyrhiza* with the DR5 and TCS synthetic reporters, which have previously been used to report cytokinin and auxin signaling output in the model plant *Arabidopsis thaliana*. The cytokinin showed highest accumulation at the initial stage of bud formation and the frond apex of *S. polyrhiza* whilst the expression of auxin was observed highest at frond with middle size. These transformed lines provide an effective way to investigate the development of *S. polyrhiza* and may shed light on the interesting way in which this specie reproduces. This is the first report of highly efficient callus induction and *Agrobacterium tumefaciens*-mediated transformation in *S. polyrhiza*.

Physiology

Cytokinin-induced growth in the duckweeds *Lemna gibba* and *Spirodela polyrhiza*

Kurepa, J; Shull, TE; Smalle, JA (2018) PLANT GROWTH REGULATION 86: 477-486

Duckweeds, quick-growing aquatic plants, have been recently recognized as promising hosts for the large-scale production of recombinant proteins and as an ideal biomass feedstock for biofuel production. These possible wide-spread industrial uses of duckweeds intensified research aimed at understanding the mechanisms that control duckweed growth. Here, we describe how the hormone cytokinin affects growth. We performed a number of standard cytokinin growth- and physiological-response assays using sterile-grown colonies of *Lemna gibba* and *Spirodela polyrhiza*. Similar to land plants, cytokinin inhibited root elongation in duckweeds. Surprisingly, and in contrast to land plants, cytokinin promoted growth of aerial organs in both duckweed species, suggesting that the cytokinin growth response fundamentally differs between aquatic and land plants.

Among-strain consistency in duckweed in the pace and shape of senescence in duckweed

Barks, PM; Dempsey, ZW; Burg, TM; Laird, RA (2018) Journal of Ecology 106: 2132-2145

Comparative studies have demonstrated extensive variation in age trajectories of mortality and fecundity, both within and among species, with many taxa exhibiting a general pattern of age-related demographic decline referred to as senescence. Whereas a considerable body of theory is devoted to explaining the origin and persistence of senescence, the evolutionary forces underlying variation in demographic trajectories more generally remain poorly understood. Studying variation in demographic trajectories is complicated by the fact that different species (or even different populations of a given species) may live and reproduce on different time-scales, which, for comparative purposes, can make it challenging to disentangle patterns of age-related demographic change (the shape of demographic age trajectories) from the time-scale on which those changes happen (the pace of demographic age trajectories). Here, we examine variation in the pace and shape of demographic trajectories among strains of the aquatic plant *Lemna turionifera* Landolt from 24 sites across Alberta, Canada. Our main objectives were to describe the shape of demographic trajectories in *L. turionifera*, and test for among-strain variation in pace and shape. We also tested whether potential variation in pace and shape is (1) constrained by trade-offs with other life-history traits, and (2) consistent with local adaptation to environmental characteristics at the sites of strain origin. The strains we examined were overwhelmingly subject to age-related increases in mortality and declines in fecundity, with increases in mortality tending to decelerate and plateau at advanced ages. Despite substantial among-strain variation in cumulative fecundity and plant size, measures of pace and shape did not in themselves vary significantly among strains. Both within and among strains, we observed a negative relationship between plant size and the shape of fecundity trajectories, but we found no other evidence for life-history trade-offs involving pace or shape, nor for local adaptation. Synthesis. Angiosperms display remarkable demographic variation. Our results suggest that the pace and shape of demographic trajectories are highly conserved within one particular angiosperm species (*Lemna turionifera*), despite substantial among-strain variation in other life-history traits.

Comparison of the populational characteristics of *Lemna minuta* (ARACEAE: LEMNOIDEAE) in three culture media

Ramírez-Babativa, Daniel Ferley; Espinosa Ramírez, Adriana Janneth (2018) Revista Colombiana de Biotecnologia 20: 84-96

Although neotropical macrophytes are considered appropriate for diverse applications due to their great reproductive capacity and high sensitivity to changing environmental conditions, research on these plants is currently scarce, especially in Colombia when compared to countries such as Brazil. The current research work intended to acclimatize and cultivate a clone of the duckweed *Lemna minuta*, which is widely distributed in Colombia and America. After keeping daughter fronds of this species for two months in APHA culture medium, their propagation was compared in three culture media: Hoagland's E+, APHA and AAP20x. Population growth variables such as growth rate, mortality, doubling time and life span. Additionally, the efficiency of the frond cleaning method proposed by Acreman to obtain axenic cultures was evaluated. The results indicated that Hoagland's E+ medium (without organic compounds) is the most suitable one when it comes to frond growing under laboratory conditions, due to its associated higher frond production rate (0.16 fronds d^{-1}) and life span (13.8 d), as well as lower mortality (0.11 fronds d^{-1}) and doubling time (4.61 d). Knowing the population growth and cultivation conditions of L. minuta allows proposing it as a relevant macrophyte and candidate for various water quality bioassays.

Phytoremediation

The treatment of duckweed with a plant biostimulant or a safener improves the plant capacity to clean water polluted by terbuthylazine

Panfili, I; Bartucca, ML; Del Buono, D (2018) Science of the Total Environment 646: 832-840

Water pollution is becoming alarming since thousands contaminants are dispersed in the aquatic environments, and agricultural practices, for the massive use of pesticides, are contributing to exacerbating this problem. In this context, a research aimed at investigating the ability of duckweed (*Lemna minor*), a free-floating aquatic species widespread throughout the world, to remediate water polluted with five different concentrations of a herbicide - terbuthylazine (TBA) - was carried out. In addition, duckweed was treated with a plant biostimulant and a safener with the aim of increasing the plant's capacity to tolerate and remove the TBA from the water. The results evidenced that the herbicide affected the duckweed already at the lower concentrations, reducing its capacity to proliferate and the area of its fronds. On the contrary, when the TBA treatments were performed in combination with the biostimulant or the safener the average area of the fronds was affected of lesser extents, compared to the plants treated with the herbicide only. Antioxidant enzymes, namely ascorbate peroxidases (APX) and catalases (CAT) were investigated and it was found that the biostimulated and safened duckweed showed increased activities of these enzymes, compared to the plants treated with TBA only. At last, some phytofiltration experiments were planned. The biostimulated and safened duckweed removed more TBA from polluted water than the plants treated with the herbicide alone. In conclusion, this research showed that duckweed is suitable for cleaning water polluted with TBA and this potential can be successfully improved by treating the species with a biostimulant or a safener.

Biomass, nitrogen uptake and content of *Wolffia arrhiza* depends on strength of swine lagoon water

Chikuvire, Tichaedza J; Muchaonyerwa, Pardon; Zengeni, Rebecca (2018) Water Environment Research 90:2066-2074

Studies focusing on manipulation of growth conditions for duckweed *Wolffia arrhiza* to promote biomass for crop nutrient supply, are scarce. The effects of swine lagoon water (SLW) concentration and its replenishment and harvest regimes on selected properties of *W. arrhiza* were investigated. Dry matter and average growth rate of *W. arrhiza* were not affected by SLW replenishment periods, whereas the properties decreased with increasing concentration of SLW. The carbon and carbon/nitrogen content increased as the period between solution replenishment increased and as SLW concentration declined from 15 to 5%. Harvesting regimes did not affect the nitrogen content and uptake of duckweed, and mineral-N of SLW. Harvesting duckweed once per week resulted in higher growth rate and biomass, compared with twice a week. Findings from this study suggest that *W. arrhiza* cultured on 10% SLW and harvested once a week yields biomass with nitrogen content suitable for crop nutrient supply.

Integrated comparisons of thorium(IV) adsorption onto alkali-treated duckweed biomass and duckweed-derived hydrothermal and pyrolytic biochar

Chen, Ting; Zhang, Nan; Xu, Zhao; Hu, Xin; Ding, Zhuhong (2018) Environmental Science and Pollution Research DOI:10.1007/s11356-018-3789-x

In order to remove aqueous radionuclides and find an appropriate method for the disposal of wild duckweed in eutrophic water body, alkali-treated duckweed biomass and duckweed-based hydrothermal biochar (hydrochar) and pyrolytic biochars of 300 and 600°C were prepared. Their physicochemical properties were characterized carefully. The adsorption isothermal data fitted well with the Langmuir model and the maximum Langmuir adsorption capacities were 104.1, 96.3, 86.7, and 63.5mg/g for hydrochar, modified biomass, and 300 and 600°C biochars, respectively. The adsorption kinetic data fitted well with the pseudo-second-order kinetic equation. The sorption data of fixed-bed column also confirmed the high efficient removal of Th(IV) and fitted well with the Thomas model. The duckweed-based hydrothermal biochar is a low-cost adsorbent for Th(IV) removal, and it is also a resource utilization technology of the duckweed collected from eutrophic water body.

The capacity of *Lemna minor* L. to accumulate heavy metals (Zinc, Copper, Nickel)

Neidoni, DG; Nicorescu, V; Andres, L; Ihos, M; Lehr, CB (2018) Revista de Chimie 69: 4153-4156

The assessment of the storage capacity of heavy metals (Zn, Cu, Ni) in *Lemna minor* L., was carried out on wastewater from galvanizing plants. Purification yield decreases with increasing metal concentration in water. The rate of bioaccumulation is high in the first three days, then decreases over the next four days.

Kinetics of arsenic absorption by the species *Eichhornia crassipes* and *Lemna valdiviana* under optimized conditions

de Souza, TD; Borges, AC; de Matos, AT; Veloso, RW; Braga, AF (2018) Chemosphere 209: 866-874

This work aimed to study the kinetics of arsenic absorption by *Eichhornia crassipes* and *Lemna valdiviana* under pre-established conditions of pH phosphate and nitrate in the nutrient solution. Additional aims were to evaluate the conversion kinetics between As(III) and As(V), and the effect of arsenic concentrations on development of the species. The plants were cultivated in nutrient solutions containing different arsenic concentrations: 0, 0.56, 0.89 and 1.38 mg L^{-1} for the water-hyacinth, and 0, 0.13, 0.48, 0.99 and 1.4 mg L^{-1} for *Lemna*. Monitoring of arsenic removal by the plants was performed by sampling at intervals of 0, 4, 8, 16, 24, 48, 96, 144, 192 and 240 h for the water hyacinth, and 0, 4, 8, 16, 24, 48, 96, 144 and 168 h for *Lemna*. The samples were submitted to analysis of total arsenic, As(III), As(V) and phosphorus. The first-order kinetics was fit to the arsenic removal kinetics by the plants, and it was observed that the decay coefficient (k) decreased with the increase of its initial concentration in the nutrient solution. For the, absorption was observed after 96 h of culture, the time coinciding with the greatest As(V) concentrations. For *Lemna*, the metal was only absorbed by the plant after decay of the phosphate levels of the medium, which occurred at 48 h. Concentrations above 1 mg L^{-1} implied deleterious effects in both plant species and in the

phytoremediation process, and the bioaccumulation factor decreased for concentration above this for both *E. crassipes* and *L. valdiviana*.

Phytoremediation of seleniferous soil leachate using the aquatic plants *Lemna minor* and *Egeria densa*

Ohlbaum, M; Wadgaonkar, SL; van Bruggen, JJA; Nancharaiah, YV; Lens, PNL (2018) Ecological Engineering 120: 321-328

Phytoremediation of selenium (Se)-containing Hoagland solution and seleniferous soil leachate using two aquatic plants Lemna minor and *Egeria densa* was evaluated. *L. minor* showed the highest Se removal efficiency (97%) in the Hoagland solution with a bioconcentration factor (BCF) of 504.35 ± 0.83. In artificial soil leachate with addition of 2 mg L^{-1} $MnSO_4$, *L. minor* and *E. densa* showed a Se removal efficiency of 77% and 60%, respectively. The addition of $K_2S_2O_8$ decreased the Se uptake by both plants by 40% and the medium pH decreased from 7 to 3, whereas the addition of SO_4^{2-} decreased the removal efficiency of both aquatic plants by 30%, in which only 3% of Se was taken up by the plants. *L. minor* was selected to remove Se from a real seleniferous soil leachate which contained 74 µg L^{-1} Se and a 76% efficiency was achieved, with a Se uptake of 29 µg g^{-1} dry weight and a BCF of 393.2 ± 13.6. This study demonstrates that aquatic plants such as *Lemna minor* and *Egeria densa* can be used to remove Se from seleniferous soil leachate and that the phytoremediation efficiency depends on the composition of the extractant used for soil washing.

Wastewater treatment by *Lemna minor* and *Azolla filiculoides* in tropical semi-arid regions of Ethiopia

Amare, E; Kebede, F; Mulat, W (2018) Ecological Engineering 120: 464-473

In this study, wastewater blended from textile, distillery, and domestic sources at a corresponding volumetric ratio of 3:1:18, was treated using *Lemna minor* and *Azolla filiculoides* for 28 days in a batch system installed in a shade house. Analysis of variance between the two macrophytes showed no statistical differences in removals of all tested parameters ($p < 0.05$) except for the biochemical oxygen demand where removal was higher in the *L. minor*. Electrical conductivity, pH, total dissolved solids, the studied heavy metals, and sulfate met the agricultural reuse and discharge limits. The removal of chemical oxygen demand by *A. filiculoides* (96%) was slightly higher than the *L. minor* (92%), but the biochemical oxygen demand removal by *L. minor* (92%) was significantly higher than *A. filiculoides* (90%). Despite the high removals of chemical and biochemical oxygen demands, total phosphorus and total nitrogen attained, the concentrations were found exceeding the discharge and agricultural reuse limits. Finally, while the number of total coliform in both macrophyte populated chambers were too numerous to count, the number of colonies of fecal coliform were 400 in the *L. minor* and 267 in the *A. filiculoides* treatments. In conclusion, the higher removals of all the studied parameters in the macrophyte populated chambers compared with the control might be attributed to the contributions of the macrophytes.

Bioprocess technology for coal washery effluent treatment by *Lemna minor*

Selvi, VA; Kumar, A (2018) Remediation 28: 73-79

Duckweed species are promising macrophytes that can be used in wastewater treatment due to their rapid growth, ease of harvest, low fiber feed potential, and high protein contents. Wastewater contaminants that are likely to be generated during washing of coal are total suspended solids (TSS), chemical oxygen demand (COD), acidity or alkalinity (pH), and metallic contaminants. Bioprocesses were developed to evaluate the potential of duckweed (*Lemna minor*) to treat coal washery effluent (CWE) as well as to study the impact on the biochemical changes of the *Lemna minor*. CWE samples were diluted with distilled water (DW) in different ratios as follows: T1-CWE:DW (20:80%), T2-CWE:DW (40:60%), T3-CWE:DW (60:40%), T4-CWE:DW (80:20%), T5-CWE (100%), and Control-DW(100%). The electrical conductivity of the effluent treated with *Lemna minor* was 0.035 dS/m in the control at day 10 and substantially higher at treatment ratios of 100, 80, and 60 percent (1.754, 1.842, and 1.631dS/m). The highest amount of TSS was observed at test ratio T5 (38,834 mg/L), followed by T4 (28,816 mg/L), T3 (26,970 mg/L), T2 (15,320 mg/L), T1 (4,524 mg/L), and control (424 mg/L). Total hardness was higher (820 mg/L) in T4 compared to the control (220 mg/L). Total hardness of the effluent decreased after 30 days of incubation in all the treatment aliquots. The CWE at 20 percent concentration increased the duckweed population and no adverse impacts on its growth were observed. At higher concentration (T5) total mortality of *Lemna minor* was observed. The chlorophyll production was determined to be inversely proportional to the effluent concentration. Based on this study, it is concluded that *Lemna minor* can be used for treating CWE after dilution with fresh water.

Assessment of duckweed (*Lemna gibba* L.) growth on dam water surface as green cost-effective process to improving water quality

Soltani, Z; Khani, A; Mahanpour, K; Marjani, A (2018) Desalination and Water Treatment 118: 79-86

Using living plants such as duckweed to remove pollutants from water is a cost-effective green technology. In this work, we investigated the ability of *Lemna gibba* L. to enhance water quality of Aydoghmush dam located in Miyaneh, Iran as target. Some water quality indicators that are investigated include the following: content of nitrate, nitrite, and phosphate, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). For this purpose, the pilot system was designed that consisted of five ponds. Three ponds as duckweed culture medium filled with raw water of the dam without any addition of duckweed nutrients (culture pond), one pond filled with raw water of the dam without duckweed (unplanted pond), and another pond was used as plant growth control pond that filled with distilled water (control pond). Approximately 50 g of fresh *L. gibba* were grown for 1 week in the pilot system and some operational parameters such as aeration, temperature, and pH on the plant growth, and consequently on indicators of water quality were investigated. The results showed that the amount of duckweed became 1.32 times in the culture pond but did not grow tangibly in the control pond, which indicated raw water of the dam can be a suitable culture medium for the duckweeds growth. According to the quality indicators, water quality of the dam improved by plant growth, thus suggest that *L. gibba* can be a suitable candidate to improving the quality of the dam water. Under experimental conditions, the contents of nitrite, nitrate, phosphate, COD, and BOD decreased by 55.03%, 45.45%, 46.16%, 62.06%, and 74.23%, respectively. Also, the removal ability of the used duckweeds that are dead (nonliving duckweeds) in high concentration of nitrate from the aqueous solution was examined. The highest removal degree was obtained at initial concentration of 25 mg L^{-1}, which was 93.60%. The experimental data that obtained at different initial concentrations were fitted by the pseudo-first-order rate model with R^{-2} higher than 0.99. The rate constants of biosorption process for nonliving duckweeds at different initial concentrations of 25, 50, and 75 mg L^{-1} were 0.0312, 0.0164, and 0.0119 min^{-1}, respectively.

Phytotoxicity

Duckweed biomarkers for identifying toxic water contaminants?

Ziegler, P; Sree, KS; Appenroth, KJ (2018) Environmental Science and Pollution Research DOI:10.1007/s11356-018-3427-7

Surface or ground waters can be contaminated with numerous toxic substances. The duckweeds *Lemna minor* and *Lemna gibba* are widely used for assaying waterborne toxicity to higher plants in terms of growth inhibition and photosynthetic pigment reduction. These tests cannot, however, in themselves determine the nature of the agents responsible for toxicity. Morphological, developmental, physiological, biochemical, and genetic responses of duckweeds to exposure to toxic water contaminants constitute biomarkers of toxic effect. In principle, the very detection of these biomarkers should enable the contaminants having elicited them (and being responsible for the toxicity) to be identified. However, in practice, this is severely compromised by insufficient specificity of biomarkers for their corresponding toxicants and by the lack of documentation of biomarker/toxin relationships. The present contribution illustrates the difficulties of using known water contaminant-related duckweed biomarkers to identify toxins, and discusses possibilities for achieving this goal.

Real-time CO2 uptake/emission measurements as a tool for early indication of toxicity in *Lemna*-tests

Persic, Vesna; Derd, Tamara; Varga, Martina; Hackenberger, Branimir K (2018) Aquatic Toxicology 206:154-163

This paper presents an application of continuous monitoring of the emission and uptake rate of CO_2 in Lemna toxicity test. On a real-time basis, the CO_2 concentration data were collected by the Arduino platform-based respiratory activity measuring system (ResTox) and reported as CO_2 concentration dynamic curves. The results of CO_2 measurements demonstrated that tested metals (Co, Cu, Hg, and Cd), as well as herbicides (nicosulfuron, diquat, and tembotrione), stimulated the CO_2 exchange rates at low doses, while at high doses CO_2 exchange rates were inhibited. The addition of higher concentrations of clopyralid stimulated photosynthetic activity and caused a higher increase in respiration rates indicating its mode of action as auxin mimic herbicide. The results obtained underline the necessity of considering other biological endpoints like continuous measurements of gas exchange from the very beginning of exposure to toxicants. Simultaneous measurements of real-time CO_2 concentrations, as the primary effect of toxicant mode of action, and processes that are supported by carbon flux, as the secondary effect or endpoint, are needed to relate actual and substrate-induced or inhibited respiration and photosynthesis to those processes. Therefore, continuous measurements of CO_2 exchange rates can be implemented for the initial screening of potential toxicity to give valuable information that is needed for further examination of toxicity mechanisms and risk assessment.

The use of multiwell culture plates in the duckweed toxicity test - A case study on Zn nanoparticles

Kalcikova, G; Marolt, G; Kokalj, AJ; Gotvajn, AZ (2018) New Biotechnology 47: 67-72, SI

Extensive production of nanomaterials of various properties needs to be coupled with rapid toxicity testing in order to provide information about their potential risks to the environment and human health. Miniaturization of toxicity tests may accelerate economical testing of nanomaterials, but is not a common practice. We describe a case study to miniaturize a commonly used toxicity test with plant duckweed *Lemna minor*. 6-well, 12-well and 24-well culture plates were used to assess their potential use for the duckweed toxicity test with potassium chloride as reference material. The results were compared to the standard test design using 100 mL glass beakers. The comparison showed that the best agreement was with the 6-well vessels. This set-up was further used for toxicity testing of zinc oxide nanoparticles (ZnO NP) and zinc chloride. Zinc was not adsorbed onto either glass or plastic walls of the miniaturized system. We assume that in both vessels a fast agglomeration and settling of ZnO NP took place. Linear regression and statistical testing indicated a good correlation between the toxicity results obtained in the standard test and miniaturized 6-well vessels. The miniaturization of the test system for assessing the biological effect of nanomaterials on *Lemna minor* could become an appropriate alternative to the traditionally used high volume vessels.

Simultaneous boron removal and electricity generation from domestic wastewater using duckweed-based wastewater treatment reactors coupled with microbial fuel cell

Turker, OC (2018) Journal of Environmental Management 228: 20-31

Boron removal from water environment is a critical issue for scientific spotlight because its removal from wastewater is difficult and costly with conventional treatment method. Herein, an innovative, cost effective and attractive method which depends on duckweed-based wastewater treatment systems coupled with microbial fuel cell reactor (DWWT-MFC) was investigated for B-polluted domestic wastewater treatment and simultaneous electricity generation for the first time in an eco-technological study. *Lemna gibba* L. was selected as a model duckweed species, and different reactors were also designed to identify which mechanisms are dominant for B removal in a DWWT-MFC reactor matrix. DWWT-MFC reactor achieved 71% B removal in experiment period, and the plant effect on B removal mechanisms in the reactor matrix was recorded as 37.7 ± 4.92% ($F = 2.543$, $p < 0.05$). However, supplementary aeration and microbial effects on B removal were determined as negligible. Average maximum voltage output was found as 1.47 V, and maximum power density was 34.8 mW m^{-2} at a current density of 43.9 mAm^{-2} with supplementary aeration. Moreover, DWWT-MFC reactor achieved 84%, 81% and 76% of COD, $NH_4^+$and PO_4^{3-} removal efficiencies, respectively. Moreover, *L. gibba* grew well in the anode chamber of DWWT-MFC with an average biomass yield of 218 ± 43 g m^{-2} and a total chlorophyll (a + b) concentration of 30.2 mg g^{-1}, which indicates that anolyte environment was not toxic for *L. gibba* growth. Consequently, it can be suggested that environmental experts may use DWWT-MFC as an efficient removal method to treat B from domestic wastewater and to produce bioelectricity.

Responses of duckweed (*Lemna minor* L.) to aluminum stress: Physiological and proteomics analyses

Su, Chunlei; Jiang, Yuji; Yang, Yaru; Zhang, Wei; Xu, Qinsong (2018) Ecotoxicology and Environmental Safety 170:127-140

Aluminum (Al) is commonly considered an abiotic stress factor under acidic conditions. Duckweed (*Lemna minor* L.) has wide application in ecotoxicological research as a model organism and, in this study, its response to Al bioaccumulation was evaluated at morphological, physiological and proteomic levels. The Al accumulation in *L. minor* was accompanied by chlorosis and growth inhibition. Overproduction of superoxide and hydrogen peroxide, and decreased chlorophyll and protein contents, suggested that Al exposure induced oxidative stress. Inhibition of photosynthesis was evident in a significant decrease in maximum photosystem II quantum yield. There were 261 proteins, with significant changes in expression, successfully identified and quantified through isobaric tags for relative and absolute quantification (iTRAQ) analysis. Among the KEGG pathway enrichment proteins, those related to the citrate cycle and amino acid metabolism were predominantly up-regulated, whereas those associated with energy metabolism and glyoxylate and dicarboxylate metabolism were predominantly down-regulated. In addition, antioxidant enzyme related proteins played an important role in the response of *L. minor* to Al. The western blot analysis further validated the changes in photosynthetic related proteins. These results provide comprehensive insights into the physiological and molecular mechanisms of Al toxicity and tolerance in *L. minor*.

Interaction of CuO nanoparticles with duckweed (*Lemna minor* L.): Uptake, distribution and ROS production sites

Yue, L; Zhao, J; Yu, XY; Lv, KM; Wang, ZY; Xing, BS (2018) Environmental Pollution 243: 543-552

CuO engineered nanoparticles (NPs) are of increasing concern due to their extensive use in daily life and adverse effect on aquatic organisms. The investigations on the toxicity of CuO NPs to aquatic plants through uptake from roots versus fronds are limited. This paper discusses the interactions of CuO NPs with *Lemna minor*, a floating plant. After CuO NPs (150 μg L^{-1}) exposure for 7 days, the frond number, frond surface area and dry weights of whole plants significantly decreased by 32%, 47% and 33%; the responses were dose-dependent. Microscopy imaging showed that the epidermis was severely damaged in fronds, edges were severely sloughed off and cell integrity was damaged in roots. Shrinkage of both chloroplast and starch grains were observed in the frond cells. Internalization of CuO NPs in root and frond cells during CuO NPs (1 mg L^{-1}) exposure was confirmed with the root Cu levels of *Lemna minor* being three times higher than the fronds by using transmission electron microscopy and flame atomic absorption spectrophotometry. Reactive oxygen species, mainly H_2O_2 (increased by 56%) and (OH)-O-center dot (increased by 57%), accumulated in *Lemna minor* tissues in response to CuO NPs exposure. Moreover, chloroplasts were confirmed as a site of ROS production. These findings are helpful for better understanding the biological responses of aquatic plants upon NPs exposure.

Physiological responses in *Lemna minor* frond to high concentrations of zinc, lead, copper and chromium

Peng, X; Guo, XY; Ding, ZH; Xin, GR (2018) Pakistan Journal of Botany 50: 2151-2157

Robust, rapid bioindicators of heavy metal water pollution, which are responsible for increasing environmental threats globally, are required. In the present study, we investigated the possibility of using short-term ≤12 hour) physiological responses of *Lemna minor* to high concentrations (up to 10 mmol L^{-1}) of zinc (Zn), lead (Pb), copper (Cu) and chromium (Cr) for this purpose. The Results showed that (a) increase in Pb, Zn, Cu, and Cr levels increased fronds' malonaldehyde (MDA) contents, whereas increase in Pb, Cu, and Cr levels also reduced peroxidase activity (POD), although some of these effects were only observed at high concentrations, (b) high Cu and Cr levels reduced fronds' chlorophyll contents, but Zn increased chlorophyll content from 0.0016 mmol L^{-1}; (c) all four heavy metals induced frond abscission, and the percentage of frond abscission remain stable (except for Pb) after exposure for 10 h. The maximal concentrations of Zn, Cu and Cr resulted in > 50% frond abscission rates ($EFAC_{50}$) within 10 h, but Pb induced much weaker responses. Hence frond abscission would not be a suitable short-term indicator of Pb pollution.

Physiological and biochemical effect of silver on the aquatic plant *Lemna gibba* L.: Evaluation of commercially available product containing colloidal silver

Varga, Martina; Horvatic, Janja; Barisic, Lara; Loncaric, Zdenko; Dutour Sikiric, Maja; Erceg, Ina; Kocic, Aleksandra; Stolfa Camagajevac, Ivna (2018) Aquatic Toxicology 207:52-62

This paper aims to evaluate the effects of a product containing colloidal silver in the aquatic environment, using duckweed *Lemna gibba* as a model plant. Therefore, growth parameters, photosynthetic pigments content and protein content as physiological indices were evaluated. Changes in the content of non-enzymatic antioxidants and activity of several antioxidant enzymes, alongside with the accumulation of hydrogen peroxide and lipid peroxidation end-products were assessed to explore the potential of colloidal silver to induce oxidative stress. The commercially available colloidal silver product contained a primary soluble form of silver. The treatment with colloidal silver resulted in significant physiological and biochemical changes in *L. gibba* plants and a consequent reduction of growth. Accumulation of silver caused altered nutrient balance in the plants as well as a significant decrease in photosynthetic pigments content and protein concentration. The antioxidative response of *L. gibba* plants to treatment with colloidal silver was inadequate to protect the plants from oxidative stress caused by metal accumulation. Silver caused concentration-dependent and time-dependent hydrogen peroxide accumulation as well as the elevation of lipid peroxidation levels in *L. gibba* plants. The use of commercially available products containing colloidal silver, and consequent accumulation of silver, both ionic and nanoparticle form in the environment, represents a potential source of toxicity to primary producers in the aquatic ecosystem.

Impacts of the mycotoxin zearalenone on growth and photosynthetic responses in laboratory populations of freshwater macrophytes (*Lemna minor*) and microalgae (*Pseudokirchneriella subcapitata*)

Eagles, Emily J; Benstead, Rachel; MacDonald, Susan; Handy, Richard; Hutchinson, Thomas H (2018) Ecotoxicology and Environmental Safety 169:225-231

Mycotoxins are an important class of chemicals of emerging concern, recently detected in aquatic environments, potentially reflecting the influence of fungicide resistance and climatic factors on fungal diseases in agricultural crops. Zearalenone (ZON) is a mycotoxin formed by *Fusarium* spp. and is known for its biological activity in animal tissues; both in vitro and in vivo. ZON has been reported in US and Polish surface waters at 0.7-96 ng/L, with agricultural run-off and wastewater treatment plants being the likely sources of mycotoxins. As some mycotoxins can induce phytotoxicity, laboratory studies were conducted to evaluate the toxicity of ZON (as measured concentrations) to freshwater algae (*Pseudokirchneriella subcapitata*) and macrophytes (*Lemna minor*) following OECD test guidelines 201 and 221, respectively. Zinc sulphate was used as a positive control. In the OECD 201 algal static study (72 h at 24 ± 1 °C), exposure to ZON gave average specific growth rate (cell density) EC50 and yield (cell density) EC50 values of > 3.1 and 0.92 (0.74-1.8) mg/L, respectively. ZON was less toxic in the OECD 221 static study and after 7 d at 24 ± 1 °C. *L. minor* growth was significantly reduced based on frond number and frond area at 11.4 mg ZON/L, showing a higher tolerance than reported for other mycotoxins with *Lemna* spp. Chlorophyll fluorescence parameters were used as biomarkers of impacts on photosystem II efficiency, with no effect seen in algae but, with responses being observed in L. minor between 5.2-14.4 mg ZON/L. ZON toxicity seen here is not of immediate concern in context with environmental levels, but this study highlights that other freshwater organisms including algae are more sensitive to mycotoxins than Lemna sp., the only current source of toxicity data for freshwater plants.

Physiological and biochemical effect of silver on the aquatic plant *Lemna gibba* L.: Evaluation of commercially available product containing colloidal silver

Varga, Martina; Horvatic, Janja; Barisic, Lara; Loncaric, Zdenko; Dutour Sikiric, Maja; Erceg, Ina; Kocic, Aleksandra; Stolfa Camagajevac, Ivna (2018) Aquatic Toxicology 207:52-62

This paper aims to evaluate the effects of a product containing colloidal silver in the aquatic environment, using duckweed *Lemna gibba* as a model plant. Therefore, growth parameters, photosynthetic pigments content and protein content as physiological indices were evaluated. Changes in the content of non-enzymatic antioxidants and activity of several antioxidant enzymes, alongside with the accumulation of hydrogen peroxide and lipid peroxidation end-products were assessed to explore the potential of colloidal silver to induce oxidative stress. The commercially available colloidal silver product contained a primary soluble form of silver. The treatment with colloidal silver resulted in significant physiological and biochemical changes in *L. gibba* plants and a consequent reduction of growth. Accumulation of silver caused altered nutrient balance in the plants as well as a significant decrease in photosynthetic pigments content and protein concentration. The antioxidative response of *L. gibba* plants to treatment with colloidal silver was inadequate to protect the plants from oxidative stress caused by metal accumulation. Silver caused concentration-dependent and time-dependent hydrogen peroxide accumulation as well as the elevation of lipid peroxidation levels in *L. gibba* plants. The use of commercially available products containing

colloidal silver, and consequent accumulation of silver, both ionic and nanoparticle form in the environment, represents a potential source of toxicity to primary producers in the aquatic ecosystem.

Toxicity of ZnSe nanoparticles to *Lemna minor*: Evaluation of biological responses

Tarrahi, R; Khataee, A; Movafeghi, A; Rezanejad, F (2018) Journal of Environmental Management 226: 298-307

A clear consequence of the increasing application of nanotechnology is its adverse effect on the environment. Semiconductor nanoparticles are among engineered nanomaterials that have been considered recently for their specific characteristics. In the present work, zinc selenide nanoparticles (ZnSe NPs) were synthesized and characterized by XRD, TEM, DLS and SEM. Biological aspects related to the impact of nanoparticles and Zn^{2+} ions were analyzed on the aquatic higher plant *Lemna minor*. The localization of ZnSe NPs in the root cells of *L. minor* was determined by TEM and fluorescence microscopy. Then, the entrance of ZnSe NPs into the plant cells was evaluated by a range of biological tests. The outcomes revealed that both the NPs and the ionic forms noticeably poisoned *L. minor*. In one hand, growth parameters and physiological indices such as photosynthetic pigments content were decreased. On the other hand, the activities of some antioxidant enzymes (superoxide dismutase and catalase), as well as the contents of nonenzymatic antioxidants (phenols and flavonoids) were elevated. Taken together, high concentration of ZnSe NPs and Zn^{2+} triggered phytotoxicity which in turn provoked the plants' defense system. The changes in antioxidant activities confirmed a higher toxicity by Zn^{2+} ions in comparison with ZnSe NPs. It means that the considered ions are more hazardous to the living organisms than the nanoparticles.

Effect of cerium on growth and antioxidant metabolism of *Lemna minor* L.

Zicari, MA; d'Aquino, L; Paradiso, A; Mastrolitti, S; Tommasi, F (2018) Ecotoxicology and Environmental Safety 163: 536-543

An increasing input rate of rare earth elements in the environment is expected because of the intense extraction of such elements form their ores to face human technological needs. In this study *Lemna minor* L. plants were grown under laboratory conditions and treated with increasing concentrations of cerium (Ce) ions to investigate the effects on plant growth and antioxidant systems. The growth increased in plants treated with lower Ce concentrations and reduced in plants treated with higher concentrations, compared to control plants. In plants treated with higher Ce concentrations lower levels of chlorophyll and carotenoid and the appearance of chlorotic symptoms were also detected. Increased levels of hydrogen peroxide, antioxidant metabolites and antioxidant activity confirmed that higher Ce concentrations are toxic to L. minor. Ce concentration in plant tissues was also determined and detectable levels were found only in plants grown on Ce-supplemented media. The use of duckweed plants as a tool for biomonitoring of Ce in freshwater is discussed.

Instructions to Contributors for the Duckweed Forum

The Duckweed Forum (DF) is an electronic publication that is dedicated to serve the Duckweed Research and Applications community by disseminating pertinent information related to community standards, current and future events, as well as other commentaries that could benefit this field. As such, involvement of the community is essential and the DF can provide a convenient platform for members in the field to exchange ideas and observations. While we would invite everyone to contribute, we do have to establish clear guidelines for interested contributors to follow in order to standardize the workflow for their review and publication by the Duckweed Steering Committee members.

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.
DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

In addition to invitees by a Duckweed Steering Committee member, if you are considering submitting a contribution to DF but are unsure about the fit of your idea, please feel free to contact one of the members in the Duckweed Steering Committee in order to obtain feedback as to the appropriateness of the subject for DF. Please include a few sentences describing the overall topic that you are interested to present on, and why you think it is of interest to the general duckweed community. If you have the abstract or draft text prepared, please include it. The Duckweed Steering Committee will discuss the material in one of its meetings and the decision to formally invite submission will be given shortly afterwards.

Copyright and co-author consent

All listed authors must concur in the submission and the final version must be seen and approved by all authors of the contribution. As a public forum, we do not carry out any Copyright application. If you need to copyright your material, please do so beforehand.

Formatting requirements:

- A commonly used word processing program, such as Word, is highly recommended.

- Formatting requirements: 8.5-by-11-inch (or 22 cm-by-28 cm) paper size (standard US letter).
- Single-spaced text throughout.
- One-inch (or 2.5 cm) left and right, as well as top and bottom margins.
- 11-point Times New Roman font.
- Number all pages, including those with figures on the bottom and center of each page.

Title:

- Should be intelligible to DF readers who are not specialists in the field and should convey your essential points clearly.
- Should be short (no more than 150 characters including spaces) and informative.
- Should avoid acronyms or abbreviations aside from the most common biochemical abbreviations (e.g., ATP). Other acronyms or abbreviations should either:
    - be introduced in their full form (e.g., Visualization of Polarized Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) Activity in Live Cells by Fluorescence Resonance Energy Transfer (FRET) Imaging); or
    - be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

Authors:

- All authors are responsible for the content of the manuscript.
- Provide the complete names of all authors.
- Identify which author will receive correspondence regarding the contribution.
- Provide the corresponding author's name, telephone number, and current e-mail address.

Image resolution and submission:

It is extremely important that figures be prepared with the proper resolution for publication in order to avoid inaccurate presentation of the data. The minimum acceptable resolution for all figures is 300 dpi. Excessive file compression can distort images, so files should be carefully checked after compression. Note that figures that contain both line art (such as graphs) and RGB/grayscale areas (such as photographs) are best prepared as EPS (vector) files with embedded TIFF images for the RGB/grayscale portions. The resolution of those embedded TIFF images should be at least 300 dpi. Original images should be submitted as a separate file to the text file. It would be helpful to insert the intended into the Word file as well, if desired, to indicate the location for it. The legend to the image/figure should be added at the end of the text file and labeled as "Legend to Figures".

Links for further reading

http://www.ruduckweed.org/ Rutgers Duckweed Stock Cooperative, New Brunswick, New Jersey State University. Prof. Dr. Eric Lam

http://www.InternationalLemnaAssociation.org/ Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

Note to the Reader

Know of someone who would like to receive their own copy of this newsletter? Would you like to offer ideas for future articles or have comments about this newsletter? Need to be added or removed from our contact list?

Please let us know via email to the Chair of ISCDRA, Prof. Eric Lam: ericL89@hotmail.com