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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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6th ICDRA

6th ICDRA group photo

Cover page

6th ICDRA group photo: Group photo of the participants of the 6th ICDRA 2022: IPK, Gatersleben, Germany. Photo credit: Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany.

In this issue

The 5th 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
- **Dr. K. Sowjanya Sree**, Central University of Kerala, Periye, India; ksowsree9@cukerala.ac.in
- **Prof. Marcel AK Jansen**, University College Cork, Cork, Irelend; M.Jansen@ucc.ie
- **Dr. Tsipi Shoham**, GreenOnyx Ltd., Tel Aviv, Israel; tsipi@greenonyx.biz

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

Letter from the Editor:

July 29th, 2022

Dear *Duckweed Forum* readers,

Greetings once again from New Jersey on the East Coast of the USA. I hope everyone is enjoying the summer so far this year and while the pandemic related uncertainties and inconveniences still impact on our everyday lives, my own experience in the past two months suggests that things are moving toward normalcy slowly but surely globally. As you will read in my description of the 6th ICDRA that was successfully organized and hosted 2 months ago at the IPK in Gatersleben, Germany, it was well attended and a resounding success. The in-person experience of scientific discourse and the exciting vibes that I felt in the poster sessions every day of the meeting cannot be duplicated by virtual meetings. These were also capped off every evening with great food and beverages that for sure helped in many ways to reward the attendees of this conference for their effort in the past 3 years to keep the duckweed field moving forward in spite of the pandemic complications. I would like to thank Ingo Schubert, Manuela Nagel and Klaus Appenroth yet again for a job well done in delivering such a successful meeting that they have worked hard to produce for the community. Bravo !

For those of you readers who were unable to attend the Gatersleben event, which are many, we have included the titles for all the talks and posters from this ICDRA for your viewing pleasure immediately after my report. A detailed evaluation by two of the organizers Klaus-J. Appenroth and Ingo Schubert, has been published in the ACTUALIA of the German Botanical Society in German language (https://www.deutsche-botanische-gesellschaft.de/actualia-0-1/actualia-2022). In addition, you will also find the first announcement from the newly elected organizers of the 7th ICDRA who will host this meeting in Bangkok, Thailand, in 2024. I hope all of our readers will try to attend this next meeting and start to make plans for realizing that goal soon. As Tsipi Shoham's update on duckweed commercialization effort in the globe shows, the advance to real products and market creation for duckweed is gaining steam. I venture to guess that by 2024, the ICDRA in Bangkok could become a watershed moment in the history of duckweed utilization when successful products and companies dealing in the duckweed bioeconomy will come to pass while new exciting science will be driving further exploration to expand the horizon of both research and applications with these little plants.

With these positive thoughts, I wish all of you good health and enjoy the remaining summertime wherever you may be.

Sincerely,

Eric Lam

Chair, ISCDRA

Overview and Impressions of the recent 6th International Conference on Duckweed Research and Applications (ICDRA 2022)

Hosted by The IPK, Gatersleben, Germany: May 29th to June 1st, 2022

Eric Lam
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I arrived on May 28th by train in Gatersleben, Germany, on a sunny and pleasant day. It has been more than 3 years from my last visit and this return has been postponed by almost 1 year due to the disruption by the COVID-19 pandemic which delayed the 6th ICDRA's schedule. I walked through the quiet park adjacent to the train station and found my way to the Institute of Plant Genetics and Crop Plant Research (IPK) with my luggage in tow. On my way, I spotted some duckweed on a stream as I walked over a small bridge. I thought to myself then it is a fitting start for my journey here to attend the long-awaited biennial ICDRA meeting which is dedicated to this family of tiny aquatic plants.

As we celebrated last October the 10th anniversary of the inaugural ICDRA that was held in Chengdu, China (*Duckweed Forum*, Oct' 2021), I was very gratified to see the large number of attendees arriving to the IPK for the opening of the Conference on the 29th of May. In all, there were 94 scientists registered and these attended our international Conference in spite of the still ongoing pandemic and travel restrictions remaining in parts of the globe. Coming from four continents and representing 21 countries, there were also 31 doctoral students among these attendees in addition to multiple early career researchers who are starting their own groups. These metrics are good evidence for the steady growth of our fledgling community in terms of its ability to attract a new generation of plant biologists to take advantage of the special attributes and potential that the duckweed family offers. The content of presentations over the course of 4 days of the Conference demonstrated an impressive breadth and depth for the types of research being conducted by our community. Our field has grown well over the past decade, and I am hopeful that the momentum will continue in the years to come.


Photo credit: Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben, Germany.

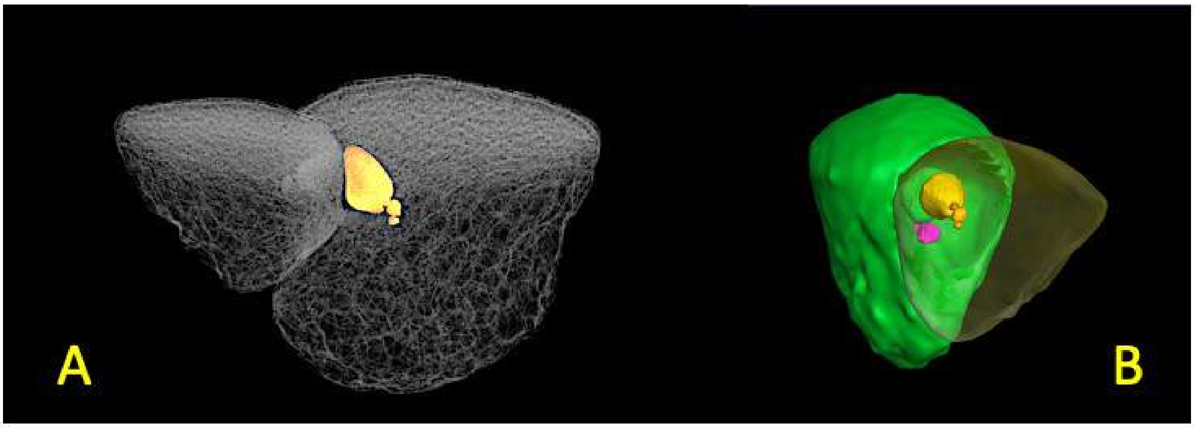
The hosting organizers (shown to the right) for the 6th ICDRA were Ingo Schubert and Manuela Nagel of the IPK, together with Klaus J. Appenroth from Jena (also a member of the Steering

Committee). Supported by an efficient and friendly team of Conference staff from the IPK, this meeting proceeded perfectly from the opening to the closing ceremony. The big round of applause that the attendees gave the organizers and staff at the end of the Conference is well deserved. In addition to financial and operational support by the IPK, the financial support from the Leopoldina (the German National Academy of Science), the AKB Stiftung, the DFG (German Research Foundation), the DBG (German Botanical Society) and Orgentis Chemicals should be acknowledged.

Presentations at the Conference consisted of 42 lectures and 31 posters. They were in the fields of genomics, evolution, development, biodiversity, ecology, microbiome biology, physiology, metabolism, stress biology, phenotyping, cryopreservation, vertical cultivation technology, applications for human and animal nutrition, wastewater treatment, and the production of recombinant proteins. Notably, the presented work in this Conference differed from those of previous ICDRA meetings in that a greater majority of the duckweed-related studies are now addressing problems of interest at the mechanistic levels instead of the early stages of descriptive observations. In many cases, this is enabled by the accumulation of a critical mass of well-curated genomic resources that are underpinned by high quality reference genomes and complementary cytogenomic studies. Several talks by Robert Martienssen, Phuong Hoang, and Todd Michael exemplified this aspect. More genomic resources are now being generated by additional groups as genome sequencing technologies continue to improve and these resources are now being leveraged to study non-genetic transgenerational inheritance of stress responses, flowering related genes using transcriptomic approaches, genomic methylation control and transposon activity modulation, to name just a few of the exciting avenues of research that were discussed.

Another rich area of exciting discoveries that is being studied is that of duckweed-microbe interaction, especially related to the microbiome and its potential impact on duckweed growth. One particularly interesting presentation was by Masaaki Morikawa (Hokkaido, Japan) which demonstrated that two bacteria, one Chryseobacterium and the other a Bacillus, can suppress a harmful microalga *Microcystis aeruginosa* under different growth context with duckweed and potentially can help improve plant health in commercial facilities. In addition to the intriguing science that may be revealed by studying this diversity of plant-microbe interactions, this type of finding can have important impact for sustainable duckweed cropping systems in the future. In this regard, it is fitting that one whole day of this Conference was dedicated to talks related to applications of duckweed in terms of services and products for society that can be generated from this plant family. In addition to updates by veteran presenters of previous ICDRAs, such as Ingrid van der Meer (Wageningen) and Tsipi Shoham (GreenOnyx, Israel) on their important work to gain broad approval for target duckweed products, multiple talks focused on the common theme of valorization of wastewater resources through the use of duckweed and aquaculture to create a circular economy (Jansen, Paolacci, Skillicorn). Another common theme in the effort to commercialize duckweed that is now in vogue is the turn to vertical farming technologies in place of the more classical raceway and pond systems. There were at least six different talks and posters which described the adaptation of this modular approach for scalable duckweed production at various stages of being deployed and optimized. Together with a number of innovative ways that duckweed products from GreenOnyx are being introduced to the marketplace, it looks likely that the effort to monetize duckweed may finally become a reality in the not-too-distant future.

Finally, I would like to point out a couple of new unpublished studies that are especially innovative. The talk by Ljudmilla Borisjuk (IPK, Germany) on the 3-D structure of Wolffia species using advanced microscopy with fine serial sections and image reconstruction provided some of the most breathtaking rendition of duckweeds that I have seen (some examples of her work are shown in the next figure). Her integrative modeling of the size and shape of different Wolffia species by comparison to various types of aquatic vessels merged engineering principles to the functional rationalization of biological structures. In contrast, the talk by Shogo Ito (University of Kyoto, Japan) is a tour de force of systematic optimization to generate the protocols required for cryopreservation of different duckweed genera. While the five genera differ in their efficacy for preservation, the results presented show that this could be a realistic approach for the long-term storage of duckweed strains with significantly less labor.



High resolution 3-D rendering of *Wolffia australiana* using image reconstruction with ultrathin serial sections. **Panel A** highlights the successive production of 3 new daughter fronds (in yellow) in addition to the oldest daughter on the left. **Panel B** highlight the granddaughter fronds (in yellow) as well as the stipe tissue (in magenta) of the mother frond (in green). Photos courtesy of Dr. L. Borisjuk and S. Ortleb (the IPK, Gatersleben, Germany).

Three poster prizes and one prize for the best lecture by participating students in the Conference were awarded, with funds donated by CLF Plant Climatics GmbH and MDPI Plants-Verlag. Finn Petersen, Osnabrück University of Applied Sciences, received the prize for the best oral contribution by a doctoral student. Martin Schäfer (University of Münster), Alexej Sonnenfeld (University of Greifswald) and Dylan Jones (University of Nottingham, UK) were awarded the prize for best posters. Members of ISCDRA worked on the jury.

Awards for student presenters. (Left) Viktor Oláh and Klaus Appenroth presented the oral presentation price to Finn Petersen. (Right) The poster award ceremony with Manuela Nagel, donor representative, lab mate of Dylan Jones, Martin Schäfer, Alexej Sonnenfeld and Eric Lam. Photo credit: Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben, Germany.

Two administrative business for the duckweed community also transpired in this Conference: the election of a new steering committee (the 5th ISCDRA) and the appointment of the new host country for the ICDRA in two years. After the open ballot election at the end of April, the newly elected members for the ISCDRA are Klaus J. Appenroth, K. Sowjanya Sree, Tsipi Shoham, Marcel Jansen and Eric Lam (picture on bottom of next page). I am honored that my fellow elected members have voted to have me continue to serve as the Chair for this committee. With their help, I hope to continue our work to maintain a high community standard and to broaden the visibility of the duckweed field in science and society. As I have expressed at the Conference, I sincerely hope that the increased membership in our community will translate into more involvement and engagement with each other and with the ISCDRA. It is through greater participation by our members that we can truly grow to our full potential together. Finally, by a unanimous vote at the close of the

6th ICDRA, the proposal by Thailand to host the next ICDRA in Bangkok, Thailand, was officially accepted. Please see the acceptance and invitation by the organizers in this issue of the DF. I look forward to seeing many, if not all, of you there.

Photo credit: Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben, Germany.

6th ICDRA: talks and posters

Sunday May 29 – Plenary Talk

Duckweed hibernation: unraveling the molecular basis of the turion induction switch in *Spirodela polyrhiza*

Eric Lam

Rutgers University, United States of America; ericL89@hotmail.com

Monday May 30 – Genomics & Evolution

Genomic and epigenomic consequences of clonal growth habit in the Lemnaceae

Evan Ernst[1], Todd Michael[2], John Shanklin[3], Eric Lam[4] and Rob Martienssen[1]

[1]Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, NY 11724, [2]Salk Institute, La Jolla, CA 92037, [3]Brookhaven National Laboratory, Upton, NY 11973, [4]Rutgers University, New Brunswick, NJ 08901

The giant duckweed, a model system for studying plant evolution in a multitrophic community

Shuqing Xu

University of Mainz, Germany; shuqing.xu@uni-mainz.de

TBP fingerprinting unveiled interspecific hybridization in the genus Lemna

Laura Morello[1], Luca Braglia[1], Floriana Gavazzi[1], Silvia Gianì[1], Massimiliano Lauria[1], Klaus-J Appenroth[2], Manuela Bog[3]

[1]Istituto Biologia e Biotecnologia Agraria CNR, Milano, Italy; [2]Matthias Schleiden Institute of Plant Physiology, University of Jena, Germany; [3]Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany; morello@ibba.cnr.it

Integrative analysis of growth dynamics in duckweed

Stefan Ortleb[1], Twan Rutten[1], Alexander Hilo[1], Andreas Guendel[1], Hardy Rolletschek[1], Eric Lam[2], Ljudmilla Borisjuk[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research, IPK-Gatersleben, Germany; [2]Department of Plant Biology Rutgers, The State University of New Jersey, USA; borisjuk@ipk-gatersleben.de

Epigenetic regulation of transposable elements in duckweeds

Rodolphe Dombey, Veronica Barragan, Arturo Marí-Ordóñez

Gregor Mendel Institute of Molecular Plant Biology. Dr Bohr-Gasse,3, 1030 Vienna, Austria; rodolphe.dombey@gmi.oeaw.ac.at

Monday May 30 – Differentiation & Stress

Nutrient stress on duckweeds

Sowjanya Sree Kandregula[1], Klaus-J. Appenroth[2]

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Non-genetic Inheritance alters Stress Resistance across Generations in the Giant Duckweed

Alexandra Chávez Argandoña, Meret Huber

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All in one: the microbiome of a rootless plant

Tarun Pal, Osnat Gillor

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Analysis on floral induction of *Wolffiella hyalina*

Minako Isoda, Tokitaka Oyama

Kyoto University, Japan; minako.isoda@gmail.com

To root or not to root: evolution of rootlessness in duckweed

Alexander Ware, Dylan Jones, Anthony Bishopp

Department of Plant Sciences, University of Nottingham, United Kingdom; alex.ware@nottingham.ac.uk

Monday May 30 – Ecology, Microbiome & Stress

Lemnaceae as key players in the circular economy; examples of wastewater valorisation in Ireland

Eamonn Walsh[1], Neil Coughlan[1], Simona Paolacci[1,2], Vlastimil Stejskal[1,3], Rachel O'Mahoney[1], Marcel AK Jansen[1]

[1]School of Biological, Earth & Environmental Sciences & Environmental Research Institute, University College Cork, Cork, Ireland; [2]Current address; Bantry Marine Research Station, Ireland; m.jansen@ucc.ie

Stress tolerance in tetraploid *Spirodela polyrhiza*

Quinten Bafort[1,2,3], Tian Wu[2,3], Annelore Natran[2,3], Yves Van de Peer[2,3,4,5]

[1]Department of Biology, Ghent University; [2]VIB-UGent Center for Plant Systems Biology; [3]Department of Plant Biotechnology and Bioinformatics, Ghent University; [4]Department of Biochemistry, Genetics and Microbiology, University of Pretoria; [5]College of Horticulture, Academy for Advanced Interdisciplinary Studies, Nanjing Agricultural University; quinten.bafort@ugent.be

Wastewater valorisation in an integrated multitrophic aquaculture system; assessing nutrient removal and biomass production by duckweed species

Simona Paolacci[1,2], Vlastimil Stejskal[1,3], Damien Toner[4], Marcel Jansen[1]

[1]University College Cork, Ireland; [2]Bantry Marine Research station, Ireland; [3]University of South Bohemia in Ceske Budejovice, Czech Republic; [4]Ireland's Seafood Development Agency, BIM, Block 2, Ireland; spaolacci@bmrs.ie

Dual function of environmental bacteria that enable the duckweed prosperity

Yeni Khairina, Shohei Kuroda, Rahul Jog, Masaaki Morikawa

Hokkaido University, Japan; morikawa@ees.hokudai.ac.jp

Eine neue Kulturpflanze mit großem Potenzial für Ernährung, Energie und Wasserreinigung

Klaus-J. Appenroth

Matthias Schleiden Institute Plant Physiology, University of Jena, Jena, Germany; Klaus.Appenroth@uni-jena.de

Tuesday May 31 – Physiology & Metabolism I

The Underwater Chemical World of Duckweed
Asaph Aharoni, Uwe Heinig, Mark Polikovsky
Weizmann Institute of Science, Rehovot, Israel; asaph.aharoni@weizmann.ac.il

Variability of chronobiological characteristics in duckweed
Tokitaka Oyama
Graduate School of Science, Kyoto University, Japan; oyama.tokitaka.8w@kyoto-u.ac.jp

Unraveling the genetic mechanisms of plant sexual reproduction in duckweed
Cristian Mateo-Elizalde[1], Evan Ernst[2], Rob Martienssen[2]
[1]Cold Spring Harbor Laboratory, NY, USA.; [2]Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, NY, USA.; mateo@cshl.edu

Using rootless duckweed *Wolffia globosa* as a model plant to clarify the effect of e[CO_2] on NO_3 photosassimilation in C3 plants.
Moshe Tuvia Halpern[1], Shimon Rachmilevitch[2]
[1]Agricultural Research Organization, Volcani Institute, Israel; [2]French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institute for Desert Research, Ben Gurion University, Sede-Boqer Campus, 84990, Israel; hmoshe@volcani.agri.gov.il

How aminomethylphosphonic acid (AMPA), the main glyphosate metabolite, affects *Lemna minor* photosynthesis?
Marcelo Pedrosa Gomes[1], Patricia Lawane Freitas[1], Rafael Shinji Akiyama Kitamura[1], Eduardo Gusmão Pereira[2], Philippe Juneau[3]
[1]Laboratório de Fisiologia de Plantas sob Estresse, Departamento de Botânica, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, Brazil; [2]Laboratório de Fisiologia do Estresse Abiótico, Instituto de Ciências Biológicas, Universidade Federal de Viçosa, Florestal, Brazil; [3]Ecotoxicology of Aquatic Microorganisms Laboratory, GRIL – EcotoQ TOXEN, Department of Biological Sciences, Université du Québec à Montréal, Montréal, QC, Canada; juneau.philippe@uqam.ca

Tuesday May 31 – Physiology & Metabolism II

The life cycle of *Spirodela polyrhiza*: a model for aquatic plant overwintering?
Paul Ziegler
University of Bayreuth, Germany; paul.ziegler@uni-bayreuth.de

Different sources of iron modulate responses in the antioxidant system in plants
Darlielva do Rosário Freitas, Pedro Henrique Santos Neves, Vinícius Melo da Silva, Gustavo Alves Puiatti, Ana Júlia Carvalho Defeo, Renata Pereira Lopes, Juraci Alves de Oliveira
Universidade Federal de Viçosa (UFV), Brazil; darlielva.freitas@ufv.br

The dynamics of $NO_3$and NH_4+ uptake in duckweed are coordinated with the expression of major nitrogen assimilation genes
Yuzhen Zhou.[1], Olena Kishchenko[1,2,3], Anton Stepanenko[1,2], Guimin Chen[1], Nikolai Borisjuk[1]
[1]Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Sciences, Huaiyin Normal University, Huaian, China; [2]Institute of Cell Biology and Genetic Engineering, National Academy of Science of Ukraine, Kyiv, Ukraine; [3]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; nborisjuk@hytc.edu.cn

Development of cryopreservation protocol for a variety of duckweed meristems by vitrification-cryo-plate method

Shogo Ito[1], Daisuke Tanaka[2], Tokitaka Oyama[1]

[1]Department of Botany, Division of Biological Sciences, Graduate School of Science, Kyoto University, Kyoto, Japan; [2]Germplasm Preservation Unit, Research Center of Genetic Resources, NARO, Tsukuba, Japan; shogoi@cosmos.bot.kyoto-u.ac.jp

Advances in droplet-vitrification and freezing of Lemna fronds for long-term preservation of duckweed

Manuela Nagel

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany; nagel@ipk-gatersleben.de

Tuesday May 31 – Genome Organisation, Diversity & Imaging

Organization and evolution of ribosomal RNA genes in duckweed

Nikolai V. Borisjuk

Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Sciences, Huaiyin Normal University, Huai'an, China; nborisjuk@hytc.edu.cn

Duckweed genome architecture

Todd Michael

Salk Institute for Biological Studies, United States of America; tmichael@salk.edu

Chromosome numbers and genome sizes for duckweed species and implication for genome evolution and diversity of Lemnaceae

Phuong Thi Nhu Hoang[1,2], Tram Ngoc Bao Tran[1], Jörg Fuchs[2], Ingo Schubert[2]

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Biodiversity of duckweed (Lemnaceae) in water reservoirs of Ukraine and China assessed by double chloroplast DNA barcoding

Guimin Chen[1], Anton Stepanenko[1,2], Olha Lakhneko[2], Yuzhen Zhou[1], Olena Kishchenko[1,2,3], Anton Peterson[1,2,3], Dandan Cui[1], Haotian Zhu[1], Jianming Xu[1], Bogdan Morgun[2], Dmitri Gudkov[4], Nikolai Friesen[5], Mykola Borysyuk[1]

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Family portrait of a duckweed colony: What can imaging-based phenotyping tell us about stress responses of mother and daughter fronds

Viktor Oláh[1], Kamilla Kosztankó[1], Muhammad Irfan[1], Csongor Freytag[1], Marcel A. K. Jansen[2], Ilona Mészáros[1]

[1]University of Debrecen, Hungary; [2]University College Cork, Ireland; olahviktor@unideb.hu

Wednesday June 1 – Waste Water Remediation, Feed, Food, Biofuel & Transformation

Duckweed – Conservation and conversion into biogas

Britt Schumacher[1], Meinolf Stützer[2]

[1]DBFZ Deutsches Biomasseforschungszentrum gemeinnützige GmbH, Germany; [2]GMBU Gesellschaft zur Förderung von Medizin, Bio und Umwelttechnologien e. V., Halle/S., Germany; britt.schumacher@dbfz.de

Water lentils and derived products thereof for human consumption: overview of the current status of NF approval in Europe

Ingrid van der Meer[1], Jurriaan Mes[2]

[1]Wageningen University & Research, Plant Research, The Netherlands; [2]Wageningen University & Research, Food & Biobased Research, The Netherlands; ingrid.vandermeer@wur.nl

Water lentils protein for human nutrition

Jurriaan Mes[1], Aard de Jong[1], Ingrid van der Meer[2]

[1]Wageningen Food & Biobased Research, The Netherlands; [2]Wageningen Plant Research, The Netherlands; jurriaan.mes@wur.nl

Lemna turionifera demonstrates good potential as an alternative system for transient expression of recombinant proteins

Anton Peterson[1,2,3], Olena Kishchenko[1,2,3], Yuzhen Zhou[2], Nikolai Borisjuk[2]

[1]Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany; [2]Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Science, Huaiyin Normal University, 223300 Huaian, China; [3]Institute of Cell Biology and Genetic Engineering, National Academy of Science of Ukraine, 03143 Kyiv, Ukraine; peterson@ipk-gatersleben.de

Insights into the nutritional value of duckweed as protein feed for broiler chickens

Johannes Demann[1], Finn Petersen[1], Hans-Werner Olfs[1], Andreas Ulbrich[1], Heiner Westendarp[1], Petra Wolf[2]

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Wednesday June 1 – Large Scale Cultivation & Social Aspects

Duckweeds, the engine of the New Circular Economy

Paul Skillicorn

Skillicorn Technologies, United States of America; Paul@SkillicornTechnologies.com

Securing fresh nutrition via a breakthrough technology for growing Duckweeds

Tsipi Shoham

GreenOnyx Ltd., Israel; tsipi@greenonyx.biz

Development and scale-up process from a small duckweed culture to a large indoor vertical farm for duckweed biomass production

Finn Petersen[1], Johannes Demann[1], Jannis von Salzen[1], Tim Dargatz[1], Dina Restemeyer[1], Hans-Werner Olfs[1], Heiner Westendarp[1], Andreas Ulbrich[1], Klaus-Juergen Appenroth[2]

[1]University of Applied Sciences Osnabrück, Germany; [2]University of Jena, Germany; finn.petersen@hs-osnabrueck.de

Carbon dioxide sequestration potential of two Thai duckweed species: *Wolffia globosa* and *Lemna aequinoctialis* under open-air greenhouse

Wisuwat Songnuan[1], Metha Meetam[2], Wanvisa Pugkaew[3], Somnuek Jaroonjitsathian[4], Kanidtha Jariyachawalid[4]

[1]Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand; [2]Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand; [3]Advanced Greenfarm Company Limited, Nonthaburi, Thailand; [4]Innovative Strategic Planning and Management Department, PTT Public Company Limited, Bangkok, Thailand; wisuwat.son@mahidol.edu

High-performance-Lemna minor cultivation: LemnaCore 1.0 as an innovative indoor vertical aquafarming concept

Marko Dietz

Carbon Clouds, Dresden, Germany

Posters

Putative Hybridisation between *Lemna minor* and *Lemna turionifera*

Alexej Sonnenfeld[1], Manuela Bog[2]

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A new machine learning method to quantify growth in Lemnaceae

Leone Ermes Romano, Giovanna Aronne

Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy; leoneermes.romano@unina.it

Developing approaches to investigate anatomical reduction in duckweed

Dylan Jones, Alex Ware, Brian Atkinson, Tony Bishopp, Darren Wells

School of Bioscience, University of Nottingham, Sutton Bonington, United Kingdom; dylan.jones@nottingham.ac.uk

Molecular diversity of 5S rDNA genes in eight geographic ecotypes of *Landoltia punctata*

Anton Stepanenko, Guimin Chen, Nikolai Borisjuk

Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake, School of Life Sciences, Huaiyin Normal University, Huai'an, China; stepanenko@hytc.edu.cn

Morphological variation, chromosome number, and DNA barcoding of Giant Duckweed (*Spirodela polyrhiza*) in Vietnam

Tram Ngoc Bao Tran[1], Jörg Fuchs[2], Ingo Schubert[2], Phuong Thi Nhu Hoang[1,2]

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Ionomic analysis across the duckweeds reveals pervasive accession-level variation and genus-scale trends

Kellie E. Smith[1], Min Zhou[2], Paulina Flis[3], Dylan H. Jones[4], Anthony Bishopp[5], Levi Yant[6]

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Identification and characterization of C-repeat binding factor (CBF) genes in duckweed

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Transcriptional profiles of Mn-stressed duckweed, *Spirodela polyrhiza*, are affected by the source of nitrogen

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Duckweed holobiont research in Thailand under Japan-Thai collaboration through SATREPS, JICA

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The domestication of novel crops in Israel: isolation, cultivation, and characterization of Wolffia species

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Ion specific salt stress of *Lemna minor* and the implications on biological effluent of pig manure treatment

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Effects of red-light and pre-culture periods on duckweed regeneration after cryopreservation

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How does Wolffia (Lemnaceae) silence Transposable Elements?

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Non-targeted site resistance mechanisms to diquat in *Spirodela polyrhiza*

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Cultivation of *Lemna minor* on industry derived dairy processing wastewater

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Resurrecting an ancestral gene network in duckweed
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Differential phytotoxic effect of silver nitrate ($AgNO_3$) and bifunctionalized silver nanoparticles (AgNPs-Cit-L-Cys) on Lemna plants (duckweeds)
M. Adelaide Iannelli[1], Amii Bellini[2], Iole Venditti[2], Barbara Casentini[3], Chiara Battocchio[2], Massimiliano Scalici[2], Simona Ceschin[2]
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Duckweed grown in recirculating systems on different dilution rates of cattle slurry: Productivity and protein production
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Aphid herbivory changes growth and metabolism of the aquatic plant, *Spirodela polyrhiza*
Martin Schäfer[1,2], Christoph Walcher[3], Marie Sarazova[1], Laura Böttner[1,2,4], Ramona Petrig[3], Shuqing Xu[1,2]
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Different physiological and biochemical responses of *Lemna minor* and *Spirodela polyrhiza* exposed to s-metolachlor
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A soil bacterium *Azotobacter vinelandii* contributes to growth promotion of duckweed through nitrogen fixation, bacterial synergism and EPS production
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Prevalence of vitamin B12 in a novel crop: developing methods and monitoring B12 in duckweed species
Linda Klamann[1,2], Michal Sela-Adler[1], Inna Khozin-Goldberg[2], Osnat Gillor[1]
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Efficient lighting in indoor vertical concepts – Effect of light intensity on limit density in sigmoidal growth models.
Jannis von Salzen, Finn Petersen, Dina Restemeyer, Tim Dargatz, Andreas Ulbrich
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What is the feasibility of duckweed cultivation on pig farms in Flanders? And how to put the insights in a global context?
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Phytoremediation potential of duckweed for heavy metals from polluted waterbodies; the case of Lebanon

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Simultaneous nutrient control and duckweed biomass production from wastewater using *Wolffia angusta*: A kinetic study

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FT-MIR-PLSR simultaneous determination of total nitrogen and nitrate in duckweeds

Javier Espinosa-Montiel[1], Javier Hernandez-Allica[2], Alla Mashanova[1], Alyssa Blachez[3], Stephan Haefele[2], Cristina Barrero-Sicilia[1], Steve McGrath[2]

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Biomass, protein and nitrate accumulation in duckweeds supplied with 3 different sources of nitrogen

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7th ICDRA: Announcement

The 7th International Conference on Duckweed Research and Applications (ICDRA 2024)

Venue: Bangkok, Thailand

Host: Kasetsart University, Thailand

Co-host: Science and Technology Research Partnership for Sustainable Development (SATREPS), JICA, Japan

Welcoming message

The 7th ICDRA will be hosted in 2024 by Kasetsart University together with Science and Technology Research Partnership for Sustainable Development (SATREPS), JICA under the joint research initiative between Japan and Thailand on Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy.

It is our great pleasure to welcome you to Thailand, the land of smile, rich culture, traditions and attractions, and plenty of duckweeds! In addition to our scientific forum as usual, we would like you to try *Wolffia globosa* or 'Khai-nam' cooked from traditional Thai cuisines to modern healthy cuisines, explore 'Khai-nam' local cultivation farms and meet with Agritech & Food/Beverage industries to drive innovation of duckweeds. We are looking forward to host great experts and young scientists from different countries around the world to share new and exciting results in duckweeds with prominent networking opportunities.

See you soon in Bangkok, Thailand, 2024!

Arinthip Thamchaipenet
Metha Meetam

Be-HoBiD

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Practical applications of Duckweed

On a mission to introduce duckweed as the 21st century cash crop:

An update on current active commercial and research efforts

Tsipi Shoham

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Dear readers:

Duckweed applications have grown significantly over the past 4 years (since my last report in October 2018). I hereby provide you with an updated review on active industrial and academic efforts that are on the way to realize the potential of duckweeds as promising platforms for various practical applications.

Accordingly, below you may find a table and its subsequent figures that review and map the currently active entities that are leading breakthrough research, technology, and development, towards duckweed based new products and/or services. I am happy to report that the overall number of global entities is currently 31, which represent almost a 50% growth as compared to the entities listed in my last review, October 2018[1] (table 1).

As demonstrated in figure 1, the growth in duckweed application research and development activities is mainly a result of: (1) the significant increase in the numbers of teams that are focused on the development of new duckweed growing systems, mainly indoor vertical units, addressing the need for controlled large-scale duckweed cultivation; (2) the increase in the need for alternative plant-based nutrition for human consumption, thus a significant market for duckweed as human food; (3) the growing demand for alternative solutions for animal and fish feed, highlighting the potential of the duckweed biomass as feed; along with (4) the focus on duckweed for circular economy.

It is also notable that the growth in duckweed application research and development activities is reflected by a wider global distribution, with a dramatic increase of activities in Germany and penetration into additional new regions (figure 2).

The advance in the duckweed application research and development activities results also in more companies that matured to the commercialization phase: 4 companies with different products for human food, 2 companies with ecotoxicology testing products, 1 company with supplement products for human consumption, and 1 company with a product for fish feed (table 1).

As this review and its presented table and figures aim to extend our dialogue and are by no means final, I will be very happy to receive any feedback, updates and/or inputs, providing further updated information to the benefit of you all.

1 http://www.ruduckweed.org/uploads/1/0/8/9/10896289/duckweed_forum_issue_23.pdf

Table 1: Companies, Academy and research institutions that develop duckweed based new products and applications (Sorted per application).

	Company/ Institute Name	Field of application	Focused on	Status	Country	Website
1	Rubisco Foods[2]	**Human food** **Pet food**	Sports nutrition, supplements, bakery, meat replacement, egg replacement in noodles	Product Development –Pilot scale	The Netherlands	www.rubiscofoods.com
2	Plantible Foods[3]	**Human food** **pet food**	Plant-based protein – alternative Egg proteins	Product Development –Pilot scale	USA	https://www.plantiblefoods.com
3	Lemnature AquaFarms[4]	**Human food**	Plant-based proteins and other ingredients	Commercialization- US market	USA	https://lemnatureusa.com
4	GreenOnyx	**Human food** **Duckweed growing systems**	Fresh green vegetables	Commercialization- Israel & EU markets	Israel	https://www.greenonyx.biz
5	Hinoman	**Human food**	Frozen green biomass of Wolffia globosa 'Mankai'	Achieved market penetration. Currently on hold - in the midst of ownership change	Israel	https://eatmankai.co.il/ https://eatmankai.com/
6	Space Lab Tech	**Duckweed growing systems**	Space Agriculture and Bioregenerative Space Life Support	Proof of concept	USA	www.spacelabtech.com
7	Bioponica	**Duckweed growing systems**	Commercial scalable systems of channel for growing Lemna species	Under Development	USA	https://bioponica.net
8	Drygro	**Duckweed growing systems**	Plant based proteins for: Human food, Pet food, Animal feed, Fish feed	NA	UK; Kenya	http://drygro.com
9	LemPro	**Animal Feed** **Fish feed**	Organic protein for fishmeal, feed, & fertilizer	NA	USA	http://www.lempro.net
10	OxyGenesis GmbH	**Animal feed**	Protein source for farm animals, pets, fish farming	Under Development	Germany	https://www.oxygenesis.de
11	Aqua-Light GmbH	**Duckweed growing systems** **Fish feed** **Pet food** **Water treatment** **Aquatic ecotoxicology**	A vertical cultivation system - "Symbiofilter" for growing duckweed as fish feed and for water treatment – filtering Phosphate from industrial wastewater	Product development – industrial scale and the initiation of market penetration	Germany	https://symbiofilter.de/?page_id=77 https://aqualight.de
12	Syntech Research	**Aquatic ecotoxicology**	Research, development & regulatory services	NA	Spain	https://www.syntechresearch.com/what-we-do/ecotoxicology-services/aquatic-toxicology-services/
13	TOXI-COOP ZRT. (CRO)	**Aquatic ecotoxicology**	Studies according to OECD guideline No. 221.	Product /service Development – Industrial scale	Hungary	https://toxicoop.com/
14	FMC	**Aquatic ecotoxicology**	Chemical manufacturing	Commercialization	USA	http://www.fmc.com
15	Stillmeadow	**Aquatic ecotoxicology**	Aquatic Plant Toxicity with *Lemna spp. (OCSPP 850.4400, OECD 221)*	Commercialization	USA	https://www.stillmeadow.com/toxicology/environmental-toxicology/
16	Lyndon Water	**Circular economy**	Integrating water treatment with aquaculture of plants and fish	NA	UK	http://www.lyndonwaterafrica.com
17	Duckweed USA	**Bioenergy**	NA	NA	USA	http://duckweedusa.com/index.html

2 Previous name – ABC Kroos
3 Previous name – Aquible
4 Previous name – Parabel

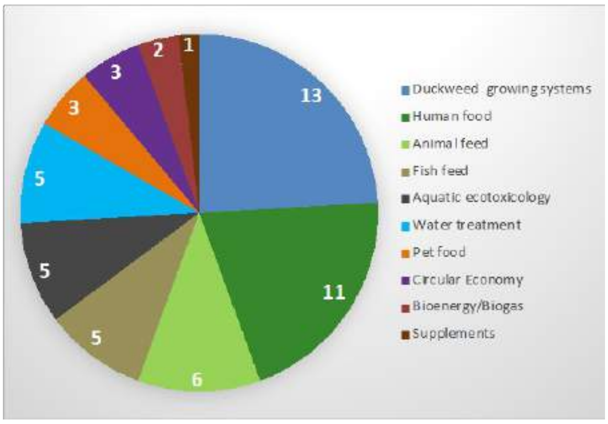
New Activities

18	Advanced Greenfarm Ltd.	**Human food**	Plant-based source of protein	Commercialization - Thailand market	Thailand	https://www.advancedgreenfarm.com/home-en
19	University of Colorado Boulder	**Human food**	Space Health, Space food	Applicative Research	USA	https://www.colorado.edu/today/2020/07/17/duckweed-incredible-radiation-fighting-astronaut-food-and-changing-how-it-grown-we-made
20	Wageningen University	**Human food**	Alternative protein	Applicative Research	The Netherlands	https://www.wur.nl/en/project/Duckweed-as-a-new-sustainable-source-of-protein-for-human-consumption-Pr0t31.htm
21	Biovolf	**Human food** **Animal feed** **Duckweed growing systems**	Plant based food ingredient, proteins and other, via vertical bioreactor	Development – Industrial scale	Russia; Singapore	https://biovolf.com/#rec414046111
22	Carbon Clouds GmbH	**Human food** **Duckweed growing systems** **Water treatment**	Dry mass products via vertical bioreactor	Development –proof of concept, demonstration	Germany	Contact-adress: info@carbon-clouds.de
23	Integrated Explorations Inc.	**Human food** **Duckweed growing systems** **Water treatment**	Producing fresh product	Development, pre-Proof of concept	Canada	www.iebiolab.com
24	SKILLICORN Technologies LLC, USA	**Duckweed growing systems** **Animal feed** **Fish feed** **Water treatment** **Circular Economy**	Wastewater Recycling for industries and Brackish water desalination, Animal Feed and Fish Feed, The New Circular Economy	Product /service Development – Industrial scale *	The HQ - Texas, USA. Duckweed facilities at: Ireland, Jordan, Egypt, India, New-Zealand:	www.skillicorntechnologies.com
25	Planet Duckweed	**Duckweed growing systems** **Animal feed** **Fish feed**	Duckweed Pods™, Duckweed-optimized fertilizer, DPM™ – vertically-integrated, mobile and scalable duckweed production modules	Commercialization - US market	USA	www.planetduckweed.com
26	Institute of Plant Genetics (IPK); Institute of Cell Biology and Genetic Engineering (ICBGE); Huaiyin Normal University	**Duckweed growing systems**	Bioreactors for expressing recombinant proteins - Oral vaccines for fish and animals	Development-Proof of concept	Germany; Ukraine; China	NA
27	University College Cork	**Duckweed growing systems** **Fish feed** **Water treatment** **Circular economy**	Growing systems for integration in agro-food wastewater treatment systems, including the dairy and biofuel industries	Research-Proof of concept Development – Industrial scale prototype	Ireland	http://www.newtrients.ucc.ie/ http://www.ucc.ie/en/brainwaves
28	Osnabrück University	**Duckweed growing systems**	Indoor vertical farm for duckweed biomass production	Development and scale-up	Germany	https://www.researchgate.net/publication/361053347_Development_and_scale-up_process_from_a_small_duckweed_culture_to_a_large_indoor_vertical_farm_for_duckweed_biomass_production

29	Osnabrück University	Animal feed	Duckweed as alternative protein feed for broiler chickens	Development – Industrial scale	Germany	
30	Lemnaceae Fermentation Inc.	Supplements	Natural astaxanthin and other carotenoids (lycopene, β-carotene, zeaxanthin, canthaxanthin) produced by fermentation	Commercialization	Taiwan	https://lemnaceae.com
31	DBFZ GmbH	Biogas	Conservation and conversion of duckweeds into biogas	Research- Proof of concept	Germany	https://www.openagrar.de/receive/dimport_mods_00002580

Not active / Not verified

	Agravis Raiffeisen GmbH	Animal feed	Raw material for animal feed	No longer active in the duckweed field	Germany	https://geschaeftsbericht.agravis.de/en/gb/gb15/wir_gestalten_zukunft/wasserlinsen/index.html
	GreenSun Products, LLC	Pet food		Company closed	USA	https://www.linkedin.com/company/greensun-products-llc/?originalSubdomain=il; http://www.GreenSunProducts.com
	Sulabh Social Service Organization	Water treatment	Local waste water treatment with direct economic returns from pisciculture	?	India	http://www.sulabhinternational.org/duckweed-based-waste-water-treatment/
	Biomass Alternative Power	Bioenergy	Produce natural gas	?	USA	https://biomassalternativepower.com
	Prosys Biorefining Systems	Duckweed growing systems	Animal feed, Human food & Bioenergy	?	USA	http://www.prosysbiorefining.com/products.html
	Jungleponics	Fish feed		?	Peru	



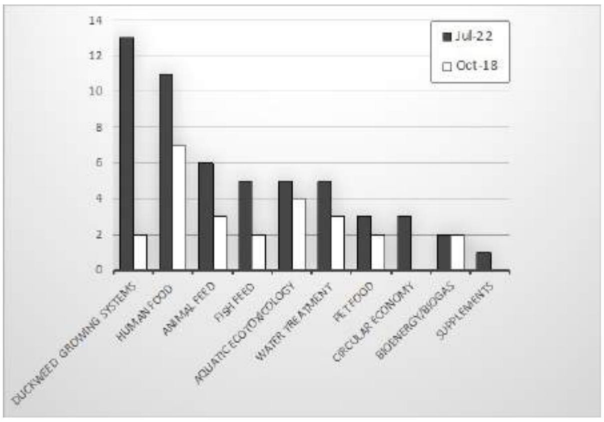
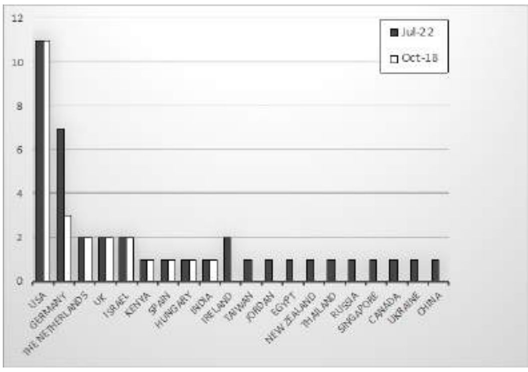


Figure 1: The potential diversity of duckweed application: Duckweeds have been studied for various applications, currently focusing on Growing systems, Human food, Animal and Fish feed, Pet food, Ecotoxicology, Water treatment, Bioenergy, Circular Economy and Supplements for human consumption. Left panel: The activity volume of each duckweed application, demonstrating that the leading fields are "Duckweed growing systems" and "Human food". Right panel: the current (July 2022) vs. the previously presented (Oct. 2018) activities show a massive growth in the "growing systems" over the last 4 years. There is also a significant growth in the "Human food", "Animal feed" and "Fish feed" along with the emergence of 2 new applications: "Supplement" for human food and "Circular economy".

Figure 2: Mapping global distribution of activities in the development of duckweed applications: Upper map – current distribution as of July 2022; Lower Map – as was presented in October 2018. The columns figure presents a comparison of the activity volume per country between the two time points (Jul 2022 vs. Oct 2018). While the highest number of activities in the duckweed application filed it is still in the USA, there is a significant growth in the activities in Germany and a significant penetration into additional new regions as Taiwan, Jordan, Egypt, New-Zealand, Thailand, Russia, Singapore, Canada, Ukraine, and China.



Student Spotlight: Sajjad Kamal Shuvro

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Farm the future

At Hokkaido university greenhouse

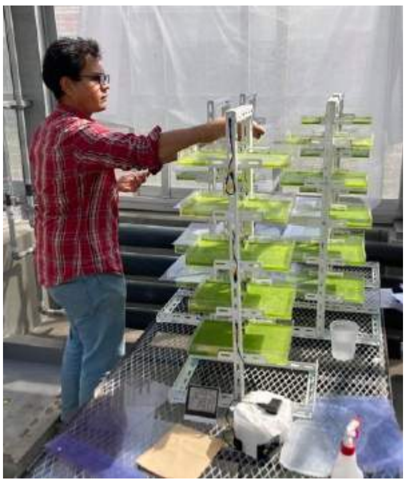
Born and raised in Bangladesh, I have experienced the dire effect of climate change and global warming in the form of frequent floods in my country. I have always wondered how scientific endeavors mitigate such adverse effects of climate change in vulnerable countries. In my undergraduate years as a microbiology student, I was mesmerized by the near infinite variations in the metabolic potential of microorganisms that act as catalysts in our ecosystems. The idea of finding a solution to issues like global warming and climate change by the utilization of microorganisms thrilled me. However, I was still missing the second component that could be used as a vehicle to enhance the utilization of the potential of microorganisms. At Hokkaido University, Japan, I was introduced to this missing component by Morikawa sensei. And that is duckweed, a perfectly suitable host for bacterial colonization and consequent application for solving many biotechnological issues. Hence, our interest lies in studying and profiling duckweed plant growth promoting bacteria that will eventually lead to the creation of an artificial duckweed holobiont for diverse biotechnological applications.

In Japan, *Lemna aequinoctialis, Lemna japonica, Lemna minor*, *Lemna gibba*, *Spirodela polyrhiza*, and *Wolffia globosa* are the most common species of duckweed found in natural lakes and ponds. Hokkaido is known for its long snow covered winter. It is not until late spring when the snow from the university ponds is completely cleared out that we find *L. minor* proliferating abundantly. The environmental samples of duckweed do contain a snapshot of the abundant species of microorganisms in the aquatic system. In order to observe the effects of single bacterial strains on the growth of the plant, isolated duckweed is sterilized using specific sterilization protocol. This removes the epiphytic bacteria from the plant body. The sterilized duckweed is then subjected to a bacterial assay called plant growth promoting bacteria (PGPB) assay. Due to the rapid growth rate of duckweed, any effect elicited by the bacteria or its metabolic byproducts can be observed on the growth of duckweed within the 10 day incubation time by comparing to a non-inoculated control. Food and beverage industries in Japan and Thailand may implement sustainable remediation of wastewater using duckweed. These open wastewater systems harbor a range of bacteria that are associated closely with the duckweed and often a PGPB can be found from associated bacterial communities. To find duckweed PGPB from wastewater, at first, duckweed associated bacteria are isolated and stored. Bacterial strains are then tested for their effect on duckweed growth through PGPB assay. In this way, positive or negative effects of a particular strain of bacteria on the growth of duckweed are quantified and molecular mechanisms of their interactions are studied deeply to make more efficient water treatment technology. After the treatment of water, the duckweed biomass may be subjected to biofuel production. Any other practical use of duckweed in Japan is currently rare. Duckweed biomass produced from wastewater thus has limited applications.

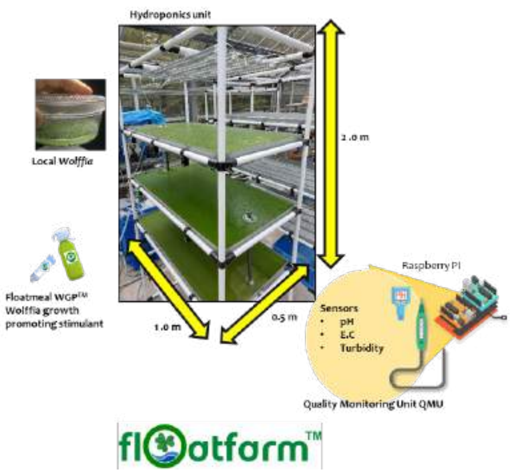
In order to add value to the duckweed biomass and render it edible to animals and even humans, there are feasibility and scaling issues that needs to be addressed. In an open air duckweed farming condition, addition of inorganic mineral nitrogen fertilizer risks increasing algal growth while use of complex organic compounds increases the bacterial load in the duckweed. Both of these extremities hamper duckweed quality and yield drastically. In my PhD research, I addressed this issue by trying to create an artificial symbiosis of a benign nitrogen fixing soil bacterium *Azotobacter vinelandii* with different duckweed species. Among the duckweed species subjected to PGPB assays, *L. minor* showed a promisingly high yield in presence of *A. vinelandii*. The PGPB effect persisted even under extremely nitrogen-limited conditions. Moreover, I have found that the bacterial cells on the fronds significantly increased during the incubation time. The symbiosis was stable for at least 30 days even in nitrogen-free condition. The *A. vinelandii*- *L. minor* artificial symbiosis was then subjected to PGPB assay in wastewater. The growth promotion effect was still observable. Bacteria associated *L. minor* significantly reduced uptake of excessive Na^+ ions from the wastewater while promoting the growth of the host plant. Finally, I have found strong evidence that the bacteria is actively fixing nitrogen in association with the *L. minor*. In conclusion, I learned that artificial holobiont may work at a laboratory scale. However, in an open mass production scale, the scenarios are different which may require further work.

I was fortunate enough to be able to gather a team consisting of water reclamation engineer, data scientist, microbiologist and food industry veterans in order to apply for a Startup research and development grant SCORE provided by the Japan Science and Technology agency (JST). Our project "Floatmeal" was accepted and we received equivalent to $40,000 dollars as R&D grant. We tried to improve the growth of duckweed *W. globosa* by optimizing the growth parameters in vertical hydroponics systems inside greenhouse. We designed a vertical hydroponics unit and optimized it for growing *W. globosa*. We have also built a prototype water quality monitoring unit using Raspberry Pi which continuously collects data into a growth optimization algorithm. The stable hydroponic system yields 2KG/week/m^2 in a greenhouse without the use of LED lights. Right now we are working on stabilizing the microorganisms of the system and our lab results have shown that we can grow up-to 3.6 times more than a completely sterile condition. The potential use of this integrated technology is the spreading of awareness of low-carbon technology amongst the duckweed farmers living in many countries of Southeast Asia. The technology can potentially increase duckweed farmers' income by 30% while contributing to alleviation of issues like food insecurity, malnourishment and climate change across the globe. After the holobiont stabilization, we will do a pilot. We have been contacted by stakeholders from Canada, Hawaii and Sri-lanka to initiate a pilot project for growing sustainable protein which I am looking forward to do.

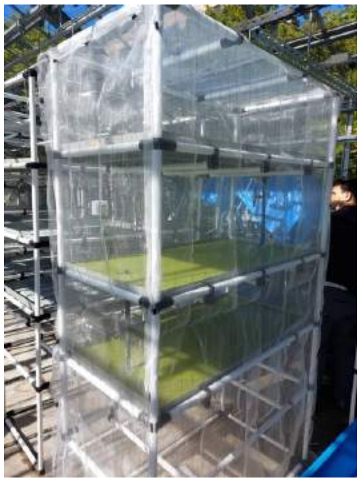
It is well documented that duckweed biomass has a very low environmental impact due to its prolific growth rate and that it is affected greatly by microbial interactions. Hence, with proper engineering and management of the duckweed rhizoplane, duckweed biomass can be rendered into sustainable biomass with a range of biotechnological applications such as human food, animal feed and biofuel. Before that, the sustainability and scalability issues of duckweed mass production has to be addressed. I consider duckweed as vehicle of change, a way to reduce greenhouse gas emission from our food and agriculture systems so it is definitely worth a try.



Floatfarm early prototype



Vertical mass production system



Floatfarm prototype V 1.0

From the Database

Highlights

Identification and expression analysis of GARP superfamily genes in response to nitrogen and phosphorus stress in *Spirodela polyrhiza*

Zhao, XY; Yang, JJ; Li, XZ; Li, GJ; Sun, ZL; Chen, Y; Chen, YM; Xia, ML; Li, YX; Yao, LG; Hou, HW. BMC Plant Biology (2022) 22: 308

GARP transcription factors perform critical roles in plant development and response to environmental stimulus, especially in the phosphorus (P) and nitrogen (N) sensing and uptake. *Spirodela polyrhiza* (giant duckweed) is widely used for phytoremediation and biomass production due to its rapid growth and efficient N and P removal capacities. However, there has not yet been a comprehensive analysis of the GARP gene family in *S. polyrhiza*. We conducted a comprehensive study of GARP superfamily genes in *S. polyrhiza*. First, we investigated 35 SpGARP genes which have been classified into three groups based on their gene structures, conserved motifs, and phylogenetic relationship. Then, we identified the duplication events, performed the synteny analysis, and calculated the Ka/Ks ratio in these SpGARP genes. The regulatory and co-expression networks of SpGARPs were further constructed using cis-acting element analysis and weighted correlation network analysis (WGCNA). Finally, the expression pattern of SpGARP genes were analyzed using RNA-seq data and qRT-PCR, and several NIGT1 transcription factors were found to be involved in both N and P starvation responses. The study provides insight into the evolution and function of GARP superfamily in *S. polyrhiza*, and lays the foundation for the further functional verification of SpGARP genes.

Circadian-period variation underlies the local adaptation of photoperiodism in the short-day plant *Lemna aequinoctialis*

Muranaka, T; Ito, S; Kudoh, H; Oyama, T. iScience (2022) 25: 104634

Phenotypic variation is the basis for trait adaptation via evolutionary selection. However, the driving forces behind quantitative trait variations remain unclear owing to their complexity at the molecular level. This study focused on the natural variation of the free-running period (FRP) of the circadian clock because FRP is a determining factor of the phase phenotype of clock-dependent physiology. *Lemna aequinoctialis* in Japan is a paddy field duckweed that exhibits a latitudinal cline of critical day length (CDL) for short-day flowering. We collected 72 strains of *L. aequinoctialis* and found a significant correlation between FRPs and locally adaptive CDLs, confirming that variation in the FRP-dependent phase phenotype underlies photoperiodic adaptation. Diel transcriptome analysis revealed that the induction timing of an FT gene is key to connecting the clock phase to photoperiodism at the molecular level. This study highlights the importance of FRP as a variation resource for evolutionary adaptation.

Aquaculture/ Agriculture

Influence of light Intensity and spectrum on duckweed growth and proteins in a small-scale, re-circulating indoor vertical farm

Petersen, F; Demann, J; Restemeyer, D; Olfs, HW; Westendarp, H; Appenroth, KJ; Ulbrich, A. (2022) Plants 11: 1010

Duckweeds can be potentially used in human and animal nutrition, biotechnology or wastewater treatment. To cultivate large quantities of a defined product quality, a standardized production process is needed. A small-scale, re-circulating indoor vertical farm (IVF) with artificial lighting and a nutrient control and dosing system was used for this purpose. The influence of different light intensities (50, 100 and 150 µmol m^{-2} s^{-1}) and spectral distributions (red/blue ratios: 70/30, 50/50 and 30/70%) on relative growth rate (RGR), crude protein content (CPC), relative protein yield (RPY) and chlorophyll a of the duckweed species *Lemna minor* and *Wolffiella hyalina* were investigated. Increasing light intensity increased RGR (by 67% and 76%) and RPY (by 50% and 89%) and decreased chlorophyll a (by 27% and 32%) for *L. minor* and *W. hyalina*, respectively. The spectral distributions had no significant impact on any investigated parameter. *Wolffiella hyalina* achieved higher values in all investigated parameters compared to *L. minor*. This investigation proved the successful cultivation of duckweed in a small-scale, re-circulating IVF with artificial lighting.

Dilution rates of cattle slurry affect ammonia uptake and protein production of duckweed grown in recirculating systems

Stadtlander, T; Bandy, J; Rosskothen, D; Pietsch, C; Tschudi, F; Sigrist, M; Seitz, A; Leiber, F. Journal of Cleaner Production (2022) 357: 131916

Duckweed is a potential collector of nitrogen from animal liquid manure and a source of protein suitable as feed for livestock and fish. Therefore, it provides opportunities for circular economy systems. Two duckweed species, *Spirodela polyrhiza* and *Landoltia punctata*, were grown in five recirculating systems each connected to a reservoir tank filled with water and graded organic cattle slurry concentrations. Fresh and dry biomass, protein production and amino acid profiles among the nitrogen removal were evaluated. *Spirodela polyrhiza* showed a significantly higher fresh biomass production but *L. punctata* dry matter content was generally higher resulting in similar dry biomass production for both species This study shows clearly that the crude protein content, ranging between 29.3 and 37.9% of dry matter, was positively correlated to slurry and total ammonia nitrogen (TAN) concentration of the substrate, independent of the duckweed species. Total crude protein yield was in the range of 1.37-1.95 g m^{-2} d^{-1}, following a quadratic function regarding slurry and TAN concentrations, with marginal differences between species. Biomass and crude protein yields were optimal for both duckweed species at a TAN concentration of 19 mg l^{-1}, which corresponded to a slurry dilution of 1:8. The results of this study provide important information for operation of recirculating duckweed production systems on slurry and operators should aim to keep TAN concentrations in that range for optimization of protein production in conjunction with TAN removal.

A non-chemical weed control strategy, introducing duckweed into the paddy field

Wang, F; Wang, S; Xu, SH; Shen, JY; Cao, LK; Sha, ZM; Chu, QN. (2022) Pest management Science 78: 3654-3663

Herbicide resistance in weeds and environmental pollution resulting from excessive application of chemical herbicides keeps increasing. Development of environment-friendly and effective weed management strategies are required for sustainable agricultural production. In this study we investigated the effects of duckweeds (*Landoltia punctata* (G. Meyer) Les & D. J. Crawford and *Spirodela polyrhiza* (Linnaeus) Schleiden) introduction on the weed community and rice growth in paddy fields. The study was conducted in the two rice-growing seasons (2018 and 2019) with three treatments: rice grown without duckweed introduction (CK), with *L. punctata* introduction (LP), and with *S. polyrhiza* introduction (SP). On average, LP and SP significantly reduced total weed density by more than 90% and 97%, respectively. Early in the rice-growing season, both duckweed species completely prevented weed growth. Further, both species significantly promoted rice plant growth in the advanced stages. SP significantly improved grain yield of rice by 23%. Light transmittance, temperature of the floodwater and soil, floodwater pH, and dissolved oxygen content significantly decreased following introduction of the duckweeds, indicating that the duckweeds introduction might inhibit weeds growth by altering environmental factors. This study provides a possible environment-friendly way to inhibit weed biomass in the paddy field by introducing duckweeds and interpreted the possible reasons of the impacts of duckweed on environmental variables. Weed control is beneficial for rice growth. Duckweed coverage might be limited in open fields and the associated practice requires additional investigation.

Fate of chlorpyrifos bound residues in paddy soils: Release, transformation, and phytoavailability

Zhong, J; Shen, D; Li, H; He, Y; Bao, Q; Wang, W; Ye, Q; Gan, J. (2022) Environment International 166: 107338

Chlorpyrifos (CPF) is a widely used organophosphorus insecticide that tends to form bound residues (BRs) in soils. However, the stability and biological activity of CPF-BRs remain to be explored. Facilitated by carbon-14 tracing, this study obtained CPF-BRs initially formed in two typical paddy soils (14C-CPF-BRin), and further investigated their release, transformation and phytoavailability using duckweed (*Lemna minor*) as a model aquatic organism. Most 14C-CPF-BRin in soils were composed of the parent CPF and its metabolite 3,5,6-trichloro-2-pyridinol (2-OH-TCP), which was mainly formed through reversible entrapment by soil fulvic acids and humin (>80%). At 36 d, 66.67-80.90% of the 14C-CPF-BRin was released, of which only 2-OH-TCP could be released into the water and absorbed by the duckweed, with bioconcentration factors ranging from 247.99 to 324.68 L kg-1. The subsequent metabolism of released 14C-CPF-BRin in duckweed included phase I metabolism from 2-OH-TCP to 4-OH-TCP and phase II metabolism of conjugation of TCP with plant endogenous amino acids. The study suggested that CPF bound residues have high bioavailability in paddy field environments. Given that many pesticides and non-pesticide chemicals share structures analogous to CPF, the findings have important implications for better understanding the environmental and human health risks of man-made chemicals.

Biochemistry

Metabolome and transcriptome analyses of the flavonoid biosynthetic pathway for the efficient accumulation of anthocyanins and other flavonoids in a new duckweed variety (68-red)

Liu, Y; Li, C; Yan, R; Yu, R; Ji, M; Chen, F; Fan, S; Meng, J; Liu, F; Zhou, G; Tang, X. Journal of Plant Physiology (2022) 275:153753

Duckweed is a kind of aquatic plant with the characteristics of high nutritional value and medicinal benefits. However, most researches focused on the natural germplasms. The underlying metabolic pathway remains to be systematically elaborated in duckweed. In our laboratory, one reddish-purple mutant with high-flavonoids was screened from a mutant library of *Spirodela polyrhiza* 6068, named 68-red. The content of anthocyanins and proanthocyanidins in 68-red mutant increased by 563.47% and 231.19%, respectively, compared to wild type. It is interesting that cynaroside and orientin content were significantly increased, in contrast, apigetrin and vitexin were decreased in 68-red mutant. Considering this, metabolome and transcriptome were employed to explore the flavonoids biosynthetic pathway. Here, a total of 734 metabolites were identified in the wild type and 68-red mutant. Among which, cyanidin-3-O-glucoside, cyanidin-3-O-galactoside, pelargonidin-3-O-glucoside and pelargonidin-3-O-(6-O-malonyl)glucoside were significantly accumulated, which were positively correlated with deep reddish-purple of 68-red mutant. In addition, proanthocyanidins (B1, B2, B3, B4, C1, C2), flavonoid and its glycosides (11 luteolin and its glycosides, 14 quercetin and its glycosides, 14 kaempferol and its glycosides, 2 apigenin glycosides) were significantly accumulated, 2 apigenin glycosides were down-regulated in 68-red mutant. The transcriptome data and qRT-PCR indicated that 16 enzyme genes in flavonoids biosynthetic pathway (PAL, C4H, CHSs, F3H, ANS, ANR, F3'Hs, DFRs, LAR, GT1, BZ1) were significantly up-regulated in 68-red mutant. Correlation analysis found that three copies of F3'H gene play important roles in the synthesis of anthocyanins, luteolin and apigenin glycosides. In conclusion, the 68-red mutant is a high quality germplasm resources for food and medical industry. Metabolome and transcriptome provide new insight for exploring the enzyme genes and functional metabolites in duckweed.

Biotechnology

Starch accumulation in duckweeds (Lemnaceae) induced by nutrient deficiency

Sree, KS; Appenroth, KJ. Emirate Journal of Food and Agriculture (2022) 34: 204-212

The aim of the present project is to demonstrate the potential of duckweeds in fast production of starch-rich biomass that could render these plants as promising next generation crop plants. Starch-rich biomass can be produced by several methods of starch induction. In the present project the effects of nutrient deficiency (phosphate, nitrate and sulphate) on starch accumulation were investigated in 21 clones (strains) of duckweeds covering 11 species and all 5 genera of the duckweed family encompassing a wide geographic area. The magnitude of change in the starch content after treatment with nutrient deficient medium varied widely within the genera, the different species and different clones of the same species. Highest values, between 40 and 50 %, of starch on dry weight basis of the whole plant biomass were obtained after two weeks of application of phosphate- or nitrogen-lacking nutrient media whereas sulphate deficiency showed minor effects. It is concluded that various clones of the species *Landoltia punctata*, *Lemna minor*, and *Spirodela intermedia* proved to be promising candidates for starch rich biomass production.

Sulfur limitation boosts more starch accumulation than nitrogen or phosphorus limitation in duckweed (*Spirodela polyrhiza*)

Sun, ZL; Guo, WJ; Zhao, XY; Chen, Y; Yang, JJ; Xu, SQ; Hou, HW. Industrial Crops and Products (2022) 185: 115098

Duckweeds contain high levels of starch and are environmentally sustainable and economically viable feedstock for biofuel production. Here, the biomass and starch yield of three duckweed species under three different nutrient-limited conditions were analyzed to investigate the possible ways of further increasing the efficiency of starch production. The results showed that sulfur limitation resulted in the highest starch yield, which was 42% and 73% higher than in nitrogen or phosphorus limitation conditions, respectively. The high yield of sulfur-limited duckweed is largely due to the combinations of little effects on biomass and high accumulations of starch. Although nitrogen limitation led to higher starch content (67.4%), it severely reduced biomass production. Besides, this work revealed the mechanism of starch accumulation induced by sulfur limitation in duckweed based on transcriptomic analysis. In summary, sulfur limitation is a practical approach to increase starch yields in duckweed without affecting growth or biomass production.

Pasting properties and multi-scale structures of *Spirodela* starch and its comparison with normal corn and rice starch

Wang, X; Jin, YZ; Cheng, L; Li, ZF; Li, CM; Ban, XF; Gu, ZB; Hong, Y. Food Hydrocolloids (2022) 132: 107865

Turion is a starch source that can overcome the limitations of land and season. The starch obtained from turion had a positive impact on the development of the starch industry. In this study, a novel starch was extracted from *Spirodela polyrhiza* turion. The structure and the relationship between structure and gelatinization properties of *Spirodela* starch were studied by comparison with that of normal corn starch and rice starch and principal component analysis. The amylose contents, short-range order, hydrogen bond, chain-length distributions, the degree of polymerization, crystalline and semi-crystalline structure, and granule structure of *Spirodela* starches were investigated. The results showed that *Spirodela* starch has a medium amylose content (27.48 g/100 g), and has a lower hydrogen bond strength, double helix number, crystallinity (18.41 g/100 g), looser semi-crystalline layer, and a higher specific surface area of the holes on the surface of the granules than the other two starches. The structure of *Spirodela* starch is obviously looser than that of normal corn starch and rice starch. The loose structure of *Spirodela* starch causes the lowest gelatinization temperature (66.9 degrees C) and the highest peak viscosity (2664.50 m Pa.s) among the three starch.

Elucidating the structure and pasting properties of *Spirodela* starch will help understand its application in industrial processing.

A review on bioenergy production from duckweed

Chen, GY; Zhao, KG; Li, WQ; Yan, BB; Yu, YY; Li, J; Zhang, YX; Xia, SG; Cheng, ZJ; Lin, FW; Li, LP; Zhao, H; Fang, Y. (2022) Biomass & Bioenergy 161: 106468

Duckweed easily growing in wastewater and absorbing a large amount of nitrogen and phosphorus from wastewater is now being considered as a promising solution to treat eutrophic water. However, if it is not salvaged in time, its rapid accumulation will cause secondary pollution to the water body. Duckweed is rich in protein and starch, but the content of lignin is rather low, making it an ideal biomass feedstock for energy production. This work reviews the detailed steps for producing bio-oil, bio-ethanol, biogas and other kinds of bioenergy from duckweed through hydrothermal liquefaction, pyrolysis, anaerobic digestion and fermentation processes. The reaction pathways of duckweed to produce bio-oil and bioethanol are introduced in detail. Opportunities and challenges of each conversion technology are thereafter discussed. The viability of producing biofuels and industrial precursors in a sustainable mode is also assessed. According to the characteristics of different kinds of duckweed, appropriate utilization methods should be applied to produce bioenergy to realize environmental, economic and energy benefits.

Feed & Food

Trophic transfer of microplastics in a model freshwater microcosm; lack of a consumer avoidance response

Mateos-Cardenas, A; Moroney, AV; van Pelt, FNAM; O'Halloran, J; Jansen, MAK. (2022) Food Webs 31: e00228

Microplastics are considered freshwater pollutants of emerging concern. Although microplastics have been identified in a range of taxa, only a limited number of studies have focussed on the trophic transfer of micro plastics. The duckweed *Lemna minor* can act as a microplastic vector in a model freshwater food chain. Here we show for the first time scanning electron microscopy images of 1 µm polystyrene (PS) and 10-45 µm polyethylene (PE) microplastics adsorbed on *L. minor* surfaces. A feeding test and a novel feeding-choice test were designed to investigate microplastic transfer from freshwater plants to invertebrates. Both tests show that the freshwater amphipod *Gammarus duebeni* feeds on plant biomass irrespective of the presence of microplastics, indicating lack of avoidance of microplastics. A number of *G. duebeni* that were fed *L. minor* with adsorbed PE microplastics had accumulated microplastics in their digestive tracts at the end of the tests. Fragmentation of microplastics transferred via plant feed and using low concentrations was noted for the first time. PE fragments, including particles in the nanosized range, were observed in *G. duebeni* digestive tracts. Yet, there was no apparent acute negative effect on *G. duebeni* weight or survival. These findings demonstrate microplastic trophic transfer and fragmentation in a freshwater organism. The finding that novel nanoplastics are found in the gut does show that biological processes are co-determinants of the fate of plastics in the environment and emphasise the importance of nanoplastic impact studies.

Effects of partial substitution of conventional protein sources with duckweed (*Lemna minor*) meal in the feeding of rainbow trout (*Oncorhynchus mykiss*) on growth performances and the quality product

Fiordelmondo, E; Ceschin, S; Magi, GE; Mariotti, F; Iaffaldano, N; Galosi, L; Roncarati, A. (2022) Plants 11: 1220

Duckweed (*Lemna minor*) meal was included in the formulation of three experimental feeds (L1, L2, L3) for rainbow trout at 10%, 20%, 28% of the protein source, respectively. Increasing the duckweed inclusion, the other protein sources were adjusted to get isonitrogenous (41%) and isolipidic (20%) diets, as the control diet (LC). 540 fish (mean body weight 124.5 ± 0.7 g) were randomly allocated in 12 tanks divided equally among the four different diets. After 90 days, fish were weighed and the most important productive performances, fillet quality and fatty acid profile were determined. The final body weight in L1 (340.53 g) and L2 (339.42 g) was not different from LC (348.80 g); L3 trout significantly ($p < 0.05$) exhibited the lowest one (302.16 g). Similar trends were found in final mean length, weight gain, specific growth rate, food conversion rate. Somatic indices were affected by duckweed inclusion. Diets had not significant effects on the proximate composition and fatty acids of the fillet in L1, L2, L3, respect to LC. Based on this study, duckweed meal derived from *Lemna minor* can be included in the feed for the rainbow trout without negative effects on the growth performances at 20% of the protein substitution.

Effect of growth medium nitrogen and phosphorus on nutritional composition of *Lemna minor* (an alternative fish and poultry feed)

Ullah, H; Gul, B; Khan, H; Akhtar, N; Rehman, KU; Zeb, U (2022) BMC Plant Biology 22: 214

Duckweed (*Lemna minor* L.) is an aquatic macrophyte and grows profoundly on the surface of polluted water reservoirs of Pakistan. The plant can be used as a potential alternative for the fish and poultry industry to meet the promptly growing demand for feed. Our study investigates the effect of varying concentrations (ppm) of nutrients like N, P, and their combination, NP on biomass production, carbohydrate, lipid, protein, and mineral (Ca, Mg, Fe, Mn & Zn) contents of *L. minor*. The varying concentrations of N and P substantially affected the above-stated parameters. The highest biomass yield was recorded in the 30 ppm NP tank as 172 g/m^2 day in comparison with the control tank. Higher protein, lipid, and carbohydrate contents were recorded for 30 ppm NP, 20 ppm NP, and 10 ppm NP respectively. Minerals like Ca, Mg, Fe, Mn & Zn increased in 20 ppm P and all N concentrations. The combined application of NP was more effective in boosting the protein, carbohydrate, and lipid content whereas less effective in increasing the mineral contents. A rise in the concentration of N and P showed a positive correlation with the nutritional composition of *L. minor*.

Freshwater macrophytes: A potential source of minerals and fatty acids for fish, poultry, and livestock

Kumar, G; Sharma, J; Goswami, RK; Shrivastav, AK; Tocher, DR; Kumar, N; Chakrabarti, R. (2022) Frontiers in Nutrition 9: 869425

The freshwater macrophytes are abundant in tropical and subtropical climates. These macrophytes may be used as feed ingredients for fish and other animals. The nutritional value of twelve freshwater-cultured macrophytes was evaluated in the present study. Significantly higher crude protein (36.94-36.65%) and lipid (8.13-7.62%) were found in *Lemna minor* and *Spirodela polyrhiza*; ash content was significantly higher in *Hydrilla verticillata*, *Wolffia globosa*, and *Pistia stratiotes* (20.69-21.00%) compared with others. The highest levels of sodium, magnesium, chromium, and iron levels were recorded in *P. stratiotes*. *H. verticillata* was a rich source of copper, manganese, cobalt, and zinc; the contents of calcium, magnesium, strontium, and nickel were highest in S. polyrhiza. Selenium and potassium contents were higher in *Salvinia natans* and *W. globosa*, respectively. The n-6 and n-3 polyunsaturated fatty acids (PUFAs) contents were significantly higher in *W. globosa* and *Ipomoea aquatica*, respectively compared with others. Linoleic and alpha-linolenic acids were dominant n-6 and n-3 PUFAs. The highest value (4.04) of n-3/n-6 was found in *I. aquatica*. The ratio ranged from 0.61 to 2.46 in other macrophytes. This study reveals that macrophytes are rich sources of minerals, n-6 and n-3 PUFAs.

Duckweeds as promising food feedstocks globally

Pagliuso, D; Grandis, A; Fortirer, JS; Camargo, P; Floh, EIS; Buckeridge, MS. (2022) Agronomy 12: 796

Duckweeds are the smallest flowering plants on Earth. They grow fast on water's surface and produce large amounts of biomass. Further, duckweeds display high adaptability, and species are found around the globe growing under different environmental conditions. In this work, we report the composition of 21 ecotypes of fourteen species of duckweeds belonging to the two subfamilies of the group (Lemnoideae and Wolffioideae). It is reported the presence of starch and the composition of soluble sugars, cell walls, amino acids, phenolics, and tannins. These data were combined with literature data recovered from 85 publications to produce a compiled analysis that affords the examination of duckweeds as possible food sources for human consumption. We compare duckweeds compositions with some of the most common food sources and conclude that duckweed, which is already in use as food in Asia, can be an interesting food source anywhere in the world.

Interaction with other organisms

Allelopathic inhibition of the extracts of *Landoltia punctata* on *Microcystis aeruginosa*

Li, D; Li, P; Yan, ZQ; Li, N; Yao, LG; Cao, LL. Plant Signaling & Behavior (2022) 17: 2058256

To study the allelopathic effect of the extracts of *Landoltia punctata*, the changes of cell density of *Microcystis aeruginosa* were measured. The anti-algae allelopathic effect of different organic solvent extracts of *L. punctata* was evaluated, and the physiological, biochemical indexes were determined to discuss the mechanism of algal inhibition. The results showed that the petroleum ether, dichloromethane and ethyl acetate extracts showed various inhibitory effects on *M. aeruginosa*. Among them, ethyl acetate extract was the most strongly allelopathic part with the semi-effect concentration(EC_{50}) of 59.6 mg L^{-1}, the central polarity part of inhibitory activity. The contents of chlorophyll a(Chl a) and phycobiliproteins(PBPs) of *M. aeruginosa* were decreased under the concentration of 200 mg L^{-1} ethyl acetate extract, which indicated that the photosynthesis of *M. aeruginosa* was inhibited. The consent of microcystins was lower compared to control under 200 mg L^{-1}. The contents of superoxide dismutase(SOD), malondialdehyde(MDA) and hydrogen peroxide(H_2O_2) of cell pellets were firstly increased and then decreased, which suggested that the algal cells were seriously damaged by oxidation. The results indicated that the extracts of *L. punctata* had inhibitory effect on *M. aeruginosa*, and the ethyl acetate extract was the central part of the inhibitory substances, which affected photosynthesis and caused peroxidation damage to inhibit cell proliferation. These findings will be helpful for exploration and application of allelopathic effects of *L. punctata* in harmful algae control.

Toxic mechanism of two cyanobacterial volatiles beta-cyclocitral and beta-ionone on the photosynthesis in duckweed by altering gene expression

Du, S; Xu, H; Yang, M; Pan, N; Zheng, T; Xu, C; Li, Y; Zuo, Z. Environmental Pollution (2022) 308: 119711

Volatile organic compounds (VOCs) promote cyanobacteria dominating eutrophicated waters, with aquatic plant decrease and even disappearance. To uncover the toxic mechanism of cyanobacterial VOCs on aquatic plants, we investigated the growth, photosynthetic pigment levels, photosynthetic abilities and related gene expression in duckweed treated with beta-cyclocitral and beta-ionone, 2 main components in the VOCs. The levels of chlorophylls and carotenoids gradually declined with raising the concentration of the 2 compounds and prolonging the treatment time. Their decline should result from the down-regulation of 8 genes associated with photosynthetic pigment biosynthesis and up-regulation of 2 genes involved in carotenoid degradation. The reduction was also found in the photosystem II (PSII) efficiency and O_2 evolution rate, which should result from the lowered photosynthetic pigment levels and down-regulation of 38 genes related with photosynthetic process. The frond numbers, total frond area and fresh weight gradually decreased with raising the 2 compound concentration, which may result from the lowered photosynthetic abilities as well as down-regulated expression of 7 genes associated with growth-promoting hormone biosynthesis and signal transduction. It can be speculated that cyanobacterial VOCs may poison aquatic plants by lowering the photosynthesis and growth through altering related gene expression.

DF: The authors did not give the identity of the species

Removal of Dinotefuran, Thiacloprid, and Imidaclothiz Neonicotinoids in Water Using a Novel Pseudomonas monteilii FC02-Duckweed (*Lemna aequinoctialis*) Partnership

Cai, XY; Xu, M; Zhu, YX; Shi, Y; Wang, HW. (2022) Frontiers in Microbiology 13: 906026

Neonicotinoids (NEOs) are the most widely used insecticides in the world and pose a serious threat to aquatic ecosystems. The combined use of free-floating aquatic plants and associated microorganisms has a tremendous potential for remediating water contaminated by pesticides. The aim of this study was to determine whether plant growth-promoting bacteria (PGPB) could enhance the phytoremediation efficiency of duckweed (*Lemna aequinoctialis*) in NEO-contaminated water. A total of 18 different bacteria were isolated from pesticide-stressed agricultural soil. One of the isolates, *Pseudomonas monteilii* FC02, exhibited an excellent ability to promote duckweed growth and was selected for the NEO removal experiment. The influence of strain FC02 inoculation on the accumulation of three typical NEOs (dinotefuran, thiacloprid, and imidaclothiz) in plant tissues, the removal efficiency in water, and plant growth parameters were evaluated during the 14-day experimental period. The results showed that strain FC02 inoculation significantly ($p < 0.05$) increased plant biomass production and NEO accumulation in plant tissues. The maximum NEO removal efficiencies were observed in the inoculated duckweed treatment after 14 days, with 92.23, 87.75, and 96.42% for dinotefuran, thiacloprid, and imidaclothiz, respectively. This study offers a novel view on the bioremediation of NEOs in aquatic environments by a PGPB-duckweed partnership.

Overview of allelopathic potential of *Lemna minor* L. obtained from a shallow eutrophic lake

Gostynska, J; Pankiewicz, R; Romanowska-Duda, Z; Messyasz, B. (2022) Molecules 27: 3428

Allelopathy is an interaction that releases allelochemicals (chemicals that act allelopathically) from plants into the environment that can limit or stimulate the development, reproduction, and survival of target organisms and alter the environment. *Lemna minor* L. contains chemicals that are allelopathic, such as phenolic acids. Chemical compounds contained in *L. minor* may have a significant impact on the development and the rate of multiplication and lead to stronger competition, which may enhance the allelopathic potential. Allelopathic potential may exist between *L. minor* and *Cladophora glomerata* (L.) Kutz. because they occupy a similar space in the aquatic ecosystem, have a similar preference for the amount of light, and compete for similar habitat resources. *L. minor* and *C. glomerata* can form dense populations on the water surface. Allelopathy can be seen as a wish to dominate one of the plants in the aquatic ecosystem. By creating a place for the development of extensive mats, an interspecific interaction is created and one of the species achieves competitive success. It is most effective as a result of the release of chemicals by macrophytes into the aquatic environment. Therefore, allelopathy plays a significant role in the formation, stabilization, and dynamics of the structure of plant communities.

Physiology & Stress

Nitric oxide (NO) involved in Cd tolerance in NHX1 transgenic duckweed during Cd stress

Ren, QT; Li, N; Liu, RX; Ma, X; Sun, JG; Zeng, JY; Li, QQ; Wang, MW; Chen, XL; Wu, XY. Plant Signaling & Behavior (2022) 17: 2065114

Anthropogenic activities cause heavy metal pollution, such as cadmium (Cd). Na^+/H^+ antiporter (NHX1) transgenic duckweed showed Cd tolerance in our previous study, and the signal mechanism needs to be explored. As an important signal molecule, nitric oxide (NO) is involved in a number of functions under abiotic stress response. This study analyzed the levels of endogenous NO in wild-type (WT) duckweed and NHX1 duckweed under Cd treatment. The results showed that after 24 h Cd treatment, the endogenous NO level of WT duckweed decreased, which was significantly lower than that in NHX1 duckweed. Studies have proved that NHX1 influences pH. The level of NO in this study has been investigated at different pH. The NO level was the highest in the duckweed cultured with pH 5.3. Nitrate reductase gene expression was down-regulated and NO synthesis was decreased under Cd stress in WT duckweed. This study showed that NO level has been modified in NHX1 duckweed, which could be influenced by pH.

DF: The authors used *Lemna turionifera* 5511

Pace and shape of senescence in three species of duckweed

Paiha, AP; Laird, RA. Ecology and Evolution (2022) 12: e9038

Senescence is progressive bodily deterioration associated with declines in survival and fecundity in older age classes. There is great diversity in patterns of senescence across species, but these patterns can be difficult to compare formally due to variation in the absolute time scales in which species live and die: members of some species live for a matter of days, others for millennia. To address this issue, the "pace-shape" approach was developed to decouple absolute time from analyses and instead standardize life history traits in terms of average life expectancy, facilitating intra- and interspecific comparisons. Here, we use this approach to distinguish the generic form of demographic trajectories (shape) from the time scale on which the trajectories occurred (pace) in three species of tiny, free-floating aquatic plants known as duckweeds (*Lemna gibba* L., *L. minor* L., and L. *turionifera* Landolt), which have mean lifespans of less than a month under typical lab conditions, and exhibit age-related declines in survivorship and reproduction. Using a randomized block design in which we tracked a final total of 430 individuals, we report differences in pace and shape among the three species. Specifically, the largest, least-fecund, and typically longest-lived species, *L. gibba*, tended to exhibit more rapid decreases in time-standardized survivorship and fecundity compared with the other two species. This study emphasizes variation in aging patterns that can be found among plant species, including those in the same genus, and provides further validation for the utility of applying the pace and shape approach in interspecific comparisons.

Time course of age-linked changes in photosynthetic efficiency of *Spirodela polyrhiza* exposed to cadmium

Persic, V; Dunic, JA; Domjan, L; Zellnig, G; Cesar, V. (2022) Frontiers in Plant Science 13: 872793

Short-term assessment of adverse effects is essential for populations exposed to higher risk of environmental pollution. This study presents the time course of physiological and morphological changes attributed to cadmium, emphasizing age-linked differences in the susceptibility of photosynthetic apparatus of *Spirodela polyrhiza* fronds exposed to different cadmium concentrations. A four-frond colony represented by mother, daughter, and granddaughter plants was exposed to cadmium concentrations for 6, 24, and 72 h to establish its effect on different generations of the great duckweed. The duration of cadmium exposure accounted for the most variation in chlorophyll content as the most influential variable, and after 72 h, frond responsiveness was a function of cadmium concentration. Carotenoid contents behaved slightly differently in fronds of different ages, with the oldest mother frond exhibiting accelerated senescence. Chlorophyll fluorescence measurements showed that cadmium affects different photosynthetic electron transport segments relative to the frond's chloroplast structure level. Photosynthesis of mother fronds exposed to low cadmium and daughter fronds exposed to high cadmium was determined by the functionality of primary electron acceptance at the PSII level. Mother plants exposed to higher cadmium concentrations were characterized by closed and inactive reaction centers, dissipated energy outflux, and inhibited photosynthesis. Young fronds exposed to low and high cadmium concentrations were characterized by increased non-reducing reaction centers and thermal phase reduction, with activated dissipative mechanisms at high cadmium concentrations. Cadmium-induced changes in the ultrastructure of chloroplasts were visible after 6 h of exposure to lowest concentrations, with gradual degradation of the thylakoid system as the fronds aged. Younger fronds responded to cadmium more dynamically through molecular, physiological, and anatomical changes and tolerated a more reduced electron transport chain under given conditions than older fronds.

Phytomedicine

The construction of a duckweed expression and delivery system for grass carp reovirus VP35

Zhu, L; Yuan, GL; Wang, XR; Zhao, T; Hou, LB; Li, C; Jiang, XY; Zhang, J; Zhao, XL; Pei, C; Li, L; Kong, XH. (2022) Aquaculture 553: 738059

Haemorrhagic disease caused by grass carp reovirus (GCRV) in grass carp (*Ctenopharyngodon idella*) is the main viral disease that diminishes the survival of juvenile grass carp. The usage of vaccines, especially oral vaccines, has been considered an effective and convenient way to protect against this disease. However, oral vaccines are easily degraded and digested by digestive enzymes in the intestinal tract. Therefore, a vaccine delivery system that can protect oral vaccines from degradation is a prerequisite for oral vaccines. In this study, the S11 segment (encoding a 35 kDa protein known as VP35) of GCRV was cloned into a binary vector and then transferred into duckweed (*Lemna aequinoctialis*) to prepare an oral subunit vaccine. Subsequently, Gus staining, Polymerase chain reaction (PCR), Western blotting, and fluorescence observation were used to verify the expression of the S11 segment. The results showed that duckweed calli were successfully induced and could be used for the transformation of *Agrobacterium*. Afterwards, a plant binary expression vector (pCAMBIA1303-S11) containing GUS and GFP tags was constructed, after which transgenic duckweed lines were obtained. The results of the PCR analysis using RNA or DNA extracted from transgenic duckweed showed that the expression vector pCAMBIA1303-S11 was successfully transferred and replicated in duckweed. After GUS staining, obvious blue signals appeared at the roots, leaf bases, and frond edges of duckweed, and a strong fluorescent signal could be observed under a fluorescence microscope. A Western blot analysis of the protein samples from the transgenic plants demonstrated the expression of a fusion protein with a molecular mass of approximately 50 kDa. Taken together, the duckweed expression system was successfully constructed, and an oral vaccine based on the S11 segment of GCRV was successfully prepared. These results can provide further information for the development of a duckweed-based expression system to produce an edible vaccine for *C. idella* against GCRV.

Phytoremediation

Phytoremediation of contaminated industrial wastewater by duckweed (*Lemna minor* L.): Growth and physiological response under acetic acid application

Farid, M; Sajjad, A; Asam, ZU; Zubair, M; Rizwan, M; Abbas, M; Farid, S; Ali, S; Alharby, HF; Alzahrani, YM. Chemosphere (2022) 304: 135262

Extensive usage of heavy metals (HMs) in chemical reactions and processes eventually contaminate the environmental segments and is currently a major environmental concern. HMs such as cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr) and nickel (Ni) are considered the most harmful pollutants as they have adequate potential of bioaccumulation. The current research was carried out to assess the HMs toxicity of textile and tannery wastewater and effect of acetic acid (AA) on phytoextraction of HMs by duckweed (*Lemna minor* L.) in a hydroponic system. Plants were treated with different treatments having different hydroponic concentrations of AA (5 and 10 mM) and textile and tannery effluents, where these two effluents were equally mixed and then diluted with good quality water with different ratios (25, 50, 75, and 100%) along with three replications of each treatment. Results were recorded for growth attributes, chlorophylls, antioxidant enzymes, electrolytic leakage, reactive oxygen species and HMs accumulation in plants. HMs accumulation disrupts the growth parameters, chlorophyll contents and carotenoids contents along with increased activities of antioxidant enzyme such as catalases (CAT), superoxide dismutase (SOD), peroxidases (POD) and ascorbate peroxidase (APX). Addition of AA in the hydroponic experimental system significantly improves the antioxidant defence mechanism and alleviated the HM induced toxicity in plants. Cr, Cd, Pb, Cu and Ni concentrations were maximally increased up to 116 & 422%, 106 & 416%, 72 & 351%, 76 & 346%, and 41 & 328% respectively under AA (10 mM) application. The results revealed that duckweed can be applied as potential phyto-remedy to treat industrial wastewater.

Application of duckweed (*Lemna* sp.) and water fern (*Azolla* sp.) in the removal of pharmaceutical residues in water: State of art focus on antibiotics

Maldonado, I; Terrazas, EGM; Vilca, FZ. Science of the Total Environment (2022) 838: 156565

In recent decades, antibiotic residues in the environment have increased, affecting components of biological communities, from bacteria to plants and animals. Different methods have been used to remove these compounds, including phytoremediation with floating aquatic species such as duckweed and aquatic fern, with positive results. This study analyses information about the removal efficiency of drugs, with a focus on antibiotics, using *Lemna* and *Azolla*, which will allow a better understanding of phytoremediation processes from the perspective of plant physiology. The physiological processes of macrophytes in an environment with this type of pollutant and the phytotoxic effects on plants at high concentrations are also analysed. The metabolization of toxic compounds occurs in three phases: phase I begins with the absorption of antibiotics and the secretion of reactive oxygen species (ROS); in phase II, the effects of ROS are neutralized and minimized by conjugation with enzymes such as glutathione transferase or metabolites such as glutathione; and phase III culminates with the storage of the assimilated compounds in the vacuoles, apoplast and cell wall. In this way, plants contribute to the removal of toxic compounds. In summary, there is sufficient scientific evidence on the efficiency of the elimination of pharmaceutical compounds by these floating macrophytes at the laboratory scale, which indicates that their application under real conditions can have good results.

Naturally grown duckweeds as quasi-hyperaccumulators of rare earth elements and yttrium in aquatic systems and the biounavailability of gadolinium-based MRI contrast agents

Zocher, AL; Klimpel, F; Kraemer, D; Bau, M. The Science of the Total Environment (2022) 838: 155909

The use of rare earths and yttrium (REY) in high-technology products is accompanied by their increasing release into the environment. Concerns regarding the (eco-)toxicity and bioaccumulation of these emerging contaminants highlight the need for research on REY uptake by (aquatic) plants. Duckweeds are widespread macrophytes in lentic waters and receive increasing attention as a potential protein-rich food additive. We here provide a baseline dataset for the complete set of REY in naturally grown duckweed assemblages and ambient freshwater and coastal brackish seawater. Our results show that duckweeds strongly bioaccumulate REY and incorporate them at the µg/kg level (dry matter basis). Their shale-normalised (SN) REY patterns are mildly fractionated relative to upper continental crust, regardless of sampling location and season. In contrast, the patterns of ambient waters increase from light to heavy REY (LREY and HREY, resp.) and may show prominent positive anthropogenic GdSN anomalies due to the presence of Gd-based contrast agents (Gd-CAs) applied for magnetic resonance imaging (MRI). The lack of GdSN anomalies in the duckweed assemblages reveals discrimination against the uptake of Gd-CAs by the macrophytes, providing further evidence for the conservative behaviour of these xenobiotics in the environment. High REY concentrations and apparent bulk distribution coefficients between duckweeds and ambient waters of up to 105 show that duckweeds are quasi-hyperaccumulators of REY. Uptake of LREY is up to two orders of magnitude higher than of HREY, possibly due to stronger complexation of HREY with dissolved ligands. The REY closely correlate with Mn but not with Ca, suggesting that uptake of REY and Mn occurs via the same pathway and revealing the negligible role of calcium oxalates. Our study demonstrates that while duckweeds are quasi-hyperaccumulators of REY, there is currently no risk that anthropogenic Gd from MRI contrast agents may enter the food chain via consumption of duckweeds.
DF: The authors did not identify the duckweed species.

Engineered bacterium-binding protein promotes root recruitment of functional bacteria for enhanced cadmium removal from wastewater by phytoremediation

Feng, LA; Liang, BY; Zeng, XL; Shi, C; Yin, HD; Feng, YM; Chen, YQ; Yu, QL. Water Research (2022) 221: 118746

Functional bacteria promote the efficiency of phytoremediation by enhancing plant growth and participating in decontamination. However, their activity is frequently compromised by the weakness of their interaction with plant roots. In this study, we designed the artificial protein LcGC composed of a bacterium-binding domain, a GFP fluorescence reporter, and a carbohydrate-binding domain to function as a physical contact between functional bacteria and plant roots. This protein was then expressed in an engineered yeast cell factory and extracted to assess its effect on rhizosphere microbiome composition, plant growth, and cadmium removal in a simulated phytoremediation system containing the remediation plant *Lemna minor* and the functional heavy metal-capturing bacteria *Cupriavidus taiwanensis* and *Pseudomonas putida*. LcGC efficiently bound bacterial cell wall components and glucan, endowing it high efficiency to bind both functional bacteria and plant roots. Scanning microscopy and microbiome analysis revealed that LcGC enhanced root recruitment and colonization of functional bacteria on the root surfaces. Furthermore, LcGC with the aid of single *C. taiwanensis* or of *C. taiwanensis* and *P. putida* in combination promoted plant growth, enhanced tolerance to cadmium-induced oxidative stress, and consequently improved cadmium-removing capacity of the plants, with the percent of cadmium removal reaching up to 91% for LcGC plus *C. taiwanensis*, and to 96% for LcGC plus *C. taiwanensis* and *P. putida* on day 7. This study provided a physical contact-based strategy to enhance the interaction between functional microbes and plant roots for efficient phytoremediation.

Long-term interactions between microplastics and floating macrophyte *Lemna minor*: The potential for phytoremediation of microplastics in the aquatic environment

Rozman, U; Kokalj, AJ; Dolar, A; Drobne, D; Kalcikova, G. Science of the Total Environment (2022) 831: 154866

The presence of microplastics (MPs) in the environment has raised many concerns, and therefore approaches and technologies to remove them in situ are of high interest. In this context, we investigated the interactions between polyethylene MPs (fragments with a mean size of 149 +/- 75 mu m) and an aquatic floating macrophyte *Lemna minor* in order to assess its potential use for in situ phytoremediation. We first investigated the long-term effects of a high (100 mg/L = 9600 MPs/L), but still environmentally relevant concentration of MPs on *L. minor*. Subsequently bioadhesion of MPs was studied and the number and strength of MPs adhering to plant biomass were assessed. MPs did not adversely affect various parameters of plants (e.g., specific growth rate, chlorophyll contents, total antioxidant capacity, electron transport system activity, and contents of energy-rich molecules) throughout the duration of the experiment (12 weeks), except for the first week of the experiment, when protein content and total antioxidant capacity were affected. On the other hand, MPs affected the root length of *L. minor* during the first eight weeks of the experiment, while further exposure resulted in a decrease in the effects, indicating the ability of *L. minor* to tolerate the presence of MPs for a long period of time. MPs adhered rapidly to the plant biomass and the average percentages of strongly and weakly adhered particles were 6.5% and 20.0%, respectively, of the total MPs applied. In summary, results of this study suggest that *L. minor* can tolerate hotspot concentrations of MPs and can collect MPs from the water surface. Therefore, phytoremediation using floating plants could be considered as a potential method for in situ removal of MPs from the aquatic environment.

Elimination of toxic heavy metals from industrial polluted water by using hydrophytes

Xie, P; Zahoor, F; Iqbal, SS; Zahoor; Ullah, S; Noman, M; Din, ZU; Yang, W. (2022) Journal of Cleaner Production 352: 131358

Heavy metals (HMs) are frequently detected in wastewaters, especially in industrial discharges. Phytoremediation is one of the promising low cost, environmental friendly and efficient technique to remove HMs from industrial wastewater. The current research was carried out to investigate the ability of constructed wetland (CW) for the removal of HMs including copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni) and chromium (Cr) from local wastewater of industries by using two local common hydrophytes species *Pistia stratiotes* and *Lemna gibba*. After 45 days cultivation, the concentration of HMs reduced to 0.25-0.90 mg/L and 0.78-1.4 mg/L from 5 mg/L for *Lemna gibba* and *Pistia stratiotes*, respectively. The HMs removal efficiency ranged from 82% to 95% for *Lemna gibba* and 72%-84.4% for *Pistia stratiotes*. The accumulation of HMs in roots are always higher than that in aerial part including stems and leaves for both plants. The accumulation of HMs in roots of *Lemna gibba* ranged from 4.3 to 4.7 mg/kg and, 1.85-3.9 mg/kg in aerial part. The corresponding values were 3.9-4.7 mg/kg and, 1.75-3.3 mg/kg for *Pistia stratiotes*. Results of health risk models including the average daily intake (ADI), hazard quotient (HQ) and cancer risk (CR) models indicated lower health risk of water after treatment than before treatment water. Result indicated the selected hydrophytes could be applied for HMs treatment from contaminated water.

Duckweed potential for the phytoremediation of linear alkylbenzene sulfonate (LAS): identification of some intermediate biodegradation products and evaluation of antioxidant system

Masoudian, Z; Salehi-Lisar, SY; Norastehnia, A; Tarigholizadeh, S. (2022) Bulletin of Environmental Contamination and Toxicology DOI10.1007/s00128-022-03549-9

Duckweed (*Lemna minor* L.) has a high potential for wastewater treatment. Here, its capability for bioremoval of linear alkylbenzene sulfonate (LAS) as one of the primary contaminants of water resources was evaluated. The effect of some operational parameters on surfactant removal efficiency was determined. Also, the impact of LAS on several physiological responses of *Lemna* was investigated. LAS remediation efficiency of *L. minor* was elevated with increasing LAS concentration, duckweed weight, and temperature. Furthermore, the optimal pH for removal was 7-8.5. The benzenesulfonate ring and five homologs of sulfophenyl carboxylate were identified as intermediates in the LAS degradation pathway. A decrease in relative growth rate and pigment contents was observed by increasing LAS concentration. In contrast, an increase in hydrogen peroxide content and electrolyte leakage indicated oxidative stress by LAS. Induction of enzymatic/non-enzymatic antioxidants was observed during the surfactant remediation process, indicating their role in overcoming free radicals generated under surfactant stress.

Online control of *Lemna minor* L. phytoremediation: using pH to minimize the nitrogen outlet concentration

Sigcau, K; van Rooyen, IL; Hoek, Z; Brink, HG; Nicol, W. (2022) Plants 11: 1456

Phytoremediation technologies are employed worldwide to remove nutrient pollutants from agricultural and industrial wastewater. Unlike in algae-based nutrient removal, control methodologies for plant-based remediation have not been standardized. Control systems that guarantee consistently low outlet concentrations of nitrogen and phosphorous often use expensive analytical instruments and are therefore rarely viable. In this study, pH measurement was used as the sole input to control the nitrate outlet concentration in a continuously operated *Lemna minor* (lesser duckweed) phytoremediation tank. When grown in 20 L batches of modified Hoagland's solution, it was found that a constant ratio exists between the amount of nitrate removed and the amount of acid dosed (required for pH control), which was equal to 1.25 mol N.(mol $H^+)^{-1}$. The nitrate uptake rates were determined by standard spetrophotometric method. At critically low nitrate concentrations, this ratio reduced slightly to 1.08 mol N.(mol $H^+)^{-1}$. Assuming a constant nitrogen content, the biomass growth rate could be predicted based on the acid dosing rate. A proportional-integral controller was used to maintain pH on 6.5 in a semi-continuously operated tank covered by *L. minor*. A nitrogen control strategy was developed which exploited this relationship between nitrate uptake and dosing and successfully removed upwards of 80% of the fed nitrogen from synthetic wastewater while a constant biomass layer was maintained. This study presents a clear illustration of how advanced chemical engineering control principles can be applied in phytoremediation processes.

Removal of 17 alpha-ethynylestradiol and beta-estradiol using bench-scale constructed wetlands

Bliss, SN; Berger, K; Thieme, J; Cobos, MM; Nyman, M. (2022) Water Science and Technology 85: 3408-3418

Aquatic ecosystems have been devastated by the continued persistence of the synthetic estrogen compounds beta-estradiol and 17 alpha-ethynylestradiol. Common wastewater treatment methods do not reduce these compounds in effluent below problematic concentrations. An emerging cost-effective solution to this problem is the use of constructed wetlands to remove these estrogen compounds. This study analyzed the ability of duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*), and water cabbage (*Pistia stratiotes*) to remove beta-estradiol and 17 alpha-ethynylestradiol through the use of bench scale constructed wetlands over 15-week period. Estrogen concentration in water was collected over time along with plant nutrient content, contaminant extractions, and media extractions. Results indicated that estrogen concentration was reduced by the plants and soil media. Duckweed was the most effective at 96% removal, followed by water hyacinth at

72% removal, then water cabbage at 35% removal, and lastly sediment media at 9% removal. This study provides evidence for the ability of constructed wetlands to be used as a means to remove estrogen compounds from wastewater and demonstrates differences in plants removal efficiencies, with duckweed being the most effective of the selected plants.

The use of hydrophytes for additional treatment of municipal sewage

Pichura, V; Potravka, L; Ushkarenko, V; Chaban, V; Mynkin, M. (2022) Journal of Ecological Engineering 23: 54-63

At the current stage of discharge and treatment of municipal sewage and other types of wastewater in the territory of Ukraine, traditional technologies of biological treatment in aero-tanks by the process of aerobic oxidation involving active silt characterized by low efficiency are largely used. It was established that biological treatment and additional treatment of sewage involving hydrophytes are efficient. The research on wastewater quality and the efficiency of sewage treatment was conducted in three phases: Phase 1 - "the quality before treatment", Phase 2 - "the quality after mechanical-biological treatment" at the existing municipal treatment plants, Phase 3 - "the quality after additional treatment by hydrophytes". In order to determine the efficiency of using hydrophytes additional treatment, *Eichhornia crassipes* (water hyacinth) and the perennial aquatic plant *Lemna minor* were planted in one treatment pond. The results of the experiment made it possible to determine high efficiency of using hydrophytes for additional sewage treatment. In particular, the efficiency of additional treatment in the treatment ponds removing the residue of suspended pollutants for 40 days was 32%, toxic salts - 13.0-23.0%, oil products - 30.0%, biogenic substances - 68.5-83.3%. It caused a drop in the values of chemical and biological oxygen demand for 5 days by 89.6% and 61.2%, respectively. The efficiency of sewage treatment removing toxic salts and oil products reached 97.7%, whereas in the case of mineral and organic pollutants - up to 99%. That contributed to a considerable increase in the wastewater quality by the criteria for fisheries. In particular, high nutritional value of *Eichhornia crassipes* and *Lemna minor* allowed obtaining 12.5 tons of hydrophyte wet mass that can be used as green manure, feeds for farm animals, poultry and fish.

Phytotoxicity

Phytotoxicity of environmental norfloxacin concentrations on the aquatic plant *Spirodela polyrrhiza*: Evaluation of growth parameters, photosynthetic toxicity and biochemical traits

Zhao, XL; Li, P; Qu, CF; Lu, R; Li, ZH. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology (2022) 258: DOI10.1016/j.cbpc.2022.109365

As an emerging pollutant, the increasing use of antibiotics in wastewater posed a serious threat to non-target organisms in the environment. Duckweed (*Spirodela polyrrhiza*) is a common higher aquatic plant broadly used in phytotoxicity tests for xenobiotic substances. The aim of this study was to evaluate the chronic toxicity of norfloxacin (NOR) on *Spirodela polyrrhiza* during 18 days of exposure. Our study investigated the addition of NOR into the medium with environment-related concentrations (0, 0.1, 10, and 1000 μg L^{-1}). Subsequently, biomarkers of toxicity such as growth, pigment, chlorophyll fluorescence parameters, indicators of oxidative stress, and osmotic regulatory substances content were analyzed in duckweed. In response to NOR exposure, obvious chlorosis, declines in growth and photosynthetic pigment, and photosystem II inhibition were noted in a concentration dependent manner. Reactive oxygen species (ROS) and antioxidant activity content increased in the treated fronds, which indicated that oxidative stress was specifically affected by NOR exposure. A slight increase in osmotic regulatory substances in NOR treated setups than in the control represented the increasing stress resistance. These results suggest NOR exerts its toxic effects on the aquatic plant *Spirodela polyrrhiza*.

DF: The correct spelling is *S. polyrhiza*

Large-scale screening and characterization of Cd accumulation and ultrastructural deformation in duckweed

Wang, XL; Hu, L; Wu, DS; Huang, T; Zhang, BJ; Cai, GJ; Gao, GQ; Liu, ZM; Huang, XP; Zhong, ZY. Science of the Total Environment (2022) 832: 154948

Cadmium (Cd) pollution in soil, rivers and lakes is a serious problem due to the current industrialization and urbanization in China. Duckweeds are recognized as promising species for Cd phytoremediation. However, intraspecific variations in Cd accumulation in duckweeds remain largely unknown. In this study, 16 accessions selected from 39 geographically isolated duckweed strains were chosen to investigate their Cd remediation abilities. The optimal accession *Landoltia punctata* named 07SGZP01 (*L. punctata* 0701) was identified and shown to accumulate maximal Cd in the body while maintaining the highest biomass. The dominant variety treated with different Cd concentrations showed that the biomass of *L. punctata* 0701 was significantly lower than that of the control group (CK). Cd contents in *L. punctata* 0701 were substantially increased from 2511.1 to 30,641.01 mg $kg^{-}1$ with an increase in Cd treatment levels from 0.3 to 20 mg L^{-1}. The transport coefficient (TF) increased as Cd levels increased from 0.3 to 2 mg L^{-1}. In addition, the Cd content in leaves was greater than that in roots (TF > 1) within this Cd concentration range, whereas the Cd content in roots was greater than that in leaves (TF < 1) when the concentration of the Cd treatment was greater than 5 mg L^{-1}. The bioaccumulation factor (BCF) decreased significantly with increasing Cd levels (P < 0.05). The rate of Cd removal in the solution gradually decreased with increasing Cd concentrations, and the removal rate achieved the highest value (75%) when the Cd concentration was 0.5 mg L^{-1}. In addition, Cd treatment (2 mg L^{-1}) not only damaged the ultrastructure of *L. punctata* 0701, as characterized by chloroplast deformation and cell vacuolation but also caused most of the stomata to close, and the leaf epidermal cells were damaged and ruptured.

Dualistic effects of bisphenol A on growth, photosynthetic and oxidative stress of duckweed (*Lemna minor*)

Liang, J; Li, Y; Xie, P; Liu, C; Yu, L; Ma, X. Environmental Science and Pollution Research International (2022) DOI10.1007/s11356-022-21785-8

In this study, we exposed duckweed (*Lemna minor*), a floating freshwater plant, to BPA at different concentrations (0, 1, 5, 20, and 50 mg/L) for 7 days so as to investigate the effects of BPA on its growth, photosynthesis, antioxidant system, and osmotic substances. It was found that BPA had the acute toxic effects of "low promotion and high inhibition" on growth and photosynthesis. Specifically, BPA at a low concentration (5 mg/L) significantly promoted the plant growth and improved the concentration of photosynthetic pigments (chlorophyll a, b, and total Chl) of *L. minor*. However, BPA at a high concentration (50 mg/L) significantly inhibited the plant growth, the Chl content, and the maximal photochemical efficiency (Fv/Fm). Furthermore, BPA with high concentration (50 mg/L) induced ROS accumulation and increased the activities of antioxidant enzymes (SOD, CAT, POD, APX, and GR) and the contents of antioxidant substances (GSH, proline, and T-AOC), which indicated that *L. minor* might tolerate BPA toxicity by activating an antioxidant defense system. The correlation analysis revealed that the fresh weight of *L. minor* was significantly and positively correlated with photosynthesis and the contents of soluble protein and sugar, while it was negatively correlated with the content of H_2O_2. Totally, these results showed that BPA at different concentrations had dualistic effects on the growth of *L. minor*, which was attributed to the alterations of photosynthesis, oxidative stress, and osmotic regulation systems and provided a novel insight for studying the effects of BPA on aquatic plant physiology.

Effect of 17beta-estradiol on growth and biosynthesis of microalgae *Scenedesmus quadricauda* (CPCC-158) and duckweed *Lemna minor* (CPCC-490) grown in three different media

Kozlova, TA; Levin, DB. Plants 11: DOI10.3390/plants11131669

As fish farm wastewaters have detectable levels of fish hormones, such as 17beta-estradiol (E2), an understanding of the influence of fish steroids on algal (*Scenedesmus quadricauda*) and duckweed (*Lemna minor*) physiology is relevant to the potential use of fishery wastewaters for microalgae and plant biomass production. The study was conducted using three types of media: Bold Basal Medium (BBM), natural fishery wastewater (FWW), and reconstituted fishery wastewater (RFWW) with the nutrient composition adjusted to mimic FWW. During the experiment, the media were aerated and changes in the pH and conductivity of the water were closely monitored. E2 promoted the growth of *S. quadricauda* and *L. minor*, with significant accumulation of high-value biomolecules at very low steroid concentrations. However, clear differences in growth performance were observed in both test cultures, *S. quadricauda* and *L. minor*, grown in different media, and the most effective hormone concentrations were evidently different for the algae and the plant.

Performance of chlorophyll a fluorescence parameters in *Lemna minor* under heavy metal stress induced by various concentration of copper

Singh, H; Kumar, D; Soni, V. Scientific Reports (2022) 12: 10620

The objective of the present investigation was to understand the efficacy of chlorophyll fluorescence analysis and to identify the specific photosynthetic parameters for early and rapid detection of Cu-induced HM-stress in plants. Aquatic angiosperm *Lemna minor* was exposed to various concentrations (0-40 µM) of Cu. We observed that the F-V/F-O (Efficiency of the water-splitting complex on the donor side of PSII), quantum yield for electron transport, and quantum yield of primary photochemistry were decreased however, dissipated quantum yield was increased with Cu concentration. ABS/CSM, TRO/CSM, ETO/CSM and maximum quantum yield were displayed the dose-response relationship under Cu stress. Performance indexes were increased initially due to the beneficial effects of Cu at lower concentration while decreased significantly ($p <= 0.05$) at highest concentration of Cu. The outcomes of the present research revealed that the ChlF analysis is very sensitive tool that can be used to determine the toxicity of heavy metals in plants.

Anxiety in duckweed-metabolism and effect of diazepam on *Lemna minor*

Lamaczova, A; Malina, T; Marsalkova, E; Odehnalova, K; Opatrilova, R; Pribilova, P; Zezulka, S; Marsalek, B. (2022) Water 14: 1484

The fate of pharmaceuticals in the human body, from their absorption to excretion is well studied. However, medication often leaves the patient's body in an unchanged or metabolised, yet still active, form. Diazepam and its metabolites, ranging up to 100 µg/L, have been detected in surface waters worldwide; therefore, the question of its influence on model aquatic plants, such as duckweed (*Lemna minor*), needs to be addressed. *Lemna* was cultivated in a Steinberg medium containing diazepam in three concentrations-0.2, 20, and 2000 µg/L. The activity of superoxide dismutase (SOD) and catalase (CAT), leaf count, mass, and the fluorescence quantum yield of photosynthesis were assessed. The medium was also analysed by LC-MS/MS to determine the concentration of diazepam metabolites. Our results show no negative impact of diazepam on *Lemna minor*, even in concentrations significantly higher than those that are ecotoxicologically relevant. On the contrary, the influence of diazepam on *Lemna* suggests growth stimulation and a similarity to the effect diazepam has on the human body. The comparison to the human body may be accurate because γ-Aminobutyric acid-like (GABA-like) receptors responsible for the effect in humans have also been recently described in plants. Therefore, our results can open an interesting scientific area, indicating that GABA receptors and interference with benzodiazepines are evolutionarily much older than previously anticipated. This could help to answer more questions related to the reaction of aquatic organisms to micropollutants such as psychopharmaceuticals.

Taxonomy & Geobotany

Biodiversity of duckweed (Lemnaceae) in water reservoirs of Ukraine and China assessed by chloroplast DNA barcoding

Chen, GM; Stepanenko, A; Lakhneko, O; Zhou, YZ; Kishchenko, O; Peterson, A; Cui, DD; Zhu, HT; Xu, JM; Morgun, B; Gudkov, D; Friesen, N; Borysyuk, M. (2022) Plants 11: 1468

Monitoring and characterizing species biodiversity is essential for germplasm preservation, academic studies, and various practical applications. Duckweeds represent a group of tiny aquatic plants that include 36 species divided into 5 genera within the Lemnaceae family. They are an important part of aquatic ecosystems worldwide, often covering large portions of the water reservoirs they inhabit, and have many potential applications, including in bioremediation, biofuels, and biomanufacturing. Here, we evaluated the biodiversity of duckweeds in Ukraine and Eastern China by characterizing specimens using the two-barcode protocol with the chloroplast atpH-atpF and psbK-psbI spacer sequences. In total, 69 Chinese and Ukrainian duckweed specimens were sequenced. The sequences were compared against sequences in the NCBI database using BLAST. We identified six species from China (*Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis, Lemna minor, Lemna turionifera*, and *Wolffia globosa*) and six from Ukraine (*S. polyrhiza, Lemna gibba*, *Lemna minor, Lemna trisulca, Lemna turionifera*, and *Wolffia arrhiza*). The most common duckweed species in the samples from Ukraine were *Le. minor* and *S. polyrhiza*, accounting for 17 and 15 out of 40 specimens, respectively. The most common duckweed species in the samples from China was *S. polyrhiza*, accounting for 15 out of 29 specimens. *La. punctata* and *Le. aequinoctialis* were also common in China, accounting for five and four specimens, respectively. According to both *atp*H-*atp*F and *psb*K-*psb*I barcode analyses, the species identified as *Le. aequinoctialis* does not form a uniform taxon similar to other duckweed species, and therefore the phylogenetic status of this species requires further clarification. By monitoring duckweeds using chloroplast DNA sequencing, we not only precisely identified local species and ecotypes, but also provided background for further exploration of native varieties with diverse genetic backgrounds. These data could be useful for future conservation, breeding, and biotechnological applications.

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://thecharmsofduckweed.org Comprehensive site on all things duckweed-related, By Dr. John Cross, maintained by Paul Fourounjian.

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

Community Resources - Updated Table for Duckweed Collections in the Community

For information related to the location, collection size and contact email for duckweed collections in our community, please access the website of the RDSC (Rutgers Duckweed Stock Cooperative) under the heading "List of Worldwide Duckweed Collections". This Table will be updated as new entries for duckweed collections are being supplied to members of the International Steering Committee for Duckweed Research and Applications (ISCDRA). We also plan to publish the updated table in the first issue of each Duckweed Forum newsletter volume starting in 2021.

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