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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 11 (4), issue 43, pages 135 - 173 (2023)

Duck in a Duckweed-covered pond

Cover page

Duck in a duckweed-covered pond. Photos courtesy: Ms. Jutta Schenker, Jena, Germany and Design: Mr. Jonas Appenroth, Jena, Germany.

In this issue

Letter from the Editor:.....135

Useful resources: A protocol for heavy-ion irradiation mutagenesis and mutant collections in duckweed.....136

7th ICDRA: Announcement.....141

Young Researcher Spotlight: Guili Yang.....142

Duckweed Workshop at PAG 2024: Announcement.....145

Special issue on Duckweeds published in the journal Plants.....146

Student Spotlight: Yuri Lee.....150

From the Database.....152

Instructions to Contributors for the Duckweed Forum.....171

Links for Further Reading.....173

Disclaimer: Articles in the Duckweed Forum are published in the form sent in by the authors, except for some minor language editing by members of the ISCDRA team. The authors are responsible and are liable for any legal issues concerning plagiarism and/or use of copyrighted material in any form.

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, Ireland; 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:

Oct. 30th, 2023

Dear readers,

Welcome to the final *Duckweed Forum* issue of this year. In this issue, we feature three different contributions from young researchers of China and Japan. Dr. Yu Liu, and his more senior colleagues from Qingdao, China, described their approach of using high energy ions as mutagens to generate new varieties of duckweed. Stable mutants with altered morphology and metabolism were generated and characterized with *Spirodela polyrhiza* or *Lemna aequinoctialis* as the genetic backgrounds. Their results so far appear to be quite promising as an effective means of creating novel traits in duckweeds. We also shine a spotlight on a successful young researcher, Dr. Guili Yang of Guizhou University, China, who studies the application of duckweed for phytoremediation of heavy metals. Her passion for duckweed research is eminently captured in an article describing her path to academic success. Moreover, her recently completed book on duckweed research and applications in the Chinese language will likely help to spread the knowledge and appreciation of duckweeds in China and beyond. Similarly, a young student researcher from Japan, Ms. Yuri Lee, described her passion in collecting and characterizing duckweeds from the wild, with a focus to learn more about their flowering habits.

I hope the readers will enjoy reading about these articles as well as the informative Database section with many original and interesting research reports that utilize duckweed as the model system.

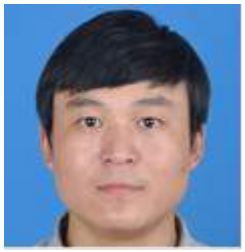
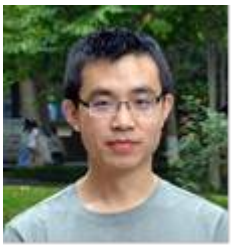
For all the ISCDRA members, I wish all of you good luck and be well, especially in these times of numerous conflicts around the world.

Sincerely,

Eric Lam

Chair, ISCDRA

Useful resources: A protocol for heavy-ion irradiation mutagenesis and mutant collections in duckweed

Gongke Zhou[1], Yubin Ma[2], Yu Liu[1]

[1] College of Landscape Architecture and Forestry, Qingdao Agricultural University, Qingdao, China. Email: zhougk@qau.edu.cn; liuyu@qau.edu.cn

[2] Ministry of Education Key Laboratory of Marine Genetics and Breeding, College of Marine Life Sciences, Ocean University of China, Qingdao, China. Email: mayubin@ouc.edu.cn

Introduction

Use of high-energy particles as a mutagen for genetic research

As an emerging radiation source, heavy ion beams, especially low energy carbon and nitrogen ion beams, have become more widely used in plant and microbial breeding research due to their strong mutagenic effects. Compared with traditional mutagenic sources, heavy ion beams have the following characteristics: 1. Having a high linear energy transfer (LET) density, it can generate high-density ionization, excitation, energy, and mass deposition in biological media, causing damage to biological material and facilitating the formation of mutants via DNA damage. 2. During the process of energy deposition, there is a sharp energy loss peak at the end of its range, known as the Bragg peak, which causes local damage to the biological sample. The extent of this local damage can vary with the level of ion energy, which is controllable and conducive to macro fixed-point and localized mutagenesis. 3. DNA damage is not easily repaired and produce a large number of stable mutants. 4. The relative biological effects can be numerous because the production efficiency of mutations is high and the mutation spectrum can be quite wide.

Application of this methodology in duckweed research – historical aspects

The creation of duckweed mutants using radiation can be traced to the 1960s. For example, production of auxotrophs from *Spirodela polyrhiza* by X-irradiation (7). A 2 mev, 500 w Van de Graaff accelerator fitted with a water-cooled gold target was employed for generating X rays. Flasks containing 40 to 50 fronds of duckweed were irradiated for periods of 3 to 24 minutes, affording radiation doses of 1,000 to 8,000 rads per flask. In other study, 7 mutants have been produced by X-irradiation of *Lemna perpusilla* 6746 (4). Strain 98-H grows slowly and on high sucrose concentrations produces high numbers of fronds per colony. Strains 1073 and 5104 grow slightly slower than wild-type and are much more radiosensitive. Strain 1073 was reported to differ in its flowering responses to light (3, 5, 6). Spectrophotometric and immunoblotting techniques were used for

localizing the site of defect. The results suggested that the cytochrome *b6-f* complex was absent in the strain 1073 (1, 2). Meanwhile, the total amount of plastoquinone-9 was reduced by 65%, compared to wild-type.

Callus tissue is composed of living thin-walled cells that can originate from various tissues within any organ of a plant. Due to its strong regeneration ability and susceptibility to *Agrobacterium* infection, callus is widely used in duckweed transgenic experiments. The structural characteristics of callus tissue are conducive to the production of mutants by heavy ion beam mutagenesis. It takes a short time and can complete radiation induced mutation in tens of seconds. Independent callus tissue can generate phenotypically stable mutant materials through regeneration. Therefore, callus tissue is an ideal material for creating new varieties of duckweed through radiation induced mutagenesis.

Method

1. Callus induction

Calli were induced from fronds on Murashige and Skoog medium containing 3% sucrose, 0.1 mg/L thidiazuron and 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). Embryogenic calli were produced after dark cultivation for 7 weeks, and developed calli were transferred to fresh induction medium for sub-cultivation.

2. Heavy-ion irradiation mutagenesis

Two to three week-old duckweed calli were irradiated by 80 MeV/μ carbon ion, which was supplied by the Heavy-Ion Research Facility in Lanzhou, Institute of Modern Physics, Chinese Academy of Sciences. The average LET value was 31 keV/μm, and a series of irradiation dosages, including 10, 25, 50, 75, 100 and 125 Gy, were used for mutagenesis *in vivo* (8).

3. Mutant collection

Afterwards, the irradiated calli were transferred to regeneration medium (Gamborg's B5 basal medium supplemented with 4.5 mg/L indole-3-acetic acid, 1 mg/L kinetin, 10 g/L sucrose, and solidified with 8.0 g/L agar), and cultivated under white light with a photoperiod of 16 (light):8 (dark). The regenerated fronds were transferred to SH solid medium supplemented with 10 g/L sucrose for conservation, and then newly grown fronds were proliferated on liquid SH medium. After 2 months of regeneration, collection, and propagation, a heavy-ion mutagenized duckweed mutant library was established.

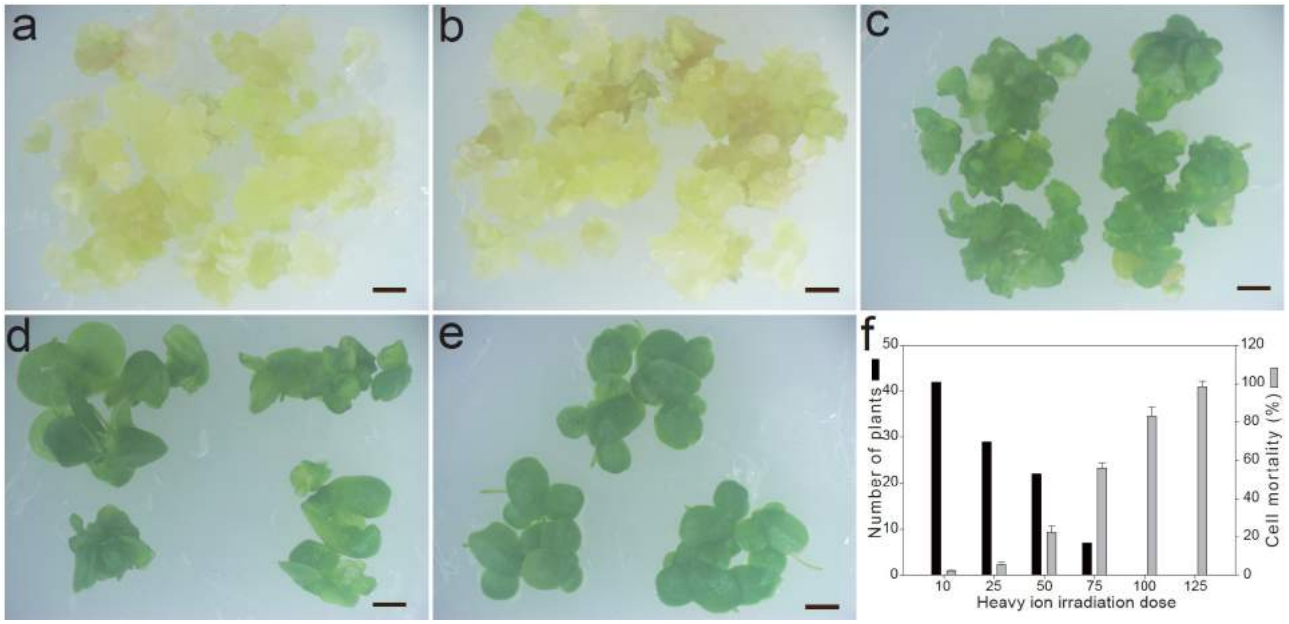


Figure 1. Regenerated mutants by heavy-ion irradiation

(a) Three-week-old calli of *Lemna aequinoctialis* 6002 before irradiation; (b) Calli irradiated by carbon ions; (c) Calli differentiation for one week; (d) Fronds regeneration; (e) Cultivation of new varieties; (f) Number of mutants regenerated from callus and cell mortality with different radiation dose. Scale bars=1 mm.

Our duckweed mutant collection:

1. *Spirodela polyrhiza* 6068 mutant lines

There are significant phenotypic differences between wild type (Figure 2a) and *68-red* mutant (Figure 2b). On the ventral surface of *68-red* mutant (9), larger area of deep reddish-purple caught our attention (Figure 2b). Quantitative analysis revealed that total anthocyanin content in *68-red* mutant was increased by 5.63 times of that of wild type (Figure 2c). In addition, DMACA staining showed that larger number of blue dots were distributed in the fronds and roots of *68-red* mutant, which indicated a lot of proanthocyanidin accumulation (Figure 2d, 2e). Quantitative analysis found that the content of proanthocyanidin was increased by 2.32 times in *68-red* mutant (Figure 2f).

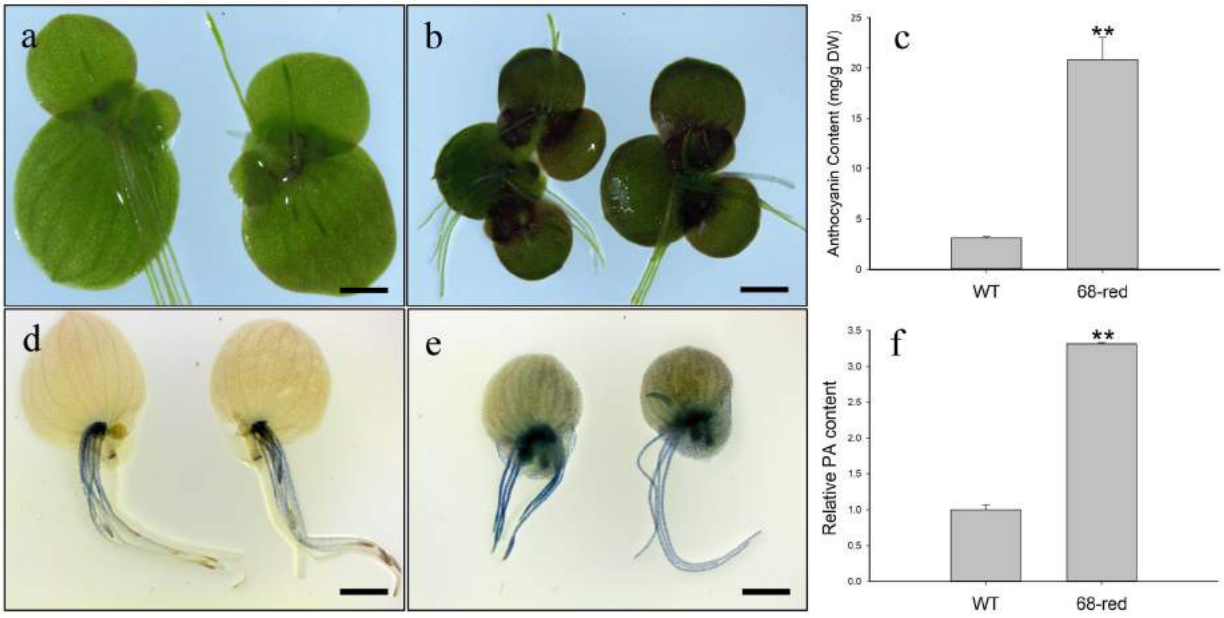


Figure 2. Phenotypes of the *68-red* mutant
(a, b): Color difference between wild-type and *68-red* mutant, (d, e): DMACA staining, (c, f): anthocyanin and proanthocyanidin content. Bars represent standard deviations (SD) from three biological repeats. Asterisks indicate the significant difference between two groups (** $P < 0.01$) using Duncan's test. Scale bars=2mm.

Using liquid chromatography mass spectrometry-mass spectrometry (LC-MS/MS) to analyze the main composition of flavonoid compounds in *68-red* mutant, the results showed that luteolin-8-C-glucoside (orientin), luteolin-7-O-glucoside (cynaroside), epicatechin and catechin content were significantly increased by 53.82%, 41.31%, 245.00% and 106.25% respectively. In contrast, apigenin-8-C-glucoside (vitexin) and apigenin-7-O-glucoside (apigetrin) were significantly decreased by 17.77% and 29.86%, compared with wild type. The above results suggested that flavonoid metabolic pathway has been remodeled in *68-red* mutant.

2. *Lemna aequinoctialis* 6002 mutant lines

The frond traits of wild type (*L. aequinoctialis* 6002) appeared to be obovate (Figure 3a), while those of the *sub-1* mutant were square (Figure 3b). Then, paraffin sections of fronds were prepared and I_2/KI staining was used to analyze starch accumulation. As shown in Figure 3, the fronds of wild-type and *sub-1* mutant were different in color, dark brown in *sub-1* mutant (Figure 3f) and light brown in wild type (Figure 3e). Compared with wild-type, the volume and quantity of starch granules in *sub-1* mutant increased significantly (Figure 3f). Starch granules were mainly distributed in the inner cavity and lower epidermal cells, while not found in the upper epidermal cells (8). It was unexpectedly found that the fronds had an additional layer of aerenchyma, resulting in a significant 72.49% increase in leaf thickness in *sub-1* mutant compared to wild type. The above result indicated that the *sub-1* mutant accumulated a large amount of starch particles, with inner cavity and lower epidermal cells being the main storage sites of starch, especially the additional layer of cells in the middle of the fronds. Sugar and starch were dyed purplish-red by PAS solution. Compared with wild type, the *sub-1* mutant showed deep purplish-red all over the fronds, reflecting the accumulation of large amounts of sugar and starch (Figure 3c,d). Based on the results of I_2/KI and PAS staining, we concluded that the upper

epidermal cells in fronds were the main sites of sugar metabolism, and the inner cavity cells were used for starch storage. Starch granules were extracted from wild type and *sub-1* mutant, and amylose content of them were detected. Compared with wild type, the content of amylose obviously decreased by 34.43%, while that of amylopectin significantly increased by 11.08% in *sub-1* mutant. The ratio of amylose to amylopectin was 0.32 and 0.19 in wild-type and *sub-1* mutant, respectively. It was particularly noted that the proportion of amylopectin in *sub-1* mutant was as high as 84.04% (10).

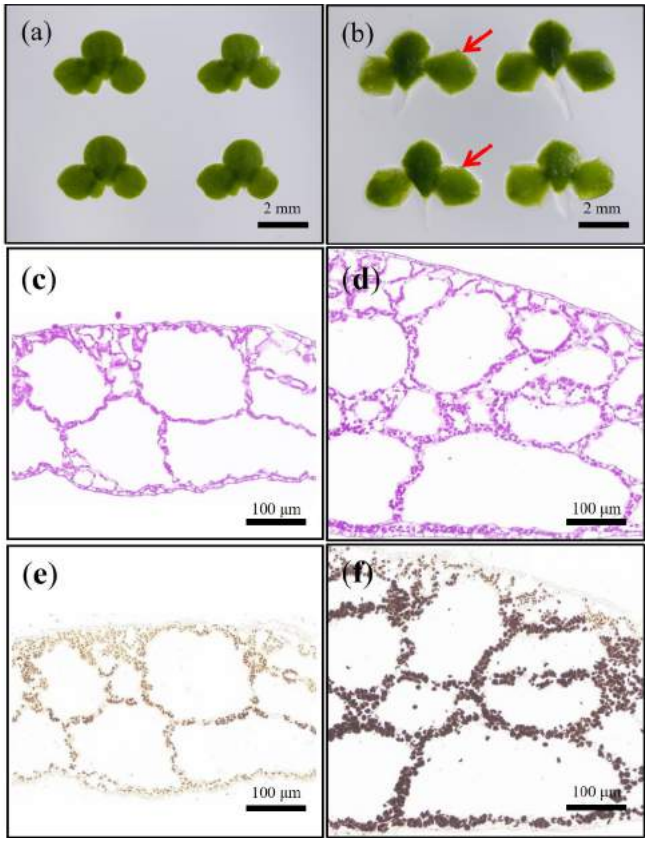


Figure 3 . Phenotypic characteristics of the sub-1 mutant.
(a,b): Frond shape difference between wild type and *sub-1* mutant. (c,d): PAS staining of wild type and *sub-1* mutant. (e,f): I_2/KI staining of wild type and *sub-1* mutant. Scale bars = 2 mm, 100 μm.

Future perspectives

For radiation mediated breeding approach, the mutagenic effects of heavy ions are mainly reflected at the nucleic acid and chromosome levels. DNA is one of the key target molecules for radiation damage, and sequence and structural changes at the DNA level are the molecular basis for gene mutations. Using constant field gel electrophoresis technology and multifunctional fluorescence imaging system to analyze the damage mode of DNA after heavy ion radiation, the results indicate that radiation-induced DNA damage includes base substitution, deletion, cleavage of hydrogen bonds between DNA strands, single and double stranded DNA breaks, DNA DNA crosslink and DNA protein crosslink (11, 12). In addition, due to the high LET of heavy ions, at least 2-5 energy deposition events can occur at sites with a diameter of 1-4 nm, which can cause clustered damages (CD) of DNA molecules and are difficult to repair. In order to maintain its normal function, organisms will initiate various repair pathways, including excision repair, recombination repair, and SOS repair, resulting in varying degrees of repair of radiation damage under certain conditions. However, if incomplete or incorrect repair occurs, it will lead to errors in the DNA replication process, leading to abnormalities in intracellular macromolecules and cellular structure. The study of DNA radiation damage and repair will contribute to a profound understanding of the mechanisms by which organisms maintain genetic stability, undergo mutations, and evolve. Meanwhile, because of heavy ion beam irradiation's effects on gene transcription and translation, metabolism, and cell signal transduction, genetically altered plants created by this method can provide a foundation for studying important biological traits in duckweed. In future research, microarray comparative genomic hybridization (array CGH), high-resolution melting curve analysis (HRM), and proteomics

can be used to comprehensively screen for chromosomal structural variations (SNPs, InDels, CNVs, etc.) induced by heavy ion radiation at the genomic level, as well as identification of differentially expressed proteins induced by heavy ion radiation at the protein level. This will further deepen the understanding of the mutagenic effects and mechanisms of heavy ion radiation on duckweed, laying the foundation for breeding and functional genomics research induced by heavy ion radiation on duckweed.

References

1. Lam, E., & Malkin, R. (1985). Characterization of a photosynthetic mutant of *Lemna* lacking the cytochrome b6-f complex. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 810(1), 106-109.
2. Bruce, B. D., & Malkin, R. (1991). Biosynthesis of the chloroplast cytochrome b6f complex: studies in a photosynthetic mutant of *Lemna*. *The Plant Cell*, 3(2), 203-212.
3. Posner, H. B. (1973). Lack of flowering responses to DCMU in a mutant of *Lemna perpusilla* 6746. *Plant and cell physiology*, 14(5), 1031-1033.
4. Posner, H. B. (1962). Characteristics of X-ray-induced aberrants of *Lemna perpusilla* 6746. *Plant and Cell Physiology*, 3(3), 275-275.
5. Gower, R. A., & Posner, H. B. (1979). Effects of Light and 3-(3, 4-Dichlorophenyl)-1, 1-Dimethylurea on Levels of ATP in *Lemna paucicostata* 6746 and a Photosynthetic Mutant with Abnormal Flowering Responses. *Plant Physiology*, 63(3), 548-551.
6. Shahak, Y., Posner, H. B., & Avron, M. (1976). Evidence for a block between plastoquinone and cytochrome f in a photosynthetic mutant of *Lemna* with abnormal flowering behavior. *Plant physiology*, 57(4), 577-579.
7. Smith, D. H., & Castle, J. E. (1960). Production of auxotrophs in a duckweed, *Spirodela polyrhiza*. *Plant Physiology*, 35(6), 809.
8. Liu, Y., Xu, H., Wang, Y., Tang, X., He, G., Wang, S., ... & Zhou, G. (2020). A submerged duckweed mutant with abundant starch accumulation for bioethanol production. *GCB Bioenergy*, 12(12), 1078-1091.
9. Liu, Y., Li, C., Yan, R., Yu, R., Ji, M., Chen, F., ... & Tang, X. (2022). 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). *Journal of Plant Physiology*, 275, 153753.
10. Liu, Y., Yan, R., Li, Z., Fan, S., Li, C., Yu, R., ... & Zhou, G. (2023). Efficient Accumulation of Amylopectin and Its Molecular Mechanism in the Submerged Duckweed Mutant. *International Journal of Molecular Sciences*, 24(3), 2934.
11. Ritter, S., & Durante, M. (2010). Heavy-ion induced chromosomal aberrations: a review. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 701(1), 38-46.
12. Tanaka, A., Shikazono, N., & Hase, Y. (2010). Studies on biological effects of ion beams on lethality, molecular nature of mutation, mutation rate, and spectrum of mutation phenotype for mutation breeding in higher plants. *Journal of radiation research*, 51(3), 223-233.

7th ICDRA: Announcement

Young Researcher Spotlight: Guili Yang

Professor of Guizhou University, head of Bioscience Department, Guiyang, China (Email: glyang3@gzu.edu.cn)

Setting Sail at the Bloom of Youth : a young researcher who exhibits excellence and loves duckweed.

I have great enthusiasm for science since my undergraduate times. Long time ago, when I was in the lab and started my first research project ----"the research on the mechanism how intercrop between soy and corn facilitates biological nitrogen fixation". Every time, while other classmates were taking a rest on weekends, I would follow my teacher Qiuzhu Li to the farmland for research instead, which started my career of scientific inquiries. When I graduated from Jinlin University with a major in agronomy. I, ranked first in my major and was then enrolled as graduate student in the Chengdu Institute of Biology, Chinese Academy of Sciences (CAS), with my research focus on "Analysis and coupling of the molecular modules responsible for high and stable yield of wheat production".

I started my story with duckweed in June, 2016, the time when my instructor Maoqun Yu was about to retire and no longer teach students. I really love scientific research and wanted to continue my further education, so for my doctoral degree I transferred to the research group of Prof. Hai Zhao in the same institute, who is a specialist in duckweed research field. I have been attracted, since I first saw this tiny hydrophyte in Prof. Zhao's lab, because I had never known that this small thing could contain so much power. At that time, our research on duckweed involved many fields, such as establishing a solid foundation for restoring bodies of polluted water, exploring the mechanism of increased starch accumulation, etc. Since there was a need to improve the molecular biology foundation in the lab in order to facilitate our desire to establish stable genetic transformation system for duckweeds, I volunteered to take up the challenge in the group to build this system up from scratch. Although without a foundation in this field, aside from my strong love to duckweed, I was able to overcame the difficulties through hard work, sometimes working in the lab late at night.

Finally, with great efforts our lab successfully built the genetic transformation platform that set a foundation for further research in the group. Only after one year of research, I published my first research paper in 2018 as a cover story in the journal *Plant Molecular Biology.* This article reported a frond-based transformation system mediated by *Agrobacterium tumefaciens* on *Lemna minor* for the first time [1] . I believe that most scientists could feel how much this meant to me and this also made me more determined to research duckweed for the rest of my life.

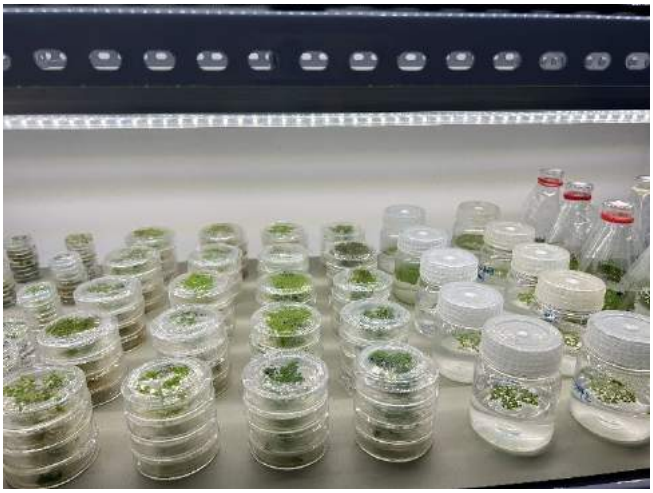
In September, 2019, with my honor as CAS's outstanding graduate and outstanding graduate of Beijing, I decided to join Guizhou University at 27 after graduated from the Zhao lab, to become a teacher which I dreamed of for years. There in Guizhou University, I continue to dedicate my research on duckweed. In order to gather more duckweed resources for further research to start my lab, I took my family, starting from Guizhou Province [2] , and set sail for our journey to collect duckweed resources nationwide.

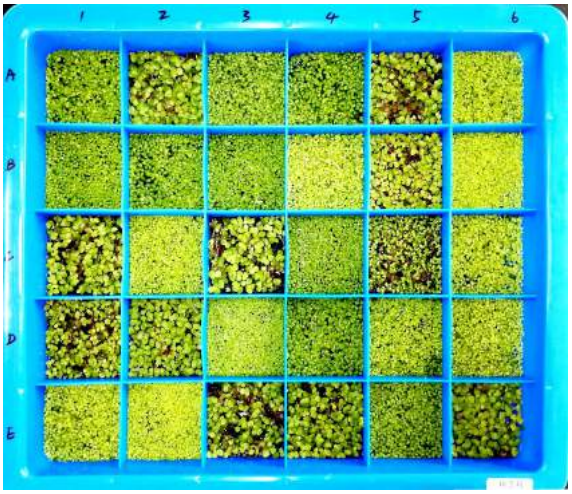
Collecting is just the very beginning of the work. We have to record and organize information for the collected duckweed. Then I would perform sterilization for each collection on my own, to keep all the collected populations preserved under aseptic conditions. After all these efforts, our own duckweed germplasm resource bank was built in Guizhou University's School of Life Sciences. Time passed by, the team of just one has now became a group of more than ten members. The team, with those duckweed germplasm resources, aimed at different targets, found many isolates with useful properties, such as duckweed materials that can quickly hyper-accumulate heavy metals [3-6] .

Prof. Yang's father collecting duckweed in the wild

After two years of work, I was unanimously elected as the head of department. In addition to my administrative work, I helped my group to extend further research directions and accomplished multiple achievements in their projects. However, I felt that duckweed has long been severely ignored and neglected domestically in China, so with all of my group, we spent 3 years to carry out data collection and organization, editing and compiling the first academic work *"Biosystematic investigations and Applications in the Lemnaceae"* [7] in Chinese. The creation of this book has taken a lot of efforts from our team since we have to carry out repeated adjustments, such as additions, deletions and modifications, in order to combine all the latest theories and practical illustrations and provide duckweed researchers at-large with the most up-to-date reference materials. Two years later, when the first draft was completed, the team continue their scientific spirits and kept improving the book through multiple iterations. In the office, heated discussions occurred almost daily on what and how to improve further. After three years, the official version was finally released. For now, I have been preparing the English version, hoping that through my effort, a larger audience across the world could learn and appreciate the importance of duckweed.

Prof. Yang introducing Eric Lam to her duckweed germplasm resource bank





Duckweed Germplasm Resources at Guizhou University

Meanwhile, as a teacher, I love my job deeply, and I would like to pass on my passion for duckweed to my students generation by generation. What I think is that " Live your life as a beam of light, because you don't know, if someone would get out of the dark under your brightness; please keep kindness in your heart, because you don't know, if someone would get out of despair for your kindness; please uphold beliefs in your heart, because you don't know, if someone would get out of confusion by virtue of your beliefs". Because of my perseverance in research along with my teaching performance, I became the youngest to be elected as "outstanding teacher in Guizhou University".

Finally, I would like to share my opinions towards the future development of duckweed as following: (1) Duckweed, containing high quantity of protein and low fibre, can be expected to become a kind of "superfood". In the future, we may be able to use duckweed to build an industrialized grain system. (2) Duckweed contains multiple active ingredients with medical values. We can explore it further and use it in manufacturing for biologic drugs. (3) Duckweed will continue to be a model organism for research on ecotoxicology and water restoration. In the future, we should be able to apply it to build a sustainable water pollution management system. (4) Duckweed is also a new kind of environmentally adaptable biomass resource. Some duckweed species has significantly minimized gene coding space, and it can offer a chassis for synthetic biology to provide a platform for highly effective bioreactors. For example, we can modify it through gene engineering, providing new opportunities for bioproducts such as biofuel.

Prof. Yang sharing the first draft to Eric Lam (left), the academic work with Prof. Yang as editor in chief (right)

I will keep on flipping through the book of duckweed and continue my further research on duckweed to make this strategically valuable biological resource beneficial for mankind. As a scientific researcher, I will be dedicated to this and hope to have the opportunity to learn and cooperate with scientists who love duckweed as well.

References

1. Yang GL, Fang Y, Xu YL, et al., Frond transformation system mediated by *Agrobacterium tumefaciens* for *Lemna minor*. *Plant Molecular Biology*, 2018, 98: 319–331

2. Feng D, Tan AJ, Lv SM, et al., Morphological identification and genetic diversity analysis of duckweed in Guizhou Province. *Acta Hydrobiologica Sinica*, 2022, 46(07): 1-15.

3. Zheng MM Feng D, Liu HJ, et al., Subcellular distribution, chemical forms of cadmium and rhizosphere microbial community in the process of cadmium hyperaccumulation in duckweed. *Science of The Total Environment*, 2023. 859: 160389.

4.Yang X, Tan AJ, Zheng MM, et al., Physiological response, microbial diversity characterization, and endophytic bacteria isolation of duckweed under cadmium stress. *Science of The Total Environment*, 2023, 902: 166056.

5. Yang GL, Huang L, Yang X, et al., Transcriptomic and Functional Analyses of Two Cadmium Hyper-Enriched Duckweed Strains Reveal Putative Cadmium Tolerance Mechanisms. International Journal of Molecular Sciences, 2023, 24(15): 12157.

6. Zheng MM, Tan AJ, Lv SM, et al., Screening, identification and enrichment effects of cadmium-tolerant duckweeds. *Chinese Journal of Environmental Engineering*, 2022, 16(02): 471-480.

7. Yang GL. Biosystematic investigations and Applications in the *Lemnaceae*[M]. *Chongqing Press*, 2023, 05.

Duckweed Workshop at PAG 2024: Announcement

Important Dates for attendees

Duckweed Research and Applications Workshop

in the

Plant and Animal Genome (PAG 31) Conference 2024

January 12-17, 2024

at the Town & Country Conference Center in San Diego, CA, USA

Eric Lam (Rutgers U.) and Todd Michael (Salk Institute) are organizing the **Duckweed Research and Applications Workshop** to take place on the afternoon of January 12th during PAG 31 in San Diego (**January 12-17, 2024**) and would encourage everyone in the community to attend. For those who are attending PAG 31 and would like to present, please contact either of us as soon as possible. We expect to have an excellent lineup of exciting speakers with new results from diverse studies with duckweed.

Please register at this link:

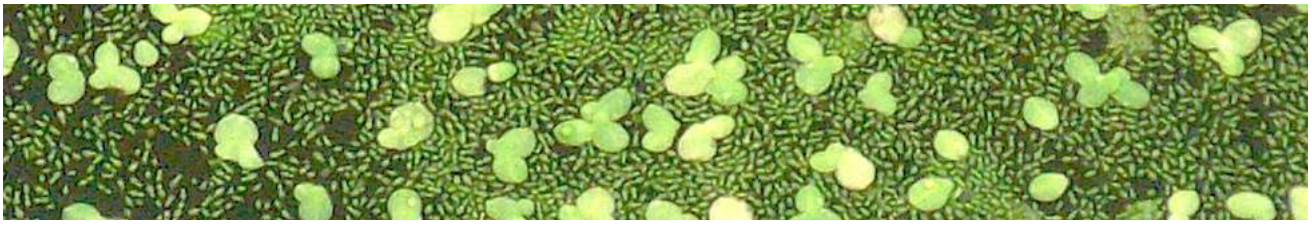
https://pag.confex.com/pag/31/registration/index.cgi?username=138578&password=624546

Please also note these deadlines:

Deadline for workshop abstract submission: December 8th, 2023
Deadline for poster abstract submission: November 3rd, 2023
Deadline for posting speakers by organizers: November 17, 2023

Confirmed Speakers and Topics:

Arturo Mari-Ordonez: "*Understanding Transposon silencing across duckweeds, tiny plants with big surprises*"
Na Wei: "*Microbial biogeography of Lemna*"
Robert A. Martienssen: "*Genomes and epigenomes in Lemnaceae: connections between clonal reproduction and triploid hybrids*"
Kelly Colt: "*Single-cell transcriptome atlas of whole Wolffia, the smallest known plant on Earth*"
Eric Lam: "*Uncovering ecological drivers for microbiome assembly in duckweeds using SynComs*"



Special issue on Duckweeds published in the journal Plants

Special Issue entitled "Duckweed: Research meets Applications" has been published in the journal PLANTS

Editors: Viktor Oláh, Klaus-J. Appenroth and K. Sowjanya Sree

In the journal Plants (ISSN 2223-7747) a Special Issue with 39 contributions has been published. We invited especially, but not exclusively, the participants of the "Sixth International Conference on Duckweed Research and Applications", held in Gatersleben, Germany from 29th May to 1st June 2022.
In the following, all papers are cited by headlines, authors and references. As all the papers are published under open source, accessing the given DOI address brings you directly to the full paper as pdf. The complete Special Issue has been published as an eBook (ISBN 978-3-0365-9069-1 hardback; ISBN 978-3-0365-9068-4 PDF) and is available at the following link: https://www.mdpi.com/books/book/8110.
We would like to thank the authors, reviewers, editors and publisher, especially Ms. Aria Li, Section Managing Editor of Plants.

Contents

About the Editors ix

1. Introduction

Viktor Oláh, Klaus-Juergen Appenroth and K. Sowjanya Sree
Duckweed: Research Meets Applications
Reprinted from: *Plants* 2023, *12*, 3307, doi:10.3390/plants12183307 1

Viktor Oláh, Klaus-Juergen Appenroth, Eric Lam and K. Sowjanya Sree
Sixth International Conference on Duckweed Research and Applications Presents Lemnaceae as a Model Plant System in the Genomics and Postgenomics Era
Reprinted from: *Plants* 2023, *12*, 2134, doi:10.3390/plants12112134 9

K. Sowjanya Sree, Klaus J. Appenroth and Ralf Oelmüller
Sustainable Stress Management: Aquatic Plants vs. Terrestrial Plants.
Reprinted from: *Plants* 2023, *12*, 2208, doi:10.3390/plants12112208 21

Paul Ziegler, Klaus J. Appenroth and K. Sowjanya Sree
Survival Strategies of Duckweeds, the World's Smallest Angiosperms
Reprinted from: *Plants* 2023, *12*, 2215, doi:10.3390/plants12112215 31

2. Molecular characterization and taxonomy

Nicholas P. Tippery, Donald H. Les, Klaus J. Appenroth, K. Sowjanya Sree, Daniel J. Crawford and Manuela Bog
Lemnaceae and Orontiaceae Are Phylogenetically and Morphologically Distinct from Araceae
Reprinted from: *Plants* 2021, *10*, 2639, doi:10.3390/plants10122639 61

Luca Braglia, Diego Breviario, Silvia Gianì, Floriana Gavazzi, Jacopo De Gregori and Laura Morello
New Insights into Interspecific Hybridization in *Lemna* L. Sect. *Lemna* (Lemnaceae Martinov)
Reprinted from: *Plants* 2021, *10*, 2767, doi:10.3390/plants10122767 81

Manuela Bog, Luca Braglia, Laura Morello, Karen I. Noboa Melo, Ingo Schubert, Oleg N. Shchepin, K. Sowjanya Sree, Shuqin Xu, Eric Lam and Klaus-Juergen Appenroth
Strategies for Intraspecific Genotyping of Duckweed: Comparison of Five Orthogonal Methods Applied to the Giant Duckweed *Spirodela polyrhiza*
Reprinted from: *Plants* 2022, *11*, 3033, doi:10.3390/plants11223033 101

Manuela Bog, Klaus-Juergen Appenroth, Philipp Schneider and K. Sowjanya Sree
Intraspecific Diversity in Aquatic Ecosystems: Comparison between *Spirodela polyrhiza* and *Lemna minor* in Natural Populations of Duckweed
Reprinted from: *Plants* 2022, *11*, 968, doi:10.3390/plants11070968 113

Guimin Chen, Anton Stepanenko, Olha Lakhneko, Yuzhen Zhou, Olena Kishchenko, Anton Peterson, Dandan Cui, Haotian Zhu, Jianming Xu , Bogdan Morgun, Dmitri Gudkov, Nikolai Friesen and Mykola Borysyuk
Biodiversity of Duckweed (Lemnaceae) in Water Reservoirs of Ukraine and China Assessed by Chloroplast DNA Barcoding
Reprinted from: *Plants* 2022, *11*, 1468, doi:10.3390/plants11111468 125

Avital Friedjung Yosef, Lusine Ghazaryan, Linda Klamann, Katherine Sarah Kaufman, Capucine Baubin, Ben Poodiack, Noya Ran, Talia Gabay, Shoshana Didi-Cohen, Manuela Bog, Inna Khozin-Goldberg and Osnat Gillor
Diversity and Differentiation of Duckweed Species from Israel
Reprinted from: *Plants* 2022, *11*, 3326, doi:10.3390/plants11233326 139

Phuong T. N. Hoang, Jörg Fuchs, Veit Schubert, Tram B. N. Tran and Ingo Schubert
Chromosome Numbers and Genome Sizes of All 36 Duckweed Species (Lemnaceae)
Reprinted from: *Plants* 2022, *11*, 2674, doi:10.3390/plants11202674 153

Lakshmi Pasricha Sarin, K. Sowjanya Sree, Károly Bóka, Áron Keresztes, Jörg Fuchs, Akhilesh K. Tyagi, Jitendra Paul Khurana and Klaus-Juergen Appenroth
Characterisation of a Spontaneous Mutant of *Lemna gibba* G3 (Lemnaceae)
Reprinted from: *Plants* 2023, *12*, 2525, doi:10.3390/plants12132525 161

3. Phytoremediation: Wastewater

Yuzhen Zhou, Anton Stepanenko, Olena Kishchenko, Jianming Xu and Nikolai Borisjuk
Duckweeds for Phytoremediation of Polluted Water
Reprinted from: *Plants* 2023, *12*, 589, doi:10.3390/plants12030589 179

Neil E. Coughlan, Eamonn Walsh, Roger Ahern, Gavin Burnell, Rachel O'Mahoney, Holger Kuehnhold and Marcel A. K. Jansen
Flow Rate and Water Depth Alters Biomass Production and Phytoremediation Capacity of *Lemna minor*
Reprinted from: *Plants* 2022, *11*, 2170, doi:10.3390/plants11162170 199

Reindert Devlamynck, Marcella Fernandes de Souza, Jan Leenknegt, Liesbeth Jacxsens, Mia Eeckhout and Erik Meers
Lemna minor Cultivation for Treating Swine Manure and Providing Micronutrients for Animal Feed
Reprinted from: *Plants* 2021, *10*, 1124, doi.org/10.3390/plants10061124 219

Marie Lambert, Reindert Devlamynck, Marcella Fernandes de Souza, Jan Leenknegt, Katleen Raes, Mia Eeckhout and Eric Meers
The Impact of Salt Accumulation on the Growth of Duckweed in a Continuous System for Pig Manure Treatment
Reprinted from: *Plants* 2022, 11, 3189, doi:10.3390/plants11233189 237

Eamonn Walsh, Neil E. Coughlan, Seán O'Brien, Marcel A. K. Jansen and Holger Kuehnhold
Density Dependence Influences the Efficacy of Wastewater Remediation by *Lemna minor*
Reprinted from: *Plants* **2021**, *10*, 1366, doi.org/ 10.3390/plants10071366 259

Rachel O'Mahoney, Neil E. Coughlan, Eamonn Walsh and Marcel A. K. Jansen
Cultivation of *Lemna minor* on Industry-Derived, Anaerobically Digested, Dairy Processing Wastewater
Reprinted from: *Plants* **2022**, 11, 3027, doi:10.3390/plants11223027 277

Simona Paolacci, Vlastimil Stejskal, Damien Toner and Marcel A. K. Jansen
Integrated Multitrophic Aquaculture; Analysing Contributions of Different Biological Compartments to Nutrient Removal in a Duckweed-Based Water Remediation System
Reprinted from: *Plants* **2022**, *11*, 3103, doi:10.3390/plants11223103 291

Yuzhen Zhou, Olena Kishchenko, Anton Stepanenko, Guimin Chen, Wei Wang, Jie Zhou, Chaozhi Pan and Nikolai Boisjuk
The Dynamics of NO_3^- and NH_4^+ Uptake in Duckweed Are Coordinated with the Expression of Major Nitrogen Assimilation Genes
Reprinted from: *Plants* **2021**, 11, 11, doi:10.3390/plants11010011 309

Olena Kishchenko, Anton Stepanenko, Tatsiana Straub, Yuzhen Zhou, Benjamin Neuhäuser and Nikolai Borisjuk
Ammonium Uptake, Mediated by Ammonium Transporters, Mitigates Manganese Toxicity in Duckweed, *Spirodela polyrhiza*
Reprinted from: *Plants* **2023**, *12*, 208, doi:10.3390/plants12010208 329

Orathai Pakdee, Shomo Tshering, Prayad Pokethitiyook and Metha Meetam
Examination of the Metallothionein Gene Family in Greater Duckweed *Spirodela polyrhiza*
Reprinted from: *Plants* **2022**, *12*, 125, doi:10.3390/plants12010125 349

Viktor Oláh, Muhammad Irfan, Zsuzsanna Barnáné Szabó, Zsófi Sajtos, Agota Zsófia Ragyák, Boglárka Dönczö, Marcel A. K. Jansen, Sándor Szabó and Ilona Mészáros
Species- and Metal-Specific Responses of the Ionome of Three Duckweed Species under Chromate and Nickel Treatments
Reprinted from: *Plants* **2023**, *12*, 180, doi:10.3390/plants12010180 361

4. Applications: Accumulation of protein or starch

Finn Petersen, Johannes Demann, Dina Restemeyer, Andreas Ulbrich, Hans-Werner Olfs, Heiner Westendarp and Klaus-Juergen Appenroth
Influence of the Nitrate-N to Ammonium-N Ratio on Relative Growth Rate and Crude Protein Content in the Duckweeds *Lemna minor* and *Wolffiella hyalina*
Reprinted from: *Plants* **2021**, 10, 1741, doi:10.3390/plants10081741 377

Finn Petersen, Johannes Demann, Dina Restemeyer, Hans-Werner Olfs, Heiner Westendarp, Klaus-Juergen Appenroth and Andreas Ulbrich
Influence of Light Intensity and Spectrum on Duckweed Growth and Proteins in a Small-scale, Re-Circulating Indoor Vertical Farm
Reprinted from: *Plants* **2022**, *11*, 1010, doi:10.3390/plants11081010 391

Leone Ermes Romano and Giovanna Aronne
The World Smallest Plants (*Wolffia* Sp.) as Potential Species for Bioregenerative Life Support Systems in Space
Reprinted from: *Plants* **2021**, *10*, 1896, doi:10.3390/plants10091896 407

Elisa Fiordelmondo, Simona Ceschin, Gian Enrico Magi, Francesca Mariotti, Nicolaia Iaffaldano, Livio Galosi and Alessandra Roncarati
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
Reprinted from: *Plants* **2022**, *11*, 1220, doi:10.3390/plants11091220 419

Barbara Demmig-Adams, Marina López-Pozo, Stephanie K. Polutchko, Paul Fourounjian, Jared J. Stewart, Madeleine C. Zenir and William W. Adams III
Growth and Nutritional Quality of Lemnaceae Viewed Comparatively in an Ecological and Evolutionary Context
Reprinted from: Plants **2022**, *11*, 145, doi:10.3390/plants11020145 431

Lei Wang, Yingying Kuang, Siyu Zheng, Yana Tong, Yerong Zhu and Yong Wang
Overexpression of the Phosphoserine Phosphatase-Encoding Gene (AtPSP1) Promotes Starch Accumulation in *Lemna turionifera* 5511 under Sulfur Deficiency
Reprinted from: *Plants* **2023**, *12*, 1012, doi:10.3390/plants12051012 455

5. Interaction with microorganisms

Kenneth Acosta, Shawn Sorrels, William Chrisler, Weijuan Huang, Sarah Gilbert, Thomas Brinkman, Todd P. Michael, Sarah L. Lebeis and Eric Lam
Optimization of Molecular Methods for Detecting Duckweed-Associated Bacteria
Reprinted from: *Plants* **2023**, *12*, 872, doi:10.3390/plants12040872 471

Sarah Gilbert, Alexander Poulev, William Chrisler, Kenneth Acosta, Galya Orr, Sarah Lebeis an Eric Lam
Auxin-Producing Bacteria from Duckweeds Have Different Colonization Patterns and Effects on Plant Morphology
Reprinted from: *Plants* **2022**, *11*, 721, doi:10.3390/plants11060721 497

Chakrit Bunyoo, Peerapat Roongsattham, Sirikorn Khumwan, Juthaporn Phonmakham, Passorn Wonnapinij and Arinthip Thamchaipenet
Dynamic Alteration of Microbial Communities of Duckweeds from Nature to Nutrient-Deficient Condition
Reprinted from: *Plants* **2022**, *11*, 2915, doi:10.3390/plants11212915 513

Martin Schäfer and Shuqing Xu
The Effects of Microbiota on the Herbivory Resistance of the Giant Duckweed Are Plant Genotype-Dependent
Reprinted from: *Plants* **2022**, *11*, 3317, doi:10.3390/plants11233317 527

6. Physiology and phytomonitoring

Magdalena Chmur and Andrzej Bajguz
Brassinolide Enhances the Level of Brassinosteroids, Protein, Pigments, and Monosaccharides in *Wolffia arrhiza* Treated with Brassinazole
Reprinted from: *Plants* **2021**, *10*, 1311, doi.org/10.3390/plants10071311 535

Tatiana A. Kozlova and David B. Levin
Effect of 17-Estradiol on Growth and Biosynthesis of Microalgae *Scenedesmus quadricauda* (CPCC-158) and Duckweed *Lemna minor* (CPCC-490) Grown in Three Different Media
Reprinted from: *Plants* **2022**, *11*, 1669, doi:10.3390/plants11131669 553

Ula Rozman and Gabriela Kalčíková
The Response of Duckweed *Lemna minor* to Microplastics and Its Potential Use as a Bioindicator of Microplastic Pollution
Reprinted from: *Plants* **2022**, *11*, 2953, doi:10.3390/plants11212953 571

Simona Ceschin, Flaminia Mariani, Dario Di Lernia, Iole Venditti, Emanuele Pelella and Maria Adelaide Iannelli
Effects of Microplastic Contamination on the Aquatic Plant *Lemna minuta* (Least Duckweed)
Reprinted from: *Plants* **2023**, *12*, 207, doi:10.3390/plants12010207 583

Viktor Oláh, Anna Hepp, Muhammad Irfan and Ilona Mészáros
Chlorophyll Fluorescence Imaging-Based Duckweed Phenotyping to Assess Acute Phytotoxic Effects
Reprinted from: *Plants* **2021**, *10*, 2763, doi:10.3390/plants10122763 597

Leone Ermes Romano, Maurizio Iovane, Luigi Gennaro Izzo and Giovanna Aronne
A Machine-Learning Method to Assess Growth Patterns in Plants of the Family Lemnaceae
Reprinted from: *Plants* **2022**, *11*, 1910, doi:10.3390/plants11151910 617

Student Spotlight: Yuri Lee

Faculty of Education, Niigata University, Ikarashi-ni-no-cho, Nishi-ku, Niigata 950-2181, Japan

(Email: yuri.lemna@gmail.com)

Yuri Lee, in front of Zurich Herbarium (ZT), Switzerland

The moment that I first recognized duckweed as an "(academic) plant" was when I happened to find a duckweed specimen-box in my supervisor's (Prof. Gyu Young Chung, Andong National University in Korea) room when I was a master's student. The specimen at the top of the box was a *Wolffia sp.*, now that I think about it. At that time, I was very shocked to see these small green dots attached to the sheet and thought "Someone may study these tiny plants. Is it possible? What a challenge…"; but now I'm the one who studies duckweed!

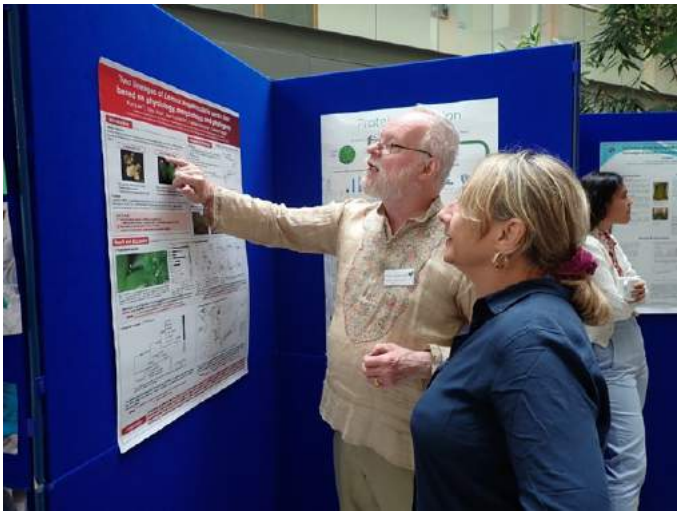
When I was a master's student, I worked in Prof. Chung's laboratory which studies the traditional taxonomy of various species of land plants. I conducted a taxonomic study of the genus *Lindernia* All., which can usually be found in paddy fields. I was the first student who studied wetland plants in Prof. Chung's group. Perhaps because of that, I had a chance to join the aquatic plant survey team of Prof. Takashi Shiga (Niigata University, Japan), an aquatic plant expert, for three weeks in Mongolia, together with Prof. Chung and Prof. Hyeok Jae Choi (Changwon National University, Korea) in 2018. Afterwards, I decided to do my PhD studies at Niigata University in Japan, starting in April 2019. At that time, I had no awareness of aquatic plants and very little experience in molecular analysis, so my new supervisor, Prof. Shiga recommended me to study *Lemna* L. a representative genus of aquatic plants which is suitable to conduct interesting evolutionary and taxonomical studies.

A happy moment collecting duckweeds in Hyogo Prefecture, Japan. Five species (4 genera) of duckweeds were collected from the population. One of them, *L. valdiviana* is kept as NGY189 in Niigata University, because it was my first time collecting *L. valdiviana,* and I was so excited.

During my PhD study, I studied the physiology, morphology, and evolution of native Japanese and Korean *Lemna* species using molecular analyses including direct sequencing and Multiplexed ISSR Genotyping by sequencing (MIG-seq). One of the three topics in my thesis was the study of *Lemna aequinoctialis* Welw., which is widely distributed across the world. I collected 70 strains of *L. aequinoctialis* in 70 localities in Japan, Korea, Thailand. Among them, flowering of 10 *L. aequinoctialis* strains and the famous *L. aequinoctialis* strain 6746 were induced using benzoic acid. At the time when I conducted the study, I really wanted to check strain 6746, and I contacted Prof. Tokitaka Oyama (Kyoto University, Japan) to ask him whether he could provide me with the strain. He kindly shared the strain, such that I could include it in my study! Over 700 flowering fronds were observed in detail, and based on physiological and morphological parameters I hypothesized that there were two groups in *L. aequinoctialis*. Chloroplast DNA-based molecular analysis supported the two groups' independence. I identified them as *L. aequinoctialis sensu* Beppu et al. (1985) and *L. aoukikusa* Beppu & Murata, respectively. Besides *L. aequinoctialis*, flowers of a few other *Lemna* species have still not been reported in detail. Identifying and studying their unknown, and hidden characteristics will be an important objective giving new insights in their lifestyle and evolution.

Due to the COVID-19 situation, that lasted for about 3 years, travelling abroad was almost impossible. However, the end of COVID-19 related travel restrictions finally allowed me to participate in the Duckweed workshop in Ireland in June 2023. At the workshop, I was able to gain new insights by listening to various presentations about the economic use of duckweed which I had not previously known about. Thanks to the host of workshop, Prof. Marcel Jansen (University College Cork, Ireland), I got a chance to show my poster. It was a wonderful experience to hear the opinions of various duckweed experts on my research.

After the workshop, I visited IBBA[1)] in Italy and saw the famous "Landolt Collection" of duckweeds, now maintained by Prof. Laura Morello's research team. We were able to discuss a lot of different topics in *Lemna* research. Afterwards, following Landolt, Welwitsch, and Hegelmaier's footsteps, I visited ZT[2)] and STU[3)] to do a specimen survey. Seeing the duckweed specimens, or living strains, collected by those scientists whom I always admired made me say a prayer of thanks to God for giving me these opportunities. Thankfully, at the end of the European journey, I had the opportunity to visit the laboratory of Prof. Klaus-J Appenroth (Friedrich Schiller University of Jena, Germany) and Prof. Ingo Schubert (IPK[4)]), both of which had bookshelves full of thick folders. I asked Prof. Schubert, "What is all this?" Prof. Schubert smiled and said proudly, "Young researchers like you always wonder what these are. These are references."



Am I dreaming? Prof. Appenroth and Prof. Morello are looking at my poster in the duckweed workshop in Cork, Ireland in June 2023!

I have now met quite a few duckweed researchers with a lot of experience, and they never hesitated to share their knowhow and give advice on research. Talking about duckweed, a common interest, and feeling their passion for research has humbled me and at the same time has made me determined to work even harder on my research. I deeply appreciate all duckweed researchers who kindly gave their precious time to me. Without their excellent research, I would never have carried out my own work.

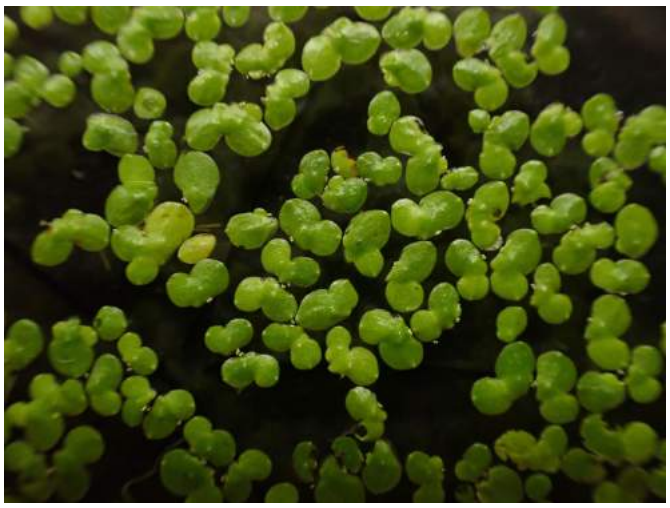
As the first person to study *Lemna* species in Prof. Shiga's laboratory I had to go through numerous basic trials, making errors when establishing axenic cultures or inducing flowering. Everything was new to me. However, after going through a lot of tears, I am currently maintaining more than 80 NGY (Niigata Yuri) axenic duckweed strains collected in Japan and Korea. In March 2023, when I got my PhD degree, my best friend asked me: "Do you love *Lemna*?" I answered: "If I don't love them, I wouldn't do this study." I am so grateful to be able to study *Lemna* species, and I want to keep studying these adorable, cute, tiny plants.

Abbreviation

1) Instituto Biologia e Biotecnologia Agraria, Italy. 2) Herbarium der Eidgenössische Technische Hochschule Zürich, Switzerland. 3) Staatliches Museum für Naturkunde Stuttgart, Germany. 4) Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Germany

Related publication

Beppu T, Yanase D, Nobuchi T, Murata G (1985) A revision of *Lemna paucicostata* group in Japan. Acta Phytotaxon Geobot 36:45–58 [in Japanese].



Flowering *Lemna aequinoctialis*

From the Database

Highlights

Cryopreservation of duckweed genetic diversity as model for long-term preservation of aquatic flowering plants

Peterson, A; Kishchenko, O; Kuhlmann, M; Tschiersch, H; Fuchs, J; Tikhenko, N; Schubert, I; Nagel, M. Plants (2023) 12: DOI10.3390/plants12183302.

Vegetatively propagating aquatic angiosperms, the Lemnaceae family (duckweeds) represents valuable genetic resources for circular bioeconomics and other sustainable applications. Due to extremely fast growth and laborious cultivation of in vitro collections, duckweeds are an urgent subject for cryopreservation. We developed a robust and fast DMSO-free protocol for duckweed cryopreservation by vitrification. A single-use device was designed for sampling of duckweed fronds from donor culture, further spin-drying, and subsequent transferring to cryo-tubes with plant vitrification solution 3 (PVS3). Following cultivation in darkness and applying elevated temperatures during early regrowth stage, a specific pulsed illumination instead of a diurnal regime enabled successful regrowth after the cryopreservation of 21 accessions of *Spirodela*, *Landoltia*, *Lemna*, and *Wolffia* genera, including interspecific hybrids, auto- and allopolyploids. Genome size measurements revealed no quantitative genomic changes potentially caused by cryopreservation. The expression of CBF/DREB1 genes, considered as key factors in the development of freezing tolerance, was studied prior to cooling but was not linked with duckweed regrowth after rewarming. Despite preserving chlorophyll fluorescence after rewarming, the rewarmed fronds demonstrated nearly zero photosynthetic activity, which did not recover. The novel protocol provides the basis for future routine application of cryostorage to duckweed germplasm collections, saving labour for in vitro cultivation and maintaining characterized reference and mutant samples.

Mechanisms of metabolic adaptation in the duckweed *Lemna gibba*: an integrated metabolic, transcriptomic and flux analysis

Shi, H; Ernst, E; Heinzel, N; McCorkle, S; Rolletschek, H; Borisjuk, L; Ortleb, S; Martienssen, R; Shanklin, J; Schwender, J. BMC Plant Biology (2023) 23: 458.

Duckweeds are small, rapidly growing aquatic flowering plants. Due to their ability for biomass production at high rates they represent promising candidates for biofuel feedstocks. Duckweeds are also excellent model organisms because they can be maintained in well-defined liquid media, usually reproduce asexually, and because genomic resources are becoming increasingly available. To demonstrate the utility of duckweed for integrated metabolic studies, we examined the metabolic adaptation of growing *Lemna gibba* cultures to different nutritional conditions. To establish a framework for quantitative metabolic research in duckweeds we derived a central carbon metabolism network model of *Lemna gibba* based on its draft genome. *Lemna gibba* fronds were grown with nitrate or glutamine as nitrogen source. The two conditions were compared by quantification of growth kinetics, metabolite levels, transcript abundance, as well as by ^{13}C-metabolic flux analysis. While growing with glutamine, the fronds grew 1.4 times faster and accumulated more protein and less cell wall components compared to plants grown on nitrate. Characterization of photomixotrophic growth by 13C-metabolic flux analysis showed that, under both metabolic growth conditions, the Calvin-Benson-Bassham cycle and the oxidative pentose-phosphate pathway are highly active, creating a futile cycle with net ATP consumption. Depending on the nitrogen source, substantial reorganization of fluxes around the tricarboxylic acid cycle took place, leading to differential formation of the biosynthetic precursors of the Asp and Gln families of proteinogenic amino acids. Despite the substantial reorganization of fluxes around the tricarboxylic acid cycle, flux changes could largely not be associated with changes in transcripts. Through integrated analysis of growth rate, biomass composition, metabolite levels, and metabolic flux, we show that *Lemna gibba* is an excellent system for quantitative metabolic studies in plants. Our study showed that *Lemna gibba* adjusts to different nitrogen sources by reorganizing central metabolism. The observed disconnect between gene expression regulation and metabolism underscores the importance of metabolic flux analysis as a tool in such studies.

Aquaculture/ Agriculture

Quantitative and qualitative traits of duckweed (*Lemna minor*) produced on growth media with pig slurry

Sonta, M; Wiecek, J; Szara, E; Rekiel, A; Zalewska, A; Batorska, M. Agronomy (2023) 13: 1951.

Duckweed is a plant with high phytoremediation abilities, which is why it is used in the process of cleaning the aquatic environment. The present study aimed to determine the effect of various concentrations of pig slurry added to the growth media used to produce duckweed (*Lemna minor*) (laboratory Warsaw University of Life Sciences-SGGW) (experimental groups 1-9, pig slurry concentration (%): 1-2.00, 2-1.50, 3-1.00, 4-0.75, 5-0.50, 6-0.25, 7-0.12, 8-0.06, 9-0.03, control group 0-0.00). The contents of nutrients in the growth media could be classified as high (gr. 1-3), optimal (gr. 4-6), and deficient (gr. 7-9). Analyses were conducted for duckweed yield and growth medium parameters (pig slurry concentration, pH, salinity, temperature, TDS, and EC) on days 0, 10, 20, and 30 of the experiment. No growth or poor growth of duckweed were noted in groups 1, 6-9, and 0. In turn, satisfactory yields of duckweed green mass were recorded in groups 3-5, which allowed choosing them for further observations and analyses, including proximate composition (including protein content); contents of Ca, Mg, K, Na, Zn, Cu, Cd, Pb, Al, Cr, and α-tocopherol; and β-carotene, α-carotene, violaxanthin, zeaxanthin, lutein, amino acids, fatty acids as well as N-NH_4 and N-NO_3. The plant material had an acceptable proximate composition and nutritionally safe analysed component contents. Appropriate, stable growth medium conditions allowed the production of satisfactory duckweed yields. The study results allowed us to conclude that it is feasible to obtain feed material meeting basic quality standards by maintaining a closed circuit of duckweed culture, and use in the agricultural environment is possible through harnessing pig slurry for its production and ensuring its optimal growth conditions.

Biochemistry

Duckweed pectic-arabinogalactan-proteins can crosslink through borate diester bonds

Tan, L; Ishihara, M; Black, I; Glushka, J; Heiss, C; Azadi, P. Carbohydrate Polymers (2023) 319: 121202.

Material containing pectin and arabinogalactan-protein (AGP) was released and purified from *Spirodela* alcohol insoluble residues. Results of carbohydrate analyses and two-dimensional NMR spectroscopy suggest that this material is composed of apiogalacturonan and rhamnogalacturonan-I covalently attached to AGPs. 11B NMR spectroscopy indicated that some of the glycoses in this complex exist as their boric acid monoesters. Borate diesters were formed when the pectic-AGPs were allowed to react at pH above 6.2 with the boron-depleted pectic-AGPs, suggesting that in vitro two pectic-AGP molecules can crosslink to one another through borate. Borate diesters also formed when the pectic-AGPs were incubated with monomeric rhamnogalacturonan-II in the presence of Pb^{2+} ion at pH 9.2. This data presents evidence of the first wall polymer after rhamnogalacturonan-II to crosslink through borate diesters. We suggest that the formation of these borate-crosslinks may help *Spirodela* respond to high-pH condition.

DF Comment: The authors used *Spirodela polyrhiza* of unknown origin

Biotechnology

High starch duckweed biomass production and its highly-efficient conversion to bioethanol

Guo, L; Fang, Y; Jin, YL; He, KZ; Zhao, H. Environmental Technology and Innovation (2023) 32: 103296.

This paper carried out a new attempt which combined nutrient starvation, uniconazole and elevated CO_2 to increase duckweed biomass and starch content. At laboratory level, the highest growth rate and starch content reached up to 9.4±0.2 g m^{-2} d^{-1} and 75.9± 3.3 %, leading to an estimated starch yield of 30.8 t ha^{-1} y^{-1}. Furthermore, without any pretreatment, up to 88.4±0.4 % of glucose from high starch duckweed biomass can be released. The enzymatic hydrolyzates can be efficiently converted to bioethanol by yeast and the ethanol yield based on duckweed biomass input (YE/B) reached up to 0.38±0.01 g g^{-1} applying simultaneous saccharification and fermentation (SSF), which, to our knowledge, is the highest duckweed-to-ethanol yield thus far reported. Based on the data above-mentioned, an annual duckweed-based ethanol yield of 16.0 x 10^3 L ha^{-1} can be achieved, about 2.6 times higher than maize-based ethanol yield. This approach has achieved quick high starch accumulation in duckweed and demonstrated that duckweed can serve as a competitive starch feedstock for bioethanol production.

Duckweed derived N, P-doped carbon microspheres supported Ru as highly active catalyst for electrocatalytic hydrodechlorination diclofenac

Wang, SX; Yang, MD; Cui, CY; Zheng, QZ; Zhou, CZ; Xin, YJ. Electrochimica Acta (2023) 462: 142707.

In order to improve degradation efficiency and reduce electrode cost, carbon microspheres (CM) particle electrode was prepared from readily available duckweed as a raw material for electrocatalytic hydrodechlorination. The surface structures and chemical characteristics of the CM were regulated by adjusting the pyrolysis temperature. When pyrolysis was performed at 650 °C, carbon microspheres (CM-650) with large surface areas that are rich in heteroatoms (N, P) and high electrical conductivity are obtained. After the Ru catalyst was supported, the Ru/CM-650 as particle electrode forming a three-dimensional (3D) electrochemical reaction system exhibits high electrocatalytic performance for diclofenac (DCF) hydrodechlorination with dechlorination efficiency of more than 90% in 150 min. Furthermore, the Ru/CM-650 has good stability for repeated use, which is attributed to the synergistic interaction between N and P elements improving the stability of the catalyst.

Optimization of water lentil (duckweed) leaf protein purification: Identification, structure, and foaming properties

Muller, T; Bernier, ME; Bazinet, L. Foods (2023) 12: DOI10.3390/foods12183424.

Water lentil, commonly known as duckweed, is an aquatic plant with great agronomic potential, as it can double its biomass in less than 24 h and contains up to 45% leaf proteins on a dry matter basis. However, extracting proteins from leaves is an arduous process due to the complexity of the matrix, which limits their uses in the food industry. In this study, water lentil protein extraction by solubilisation was maximized using response surface methodology. By heating at 80°C at pH 11 with a water lentil powder concentration of 2% or 4% for 2 h, up to 77.8% of total proteins were solubilized. Then, by precipitating the solubilized proteins at pH 4, a protein purity of 57.6% combined with a total protein yield of 60.0% was achieved. To the best of our knowledge, this is the highest leaf protein extraction yield reported in the literature with such protein purity. Proteomics analyses showed that the protein concentrate was composed of around 85.0% RubisCO, and protein structure analyses using ATR-FTIR and DSC were linked to a high protein solubility in water at pH 7. Moreover, a 1.5% protein solution of the protein concentrate at pH 7 showed excellent foaming properties compared to a 10.3% protein egg white solution. It had a superior foaming capacity (194% vs. 122%, respectively) for the same foaming stability after 60 min, which confirms water lentil proteins' potential for human nutrition and food formulation.

Optimum aquaculture and drying conditions for *Wolffia arrhiza* (L.) Wimn.

Prosridee, K; Oonsivilai, R; Tira-Aumphon, A; Singthong, J; Oonmetta-Aree, J; Oonsivilai, A. Heliyon (2023) 9: e19730.

This study aimed to investigate the effects of aquaculture and the optimum conditions for drying duckweed plants to maintain the highest nutritional value and bioactive substances. Protein quantification was used to screen duckweed plants subjected to the 14 treatments under aquaculture conditions. Proximate analysis of three aquaculture conditions showed the highest quantification of protein. Moreover, these samples were analysed for total phenolics, flavonoids, and chlorophylls. The optimal drying conditions for duckweed plants with the highest protein content were determined using a factorial design with three temperature and time parameters. The results showed that the duckweed under aquaculture conditions in an outdoor cement pond with hydroponic electrical conductivity (EC) of 0.5mS/cm contained the highest protein at 41.81±3.40%. Moreover, proximate analysis of this sample showed fat, fibre, moisture, ash, and carbohydrate contents of 1.99±0.08%, 4.46±0.71%, 3.29±0.17%, 22.06±0.07% and 14.12±1.63%, respectively. In addition, the optimum drying conditions for this sample were 50°C and a drying time of 6h. Under optimum drying conditions, this sample showed total phenolics, flavonoids, and chlorophylls contents of 55.28±1.35 (µg GAE/g dry weight), 159.84±6.65 (µg catechin equivalent [QE]/g dry weight) and 22.91±0.15 (mg/g dry weight), respectively. In conclusion, the dried duckweed under aquaculture conditions in an outdoor cement pond with hydroponic EC 0.5mS/cm contained the highest contents of proteins, total phenolics, total flavonoids, and total chlorophyll, which could be used as functional ingredients in health food products.

DF Comment: The correct term is *Wolffia arrhiza* (L.) Horkel ex Wimm.

Ecology

Core-shell Au@Nanoplastics as a quantitative tracer to investigate the bioaccumulation of nanoplastics in freshwater ecosystems

Fu, SF; Feng, LJ; Zhu, HL; Zhang, Y; Yuan, XZ; Zou, H; Guo, RB. Analytical Chemsitry (2023) DOI10.1021/acs.analchem.3c01666.

Studies on the adverse effects of nanoplastics (NPs,particle diameter <1000 nm) including physical damage, oxidative stress, impaired cell signalling, altered metabolism, developmental defects, and possible genetic damage have intensified in recent years. However, the analytical detection of NPs is still a bottleneck. To overcome this bottleneck and obtain a reliable and quantitative distribution analysis in complex freshwater ecosystems, an easily applicable NP tracer to simulate their fate and behaviour is needed. Here, size- and surface charge-tuneable core-shell Au@Nanoplastics (Au@NPs) were synthesized to study the environmental fate of NPs in an artificial freshwater system. The Au core enables the quantitative detection of NPs, while the polystyrene shell exhibits NP properties. The Au@NPs showed excellent resistance to environmental factors (e.g., 1% hydrogen peroxide solution, simulating gastric fluid, acids, and alkalis) and high recovery rates (>80%) from seawater, lake water, sewage, waste sludge, soil, and sediment. Both positively and negatively charged NPs significantly inhibited the growth of duckweed (*Lemna minor* L.) but had little effect on the growth of cyanobacteria (*Microcystis aeruginosa*). In addition, the accumulation of positively and negatively charged Ps in cyanobacteria occurred in a concentration-dependent manner, with positively charged NPs more easily taken up by cyanobacteria. In contrast, negatively charged NPs were more readily internalized in duckweed. This study developed a model using a core-shell Au@NP tracer to study the environmental fate and behaviour of NPs in various complex environmental systems.

Lemnaceae as novel crop candidates for CO_2 sequestration and additional applications

López-Pozo, M; Adams, WW III; Demmig-Adams, B. Plants (2023) 12: 3090.

Atmospheric carbon dioxide (CO_2) is projected to be twice as high as the pre-industrial level by 2050. This review briefly highlights key responses of terrestrial plants to elevated CO_2 and compares these with the responses of aquatic floating plants of the family Lemnaceae (duckweeds). Duckweeds are efficient at removing CO_2 from the atmosphere, which we discuss in the context of their exceptionally high growth rates and capacity for starch storage in green tissue. In contrast to cultivation of terrestrial crops, duckweeds do not

contribute to CO_2 release from soils. We briefly review how this potential for contributions to stabilizing atmospheric CO_2 levels is paired with multiple additional applications and services of duckweeds. These additional roles include wastewater phytoremediation, feedstock for biofuel production, and superior nutritional quality (for humans and livestock), while requiring minimal space and input of light and fertilizer. We, furthermore, elaborate on other environmental factors, such as nutrient availability, light supply, and the presence of a microbiome, that impact the response of duckweed to elevated CO_2. Under a combination of elevated CO_2 with low nutrient availability and moderate light supply, duckweeds' microbiome helps maintain CO_2 sequestration and relative growth rate. When incident light intensity increases (in the presence of elevated CO_2), the microbiome minimizes negative feedback on photosynthesis from increased sugar accumulation. In addition, duckweed shows a clear propensity for absorption of ammonium over nitrate, accepting ammonium from their endogenous N_2-fixing Rhizobium symbionts, and production of large amounts of vegetative storage protein. Finally, cultivation of duckweed could be further optimized using hydroponic vertical farms where nutrients and water are recirculated, saving both resources, space, and energy to produce high-value products.

Feed & Food

Wolffia globosa-based nutritious snack formulation with high protein and dietary fiber contents

On-Nom, N; Promdang, P; Inthachat, W; Kanoongon, P; Sahasakul, Y; Chupeerach, C; Suttisansanee, U; Temviriyanukul, P. Foods (2023) 12: 2647.

Wolffia globosa (*W. globosa*) or duckweed is a small aquatic plant with high protein, dietary fibre, and lipid contents that can be combined with food products to develop nutritious snacks as one strategy to mitigate malnutrition. Here, response surface methodology (RSM) with mixture design was used to develop snacks from *W. globosa* freeze-dried powder (WP). The physical properties, proximate analysis, amino acid profiles, sensory evaluation, phytochemical analysis, antioxidant properties, and genotoxicity (Ames test) of the snacks were evaluated. The optimal *W. globosa* snack formula was 64% glutinous rice flour, 10% tapioca flour, and 26% WP, giving a highly desirable liking score of 1.00. Addition of WP increased crude protein, essential amino acids, and dietary fibre compared with the control snack by 51%, 147%, and 83%, respectively. According to the Thai recommended daily intakes, the developed *W. globosa* snack had high protein and dietary fibre. Phytochemical contents and antioxidant activities of the *W. globosa* snack such as total phenolic contents (TPCs), total flavonoid contents (TFCs), ferric ion reducing antioxidant power (FRAP) activity, and oxygen radical absorbance capacity (ORAC) activity were significantly higher than the control snack. The novel combination of WP with snack product ingredients greatly enhanced nutritional value.

Effect of duckweed (*Spirodela polyrhiza*)-supplemented semolina on the production parameters and nutrient composition of Yellow Mealworm (*Tenebrio molitor*)

Toviho, OA; Imane, M; Tuende, P; Péter, B. Agriculture (2023) 13: 1386.

This research explores the potential of using common duckweed (*Spirodela polyrhiza*) as a feeding substrate and supplement for yellow mealworm production. Duckweed is known for its high content of protein (20-35%) and essential amino acids. This study aims to assess the effect of the combination of semolina and duckweed as a feeding substrate for yellow mealworm larvae (*Tenebrio molitor*). The experiment involved different combinations of semolina and duckweed in varying proportions. The treatments included 100% semolina (S), 75% semolina + 25% duckweed (S75D25), 50% semolina + 50% duckweed (S50D50), 25% semolina + 75% duckweed (S25D75), and 100% duckweed (D). Over a six-week period, the production parameters, nutrient composition, amino acid composition, and fatty acid composition of the yellow mealworms were measured and analysed. The results demonstrate that S75D25 and S50D50 feed combinations were recommended due to their positive effects on production parameters and nutrient composition. Although the D substrate exhibited the highest crude protein content, yellow mealworm larvae did not grow on this substrate. The inclusion of duckweed in the feed had no significant effect on the fatty acid composition of the mealworms,

while substrates S25D75 and D induced an improved amino acid composition. In conclusion, incorporating duckweed into the feeding substrate can enhance the production parameters and nutrient composition of yellow mealworms.

Effects of humic acid on nutrient removal efficiency of aquatic duckweed (*Lemna minor*) and both growth performance, and hemato-biochemical parameters of Nile tilapia (*Oreochromis niloticus*) cultured in water recirculating system

Sarkheil, M; Zahedi, S; Safari, O; Ahmadniaye Motlagh, H. International Journal of Phytoremediation (2023) DOI10.1080/15226514.2023.2250459.

This study was carried out to evaluate the effects of humic acid (HA) on the nutrient removal efficiencies of aquatic duckweed plant (*Lemna minor*) from a water recirculating system used to culture Nile tilapia (*Oreochromis niloticus*) fish for 30 days. The HA was added to water at three concentrations of 0 (Control), 1.5, and 3 mg/L in triplicate. Water quality parameters, growth performance, and some hemato-biochemical parameters of the fish in variable HA concentrations were compared. The total ammonia nitrogen (TAN) and total phosphorous (TP) removal efficiency of *L. minor* increased with increasing the HA concentration from 0 mg/L to 3 mg/L ($p<0.05$). The concentration of nitrate (NO_3^-) in the HA-3 mg/L was higher than that in the other groups on days 20 and 30 of the fish cultivation period ($p<0.05$). The growth performance of fish improved in the HA-3 mg/L compared to the other groups. The addition of different concentrations of HA to water had no adverse effect on the hematological properties of the Nile tilapia. The plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels in the HA-0 mg/L and HA-1.5 mg/L groups were higher than in the HA-3 mg/L ($p<0.05$). No significant differences in the plasma glucose and cholesterol levels were observed between the HA-groups ($p>0.05$), while the triglyceride level increased in the HA-3 mg/L compared to the control ($p<0.05$). These results indicated that adding HA to water could be an effective method to enhance the bioremediation performance of the aquatic duckweed plants as biofilter and thus improve water quality, subsequently, fish growth performance in RASs.

General articles

Duckweed: Research Meets Applications

Olah, V; Appenroth, KJ; Sree, KS. Plants 12: DOI10.3390/plants12183307.

Editorial: The Special Issue "Duckweed: Research Meets Applications" of the journal Plants (ISSN 2223-7747) presents a comprehensive update of the current progress in the field [...].

Interaction with other organisms

Evolutionary consequences of microbiomes for hosts: impacts on host fitness, traits, and heritability

O'Brien, AM; Laurich, JR; Frederickson, ME. Evolution (2023) DOI10.1093/evolut/qpad183.

An organism's phenotypes and fitness often depend on interactive effects of its genome (Ghost), microbiome (Gmicrobe), and environment (E). These GxG, GxE, and GxGxE effects fundamentally shape host-microbiome (co)evolution and may be widespread, but are rarely compared within a single experiment. We collected and cultured *Lemna minor* (duckweed) and its associated microbiome from 10 sites across an urban-to-rural ecotone. We factorially manipulated host genotype and microbiome in two environments (low and high zinc, an urban aquatic stressor) in an experiment with 200 treatments: 10 host genotypes * 10 microbiomes * 2 environments. Host genotype explained the most variation in *L. minor* fitness and traits, while microbiome effects often depended on host genotype (GxG). Microbiome composition predicted GxG effects: when

compared in more similar microbiomes, duckweed genotypes had more similar effects on traits. Further, host fitness increased and microbes grew faster when applied microbiomes more closely matched the host's field microbiome, suggesting some local adaptation between hosts and microbiota. Finally, selection on and heritability of host traits shifted across microbiomes and zinc exposure. Thus, we found that microbiomes impact host fitness, trait expression, and heritability, with implications for host-microbiome evolution and microbiome breeding.

Environment and host genetics influence the biogeography of plant microbiome structure

Wei, N; Tan, J. Microbial Ecology (2023) DOI10.1007/s00248-023-02288-6.

To understand how microbiota influence plant populations in nature, it is important to examine the biogeographic distribution of plant-associated microbiomes and the underlying mechanisms. However, we currently lack a fundamental understanding of the biogeography of plant microbiomes across populations and the environmental and host genetic factors that shape their distribution. Leveraging the broad distribution and extensive genetic variation in duckweeds (the *Lemna* species complex), we identified key factors that governed plant microbiome diversity and compositional variation geographically. In line with the microbial biogeography of free-living microbiomes, we observed higher bacterial richness in temperate regions relative to lower latitudes in duckweed microbiomes (with 10% higher in temperate populations). Our analyses revealed that higher temperature and sodium concentration in aquatic environments showed a negative impact on duckweed bacterial richness, whereas temperature, precipitation, pH, and concentrations of phosphorus and calcium, along with duckweed genetic variation, influenced the biogeographic variation of duckweed bacterial community composition. Analyses of plant microbiome assembly processes further revealed that niche-based selection played an important role (26%) in driving the biogeographic variation of duckweed bacterial communities, alongside the contributions of dispersal limitation (33%) and drift (39%). These findings add significantly to our understanding of host-associated microbial biogeography and provide important insights for predicting plant microbiome vulnerability and resilience under changing climates and intensifying anthropogenic activities.

Pseudomonas stutzeri improves the tolerance of *Lemna minor* to $Cu(OH)_2$ nanopesticide by regulating the uptake of copper, antioxidant defense mechanisms, and the expression of metacaspase-1, chlorophyllase, and stress-responsive genes

Gulmez, O; Aksakal, O; Baris, O; Bayram, E. Plant Physiology and Biochemistry (2023) 203: 108002.

This study investigated the effect of *Pseudomonas stutzeri* inoculation on *Lemna minor* treated with $Cu(OH)_2$ nanopesticide (NP). The results showed that *P. stutzeri* inoculation increased the relative growth rate (RGR) in NP-treated plants. Although chlorophyll and carotenoid contents decreased significantly in NP-treated plants, *P. stutzeri* inoculation led to an increase in chlorophyll and carotenoid contents in NP-treated plants. Copper (Cu) content increased with increasing NP concentration, but it decreased significantly in the presence of *P. stutzeri*. NP treatment caused increased H_2O_2 and TBARS levels, as well as proline levels. However, *P. stutzeri* inoculation led to decreased H_2O_2 and TBARS levels and increased SOD, POX, GST, GR, GPX, and DHAR activities. The expression of genes encoding SOD, GST, metacaspase-1, and chlorophyllase was upregulated by NP treatment alone. Additionally, when plants were inoculated with P. *stutzeri, the expression of these genes was further enhanced. In conclusion, P. stutzeri* inoculation had a positive effect on the growth and antioxidant system of *L. minor* treated with NP as it enhanced RGR, increased chlorophyll and carotenoid contents, and decreased Cu content and oxidative stress. These findings suggested that *P. stutzeri* has the potential to promote aquatic plant growth and counteract the negative impacts of NP on these plants.

Physiological response, microbial diversity characterization, and endophytic bacteria isolation of duckweed under cadmium stress

Yang, X; Tan, AJ; Zheng, MM; Feng, D; Mao, K; Yang, GL. Science of the Total Environment (2023) 902: 166056.

Duckweed is a cadmium (Cd) hyperaccumulator. However, its enrichment characteristics and physiological responses to Cd have not been systematically studied. The physiological responses, enrichment characteristics, diversity of endophytic bacterial communities, and isolation of Cd-resistant endophytes in duckweed (*Lemna minor* 0014) were studied for different durations and Cd concentrations. The results indicated that peroxidase (POD) and catalase (CAT) activities decreased while superoxide dismutase activity first increased and then decreased with increasing Cd stress duration. POD activities, CAT activities, and $O2^-$ increased as Cd concentrations increased. Malondialdehyde content and Cd accumulation in duckweed increased with increasing concentrations and time. This endophytic diversity study identified 488 operational taxonomic units, with the dominant groups being Proteobacteria, Firmicutes, and Actinobacteria. *Paenibacillus* sp. Y11, a strain tolerant to high concentrations of Cd and capable of significantly promoting duckweed growth, was isolated from the plant. Our study revealed the effects of heavy metals on aquatic plants, providing a theoretical basis for the application of duckweed in water pollution.

Plant neopolyploidy and genetic background differentiate the microbiome of duckweed across a variety of natural freshwater sources

Anneberg, TJ; Turcotte, MM; Ashman, TL. Molecular Ecology (2023) DOI10.1111/mec.17142.

Whole-genome duplication has long been appreciated for its role in driving phenotypic novelty in plants, often altering the way organisms interface with the abiotic environment. Only recently, however, have we begun to investigate how polyploidy influences interactions of plants with other species, despite the biotic niche being predicted as one of the main determinants of polyploid establishment. Nevertheless, we lack information about how polyploidy affects the diversity and composition of the microbial taxa that colonize plants, and whether this is genotype-dependent and repeatable across natural environments. This information is a first step towards understanding whether the microbiome contributes to polyploid establishment. We, thus, tested the immediate effect of polyploidy on the diversity and composition of the bacterial microbiome of the aquatic plant *Spirodela polyrhiza* using four pairs of diploids and synthetic autotetraploids. Under controlled conditions, axenic plants were inoculated with pond waters collected from 10 field sites across a broad environmental gradient. Autotetraploids hosted 4%-11% greater bacterial taxonomic and phylogenetic diversity than their diploid progenitors. Polyploidy, along with its interactions with the inoculum source and genetic lineage, collectively explained 7% of the total variation in microbiome composition. Furthermore, polyploidy broadened the core microbiome, with autotetraploids having 15 unique bacterial taxa in addition to the 55 they shared with diploids. Our results show that whole-genome duplication directly leads to novelty in the plant microbiome and importantly that the effect is dependent on the genetic ancestry of the polyploid and generalizable over many environmental contexts.

Molecular Biology

Environmental and genetic variation in an asexual plant

Jewell, MD; Bell, G. Aquatic Botany (2023) 188: 103675.

Species may respond to variation in environmental conditions by modifying their phenotype to match local levels of resource availability. This phenotypic response can be driven by plastic physiological change, or by adaptive genetic change. Here we use *Lemna minor* (lesser duckweed), a small aquatic macrophyte that is increasingly used as a model in ecology and evolution, to investigate the source and maintenance of phenotypic variation in natural environments. We found substantial phenotypic variation in *L. minor* in the field, with its frond area and root length changing predictably over natural environmental gradients of resource availability. Separating environmental and genetic variation in these traits in a common garden, we attribute the majority of phenotypic variation we observed in the field to phenotypic plasticity. Despite this, there was substantial within-site genetic variation. We found evidence of strong purifying selection in the field, that is

necessarily balanced by mutation and migration. Using measures of environmental and genetic variation in phenotype and fitness, we estimate the rates of dispersal and evolution of fitness necessary to sustain the observed levels of genetic variation.

Physiology & Stress

Open-source workflow design and management software to interrogate duckweed growth conditions and stress responses

Scott, M; de Lange, O; Quaranto, X; Cardiff, R; Klavins, E. Plant Methods (2023) 19: 95.

Duckweeds, a family of floating aquatic plants, are ideal model plants for laboratory experiments because they are small, easy to cultivate, and reproduce quickly. Duckweed cultivation, for the purposes of scientific research, requires that lineages are maintained as continuous populations of asexually propagating fronds, so research teams need to develop optimized cultivation conditions and coordinate maintenance tasks for duckweed stocks. Additionally, computational image analysis is proving to be a powerful duckweed research tool, but researchers lack software tools to assist with data collection and storage in a way that can feed into scripted data analysis. We set out to support these processes using a laboratory management software called Aquarium, an open-source application developed to manage laboratory inventory and plan experiments. We developed a suite of duckweed cultivation and experimentation operation types in Aquarium, which we then integrated with novel data analysis scripts. We then demonstrated the efficacy of our system with a series of image-based growth assays, and explored how our framework could be used to develop optimized cultivation protocols. We discuss the unexpected advantages and the limitations of this approach, suggesting areas for future software tool development. In its current state, our approach helps to bridge the gap between laboratory implementation and data analytical software for duckweed biologists and builds a foundation for future development of end-to-end computational tools in plant science.

Differential morpho-physiological and biochemical responses of duckweed clones from Saudi Arabia to salinity

Al-Dakhil, M; Ben Romdhane, W; Alghamdi, S; Ali, AAM. Plants (2023) 12: 3206.

Salinity affects the morphological, physiological, and biochemical characteristics of several plant species. The current study was conducted to investigate differential salt tolerance potentials among ten duckweed clones under different salt-stress conditions. Morphological and physiological parameters, including fronds length, fronds number, root length, root number, Na^+/K^+, chlorophyll, proline contents, and fresh harvest weight, were recorded for each of the ten duckweed clones collected from different Saudi Arabia regions. Additionally, the expression patterns of seven salt-related genes were monitored in a salt-tolerant duckweed genotype. The results show that the Madinah-2 (*Spirodela polyrhiza*) and Al-Qassim (*Landoltia punctata*) clones presented higher performances for all the tested morphological and physiological parameters compared to other genotypes under salt-stress conditions. At concentrations greater than 150 mM NaCl, these aforementioned traits were affected for all the genotypes tested, except Madinah-2 (*S. polyrhiza*) and Al-Qassim (*L. punctata*) clones, both of which exhibited high tolerance behaviour under high salt conditions (200 mM and 250 mM NaCl). The principal component analysis (PCA) showed that the first five principal components accounted for 94.8% of the total variance among the studied traits. Morphological and physiological traits are the major portions of PC1. Moreover, the expression pattern analysis of NHX, BZIP, ST, and KTrans transcript revealed their upregulation in the Al-Qassim clone under salt-stress conditions, suggesting that these genes play a role in this clone's tolerance to salt-induced stress. Overall, this study indicates that the Al-Qassim clone could be used in a brackish-water duckweed-based treatment program with a simultaneous provision of valuable plant biomass.

DF Comment: The correct term is *Spirodela polyrhiza*

Interactive effects of immediate and ancestral salt stress on fitness in duckweed

Chmilar, SL; Zink, L; Laird, RA. International Journal of Plant Sciences (2023) DOI10.1086/726083.

Organisms that reproduce asexually must respond to abiotic stresses in their environment while contending with the potential disadvantage of the reduced ability for genetic variation through recombination. Common duckweed (*Lemna minor*) is an aquatic plant that reproduces predominantly asexually through the budding of ramets. As a freshwater plant, duckweed experiences stress from salt (e.g., NaCl), which detrimentally affects growth, photosynthesis, and cellular processes. We took a demographic approach to examine whether ancestral exposure to salt stress affects the ability of duckweed offspring to tolerate immediate exposure to the same stress. We placed three consecutive clonal generations of duckweed in an environment with 2 g L^{-1} NaCl (nonlethal salt stress) or 0 g L^{-1} NaCl (control), followed by an additional zero to three generations in the control environment to vary the presence and schedule of ancestral stress. After these treatments, we used the offspring produced as focal plants for the experiment. Half were placed into the salt stress environment, and half were placed in control conditions; focal plants were tracked longitudinally. Immediate stress decreased fitness as a result of a slowing of reproduction, suggesting a cost of stress tolerance. The effects of ancestral stress, and the interaction of immediate and ancestral stress, were more complex. Specifically, recent ancestral stress induced plants to produce offspring more quickly but with the potential cost of lowered offspring quality. Our results amplify findings that responses to, and fitness consequences of, current stressors can be contingent on past exposure.

Metabolomic analysis reveals responses of *Spirodela polyrhiza* L. to salt stress

Hui, TY; Zhang, YM; Jia, R; Hu, Y; Wang, WJ; Wang, Y; Wang, Y; Zhu, YR; Yang, L; Xiang, BB. Journal of Plant Interactions (2023) 18: 2210163.

Duckweeds are particularly well suited for studies needing high output because of their quick growth and reproduction. Due to their starches and flavonoids, the potential for using duckweed as a source of food, medicine or feed has been studied. Through LC-MS analysis, 195 differential metabolites, including organic acids, phenolics, and other categories, were screened in *Spirodela polyrhiza* L. exposed to 4 days of NaCl. *S. polyrhiza* contained higher amounts of oxidized glutathione, jasmonates, and phenolic compounds but lower amounts of citric acid and glutathione under salt stress. Additionally, after 4 days of salt stress, there was an increase in the relative expression levels of genes involved in the phenolic biosynthesis pathway. Based on metabolomics, this study provides insight into the mechanism of salt-tolerant by duckweeds, as well as useful information for plant breeders and molecular biologists developing duckweed cultivars with salt tolerance and high phenolic content.

Phytomedicine

Anti-inflammatory effects of *Spirodela polyrhiza* (L.) SCHLEID. extract on contact dermatitis in mice - Its active compounds and molecular targets

Kim, K; Lee, D; Kim, HY; Kim, S; Lyu, JH; Park, S; Park, YC; Kim, H. International Journal of Molecular Sciences (2023) 24: 13271.

Spirodela polyrhiza (L.) SCHLEID. has been used to treat epidemic fever, dysuria, and various skin ailments, such as measles eruptions, eczema, and pruritus, in China, Japan, and Korea. In this study, the active compounds in *S. polyrhiza* and their target genes were identified by network-based analysis. Moreover, the study evaluated the effects of a 70% ethanolic extract of *S. polyrhiza* (EESP) on skin lesions, histopathological changes, inflammatory cytokines, and chemokines in mice with contact dermatitis (CD) induced by 1-fluoro-2,4-dinitrobenzene (DNFB), and examined the inhibitory effects of EESP on mitogen-activated protein kinase (MAPK) signalling pathways. In our results, 14 active compounds and 29 CD-related target genes were identified. Among them, tumour necrosis factor (TNF) and interleukin 6 (IL-6) were identified as hub genes, and

luteolin and apigenin showed a strong binding affinity with TNF (<-8 kcal/mol) and IL-6 (<-6 kcal/mol). Our in vivo studies showed that topical EESP ameliorated DNFB-induced skin lesions and histopathological abnormalities, and reduced the levels of TNF-a, interferon (IFN)-G, IL-6, and monocyte chemotactic protein (MCP)-1 in inflamed tissues. In conclusion, our findings suggest the potential for dermatological applications of *S. polyrhiza* and suggest that its anti-dermatitis action is related to the inhibition of TNF and IL-6 by luteolin and luteolin glycosides.

Phenolic changes in a combined herbal extract of *Lithospermum erythrorhizon*, *Houttuynia cordata*, and *Spirodela polyrhiza* and alleviation of DNCB-induced atopic dermatitis in BALB/c mice

Choi, GY; Kim, KJ; Park, HS; Hwang, ES; Cho, JM; Kim, HB; Kim, DO; Park, JH. Food Science and Biotechnology (2023) DOI10.1007/s10068-023-01329-7.

Atopic dermatitis (AD) is an inflammatory skin disease showing skin barrier dysfunction, eczematous lesions, severe itching, and abnormal immune responses. The aim of this study was to determine whether an herb combination of *Lithospermum erythrorhizon* (LE), *Houttuynia cordata* (HC), and *Spirodela polyrhiza* (SP) has a superior anti-AD effect. Forty-two compounds were identified in LE, HC, SP, and a combined herb extract of LE, HC, and SP (LHS) using ultra-high-pressure liquid chromatography (UHPLC)-Orbitrap mass spectrometer (MS). The concentration of flavonoid glycosides including orientin (luteolin-8-C-glucoside), quercetin-3-O-rhamnoside, and luteolin-7-O-glucoside in the LHS was increased than in individual extracts. Furthermore, the treatment of LHS most effectively inhibited the increase of epidermal thickness, the number of mast cells, and the release of immunoglobulin E compared with that with each extract. These results suggest that the potential anti-AD effects of the LHS are due to the changes of bioactive compounds by the combination of herbs.

Phytoremediation

Oxalic acid-assisted phytoextraction of heavy metal contaminated wastewater through *Lemna minor* L.

Hayat, U; Abbas, S. Environmental Science and Pollution Research (2023) DOI10.1007/s11356-023-29547-w.

The present research was carried out to investigate the performance of oxalic acid (OA) through *Lemna minor* L. (duckweed) in the phytoextraction of lead (Pb) from metal contaminated water. Zero, 100 µM, 250 µM, and 500 µM Pb concentration and combinations with 2.5 ml of OA were provided to the plants in the form of solution after defining intervals. Continuous aeration was provided to the plants and kept a pH level at 6.5. Results from this research depicted that increasing concentration level of Pb inhibited the overall plant growth, biomass, frond area, chlorophyll, and antioxidant enzyme activities like peroxidase (POD), superoxide-dismutase (SOD), catalases (CAT), and ascorbate-peroxidase (APX). Moreover, Pb stress enhances the concentration, hydrogen peroxide, malondialdehyde, and electrolyte leakage substances in plants. Furthermore, the addition of OA alleviated the Pb-induced toxicity in the plants, increasing the Pb accumulation and its endorsement in the *L. minor*. The OA addition increased the Pb accumulation in plants at 0, 100, 250 and 500 µM. At higher concentration, Pb showed harmful effect as related to the other low doses. Under the application of OA, Pb higher accumulation and concentration in *L. minor* were measured, which showed that it could be the most suitable plant for the phytoextraction of lead-contaminated soil and wastewater.

Degradation of emerging pharmaceutical pollutants from wastewater using *Penicillium aurantiogriseum* 2AJS

Suresh, A; Abraham, J. Applied Biochemistry and Biotechnology (2023) DOI10.1007/s12010-023-04653-0.

Approximately 3000 pharmaceutical compounds and personal care products (PPCPs) are utilized and discharged into the wastewater at low levels, and they are rarely removed or treated in wastewater treatment

facilities. The present study focused on the potential ability of *Penicillium aurantiogriseum* 2AJS to degrade pharmaceutical and personal care products of different classes of drugs: antipyretic and analgesic drugs (paracetamol, diclofenac, and ibuprofen) and hormones (estrogen, progesterone, and testosterone). Various ligninolytic extracellular enzymatic studies were also studied. A phytotoxicity assay was performed using the *Lemna minor* species procured from the Vellore Institute of Technology, Vellore. The results revealed degradation of pharmaceutical and personal care products to 95.27% (paracetamol), 94.37% (diclofenac), 89.29% (ibuprofen), 94.16% (progesterone), 91.10% (estrogen), and 82.12% (testosterone). GC-MS and NMR analyses aided in proposing the degradation pathway of all six pharmaceutical compounds. Degradation kinetics showed a first-order model for all the degradation studies with R2 values ranging between 0.89 and 0.95. A toxicological assay using *Lemna minor* showed very less toxicity of degraded compounds with a toxicity index ranging between 1.2 and 1.5 compared to the parent compounds. Hence, strain 2AJS can be used in in situ bioremediation of wastewater treatment processes.

Using wastewater treatment performance, biomass and physiological plant characteristics for selection of a floating macrophyte for phytoremediation of swine wastewater through the integrative Entropy-Fuzzy AHP-TOPSIS method

Coimbra, ECL; Borges, AC; Mounteer, AH; Rosa, AP. Journal of Eater Process Engineering (2023) 53: 103793.

Phytoremediation success depends on the selection of suitable plant species. To this end, the multiple-attribute decision-making process (MADM) is a useful tool for selecting plants to remediate wastewater. Here, macrophytes were used to treat synthetic swine wastewater (SSW) and the best plant alternative for phytoremediation of SSW was chosen based on the hybrid Entropy-Fuzzy AHP TOPSIS MADM method. The attributes used to feed the MADM method were treatment performance, physiological endpoint changes and biomass by-products. Macrophyte-treated wastewater achieved higher pollutants removal than controls. *Lemna*, *Landoltia*, *Salvinia*, *Azolla* and Control units had removals of 81 %, 79 %, 68 %, 81 %, 25 % for tCOD; 66 %, 71 %, 65 %, 68 %, 8 % for P-PO_4^{3-}; 51 %, 63 %, 40 %, 66 %, 14 % for N-NO_3^-; 36 %, 36 %; 26 %; 42 %; 7 % for N-NH_4^+; 51 %, 40 %, 52 %, 63 %, 3 % for Cu; and 25 %, 18 %, 26 %, 42 %, 14 % for Zn, respectively. Furthermore, *Azolla* and *Lemna* removed more SSW toxicity and presented the lowest phyto-stress index (0.09) and highest lipids production (14 % dw) during treatment. *Azolla* proved to be the best option for phytoremediation of swine wastewater, since it had the highest rank as determined by the proposed method. Acute toxic units of the treated effluent (0.38) and phyto-stress index (0.49) had the greatest weight in choosing the best plant while the production of biomass volatile solids and removals of P-PO_4^{3-} and tCOD were the least influential. This study provided a reliable approach to select the best plant from available macrophytes capable of improving physicochemical and ecotoxicological quality of wastewater and offer value-added plant biomass products.

Phytotoxicity

Giant Duckweed (*Spirodela polyrhiza*) root growth as a simple and sensitive indicator of copper and chromium contamination

Lee, H; De Saeger, J; Bae, S; Kim, M; Depuydt, S; Heynderickx, PM; Wu, D; Han, T; Park, J. Toxics (2023) 11: DOI10.3390/toxics11090788.

Aquatic environment are often contaminated with heavy metals from various industrial sources. However, physicochemical techniques for pollutant detection are limited, thus prompting the need for additional bioassays. We investigated the use of greater duckweed (*Spirodela polyrhiza*) as a bioindicator of metal pollution. We exposed *S. polyrhiza* to four pollutants (namely, silver, cadmium, copper, and chromium) and assessed metal toxicity by measuring its frond area and the length of its regrown roots. The plant displayed significant differences in both frond size and root growth in response to the four metals. Silver was the most toxic (EC_{50} = 23 g L^{-1}) while copper the least (EC_{50} = 365-607 g L^{-1}). Direct comparisons of metal sensitivity and

the reliability of the two endpoint assays showed that root growth was more sensitive (lower in terms of 50% effective concentration) to chromium, cadmium, and copper, and was more reliable (lower in terms of coefficient of variation) than those for frond area. Compared to conventional *Lemna*-based tests, the *S. polyrhiza* test is easier to perform (requiring only one 24-well plate, 3 mL of medium and a 72-h exposure). Moreover, it does not require livestock cultivation/maintenance, making it more suitable for repeated measurements. Measurements of *S. polyrhiza* root length may be suitable for assessment when copper and chromium in municipal and industrial wastewater exceed the environmentally permissible levels.

Combined toxic effects of polypropylene and perfluorooctanoic acid on duckweed and periphytic microorganisms

Li, Q; Jiang, J; Lan, Y; Kang, S; Yang, Y; Zhang, J. Environmental Science and Pollution Research International (2023) DOI10.1007/s11356-023-30006-9.

Microplastics and perfluorooctanoic acid coexist in the aquatic environment. Duckweed was exposed to a range of concentrations (0.1-1000 μg L^{-1}) of solutions containing polypropylene (PP) and perfluorooctanoic acid (PFOA) for 14 days to measure their toxicity. The result showed the single and combined PP and PFOA treatments did not significantly influence the growth of duckweed. The greatest PP and PFOA concentrations of combined pollution affect plant chlorophyll. Moreover, the combined treatment of duckweed consistently resulted in increased malondialdehyde (MDA) levels, indicating oxidative damage. As an antioxidant stress response, the combination-treated plants were encouraged to produce superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Meanwhile, 3519 Operational Taxonomic Units (OTUs) were identified in the duckweed rhizosphere. Proteobacteria was the most predominant microbial community. Shannon, Simpson, and Chao discovered that microbial communities changed in response to single and combination PP and PFOA treatments, with decreased diversity and increased abundance. In addition, SEM analysis also revealed that the combined treatment significantly phyllosphere microorganisms. The findings of this investigation add to our knowledge of how PP and PFOA affect duckweed and the rhizospheric microorganisms, expanding the theoretical basis for employing duckweed in complex contamination.

The regulatory effects of biochar on PSII photochemistry, antioxidant system and nitrogen assimilation in *Lemna minor* exposed to inorganic pollutants, arsenic and fluoride

Yildiztugay, E; Ozfidan-konakci, C; Arikan, B; Alp-Turgut, FN; Guenturk, C. Journal of Environmental Chemical Engineering (2023) 11: 110713.

The excessive accumulation of inorganic pollutants such as arsenic (As) and fluoride (F) are destructive contaminants for plants. There are some reports about the toxicity mechanisms of individual exposure of As or F. Biochar (BC), carbon-rich organic material, can sequester pollutants and help in increasing growth, nutrient uptake and water holding capacity in soils. However, the defence responses of plants to a combination of two stresses and the interactions between BC and combined exposure of As and F are not explicit. In order to fill this gap, different BC concentrations (BC1; 1 g L^{-1} and BC2; 5 g L^{-1}) were hydroponically applied to *Lemna minor* subjected to sodium fluoride (F, 15 mM NaF) and sodium arsenate (As, 100 μM Na_2HAsO_4). As or F pollutants caused a reduction in the chlorophyll fluorescence (Fv/Fm and Fv/Fo) but BC application under stress conditions improved the fluorescence levels. The photochemical reactions of photosystems and fluorescence kinetics impaired by stress were re-regulated through all BC concentrations. The relative expressions of genes belonging to proteins in PSI-PSII (*psa*A, *psa*B, *psb*A and *psb*D) were up-regulated in both BCs and As/F treatments. As-treated *L. minor* had only induction in superoxide dismutase-SOD- and ascorbate peroxidase-APX-, the enhancement in SOD, catalase-CAT-, glutathione reductase-GR- and glutathione S-transferase-GST- was observed under F toxicity. Also, the joint stress (As+F) caused declines in all antioxidant capacities except with SOD activity. Due to the sufficient antioxidant activation, hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) increased in *L. minor*. While, 1 mg L^{-1} BC induced the regeneration of ascorbate (AsA) under individual application of As or F, 5 mg L^{-1} BC increased APX, MDHAR, DHAR, total ascorbate (tAsA), and DHA against F stress. As- and NaF-mediated stress suppressed the nitrogen assimilation of *L. minor*. Interestingly, the induced accumulation of NH_4^+ was prevented in BC plus stress-

applied plants through glutamate dehydrogenase (GDH) activity rather than glutamine synthetase-glutamate synthase (GS-GOGAT) pathways. All findings indicated that BCs reduced lipid peroxidation and ROS accumulation by modulating electron fluxes and photochemistry in PSI and PSII, gene expression, nitrogen metabolism as well as the antioxidant system under stress conditions (As, F, and As+F).

Acetylcholine (ACh) enhances Cd tolerance through transporting ACh in vesicles and modifying Cd absorption in duckweed (*Lemna turionifera* 5511)

Yang, L; Ma, X; Guo, YH; He, YM; Yang, YW; Wang, WQ; Xu, ZY; Zuo, ZJ; Xue, Y; Yang, R; Han, B; Sun, JS. Environmental Pollution (2023) 335: 122305.

Acetylcholine (ACh), an important neurotransmitter, plays a role in resistance to abiotic stress. However, the role of ACh during cadmium (Cd) resistance in duckweed (*Lemna turionifera* 5511) remains uncharacterized. In this study, the changes of endogenous ACh in duckweed under Cd stress has been investigated. Also, how exogenous ACh affects duckweed's ability to withstand Cd stress was studied. The ACh sensor transgenic duckweed (ACh 3.0) showed the ACh signal response under Cd stress. And ACh was wrapped and released in vesicles. Cd stress promoted ACh content in duckweed. The gene expression analysis showed an improved fatty acid metabolism and choline transport. Moreover, exogenous ACh addition enhanced Cd tolerance and decreased Cd accumulation in duckweed. ACh supplement reduced the root abscission rate, alleviated leaf etiolation, and improved chlorophyll fluorescence parameters under Cd stress. A modified calcium (Ca^{2+}) flux and improved Cd^{2+} absorption were present in conjunction with it. Thus, we speculate that ACh could improve Cd resistance by promoting the uptake and accumulation of Cd, as well as the response of the Ca^{2+} signaling pathway. Also, plant-derived extracellular vesicles (PDEVs) were extracted during Cd stress. Therefore, these results provide new insights into the response of ACh during Cd stress.

Physical and chemical effects of conventional microplastic glitter versus alternative glitter particles on a freshwater plant (Lemnaceae: *Lemna minor*)

Boots, B; Green, DS; Olah-Kovacs, B; De Falco, F; Lupo, E. Ecotoxicology and Environmental Safety (2023) 263: 115291.

Glitters are primary microplastics which are directly littered into the environment, yet the ecological effects have seldom been tested. When microplastics enter the environment, their physical presence and chemical leachate may alter the physiology of primary producers. Glitter can be composed of plastic or natural and/or biodegradable materials, often with additives. Three experiments were run for 14 days to separate chemical and physical effects of different types of glitter: polyethylene terephthalate (PET), biodegradable modified regenerated cellulose (MRC), synthetic mica, and a natural particle control (kaolinite) on several physical characteristics of *Lemna minor* (common duckweed). *L. minor* was exposed to either fresh (chemical and physical effects), leachate from glitter (chemical) or aged glitter (physical). Overall, there was little effect of PET, synthetic mica, kaolinite or of any aged glitter. High concentrations of fresh MRC glitters, however, decreased root length, biomass and chlorophyll content of *L. minor*. Some of these effects were also present when exposed to leachate from MRC glitters, but were less pronounced. Elemental analysis revealed the presence of metals in MRC glitters which may explain these responses. Short-term ecotoxicity of biodegradable glitters can arise due to their physical and chemical properties, but may lessen over time as their surface coating degrades.

Interaction of cerium oxide nanoparticles and ionic cerium with duckweed (*Lemna minor* L.): Uptake, distribution, and phytotoxicity

Liu, Y; Zhao, X; Ma, Y; Dai, W; Song, Z; Wang, Y; Shen, J; He, X; Yang, F; Zhang, Z. Nanomaterials (2023) 13: DOI10.3390/nano13182523.

As one of the most widely used nanomaterials, CeO_2 nanoparticles (NPs) might be released into the aquatic environment. In this paper, the interaction of CeO_2 NPs and Ce^{3+} ions (0~10 mg/L) with duckweed (*Lemna minor* L.) was investigated. CeO_2 NPs significantly inhibited the root elongation of duckweed at concentrations higher than 0.1 mg/L, while the inhibition threshold of Ce^{3+} ions was 0.02 mg/L. At high doses, both reduced photosynthetic pigment contents led to cell death and induced stomatal deformation, but the toxicity of Ce^{3+} ions was greater than that of CeO_2 NPs at the same concentration. According to the in situ distribution of Ce in plant tissues by μ-XRF, the intensity of Ce signal was in the order of root > old frond > new frond, suggesting that roots play a major role in the uptake of Ce. The result of XANES showed that 27.6% of Ce(IV) was reduced to Ce(III) in duckweed treated with CeO_2 NPs. We speculated that the toxicity of CeO_2 NPs to duckweed was mainly due to its high sensitivity to the released Ce^{3+} ions. To our knowledge, this is the first study on the toxicity of CeO_2 NPs to an aquatic higher plant.

Single and combined toxicity of polystyrene nanoplastics and PCB-52 to the aquatic duckweed *Spirodela polyrhiza*

Pan, T; Chen, XK; Kong, CM; Gao, DD; Liu, WJ; Liao, HP; Junaid, M; Wang, J. Science of the Total Environment (2023) 901: 166482.

As nanoplastics and persistent organic pollutants are broadly distributed in aquatic ecosystems and pose a potential threat to ecosystem, most pertinent studies have focused on aquatic animals, while studies on freshwater plants have been rarely reported. Therefore, we analysed the single and combined toxicological impacts of various concentrations of 80 nm polystyrene nanoplastics (PS-NPs) including 0.5, 5, 10, and 20 mg/L and polychlorinated biphenyl-52 (PCB-52, 2,2',5,5'- tetrachlorobiphenyl) at 0.1 mg/L on the aquatic plant *Spirodela polyrhiza* (*S. polyrhiza*) after a 10-day hydroponic experiment. Laser confocal scanning microscopy (LCSM) showed the accumulation of PS-NPs mainly in the root surface and the lower epidermis of leaves, and the enrichment of PS-NPs was aggravated by the presence of PCB-52. PS-NPs at 10 mg/L and 20 mg/L alone or in combination with PCB-52 notably inhibited the growth of *S. polyrhiza*, reduced the synthesis of chlorophylls a and b, and increased the activities of superoxide dismutase (SOD) and peroxidase (POD) as well as malondialdehyde (MDA) levels, and induced osmotic imbalance (soluble protein and soluble sugar contents) ($p < 0.05$). However, a single treatment with low levels of PS-NPs had positive effects on the growth (0.5 mg/L) and photosynthetic systems (0.5, 5 mg/L) of *S. polyrhiza*, while co-exposure exacerbated the damaging impacts of PSNPs on the antioxidant defence system of *S. polyrhiza*, which was more pronounced in the roots. Furthermore, correlation analysis revealed that plant growth parameters were positively correlated with chlorophyll a and b content and negatively correlated with soluble sugars, antioxidant enzymes, lipid peroxidation, and carotenoid content ($p < 0.05$). These results provide data to improve the understanding of the single and combined ecotoxicological effects of nanoplastics and polychlorinated biphenyls (PCBs) in aquatic plants and their application in phytoremediation measures.

Exposure of *Lemna minor* (Common Duckweed) to mixtures of uranium and perfluorooctanoic acid (PFOA)

Gonzales, AK; Donaher, SE; Wattier, BD; Martinez, NE. Environmental Toxicology and Chemistry (2023) DOI10.1002/etc.5720.

A variety of processes, both natural and anthropogenic, can have a negative impact on surface waters, which in turn can be detrimental to human and environmental health. Few studies have considered the ecotoxicological impacts of concurrently occurring contaminants, and that is particularly true for mixtures that include contaminants of emerging concern (CEC). Motivated by this knowledge gap, the present study considers the potential ecotoxicity of environmentally relevant contaminants in the representative aquatic plant Lemna minor (common duckweed), a model organism. More specifically, biological effects associated with exposure of *L. minor* to a ubiquitous radionuclide (uranium [U]) and a fluorinated organic compound (perfluorooctanoic acid [PFOA], considered a CEC), alone and in combination, were monitored under controlled laboratory conditions. *Lemna minor* was grown for 5 days in small, aerated containers. Each treatment consisted of four replicates with seven plants each. Treatments were 0, 0.3, and 3 ppb PFOA; 0, 0.5, and 5 ppb U; and combinations of these. Plants were observed daily for frond number and signs of chlorosis and

necrosis. Other biological endpoints examined at the conclusion of the experiment were chlorophyll content and antioxidant capacity. In single-exposure experiments, a slight stimulatory effect was observed on frond number at 0.3 ppb PFOA, whereas both concentrations of U had a detrimental effect on frond number. In the dual-exposure experiment, the combinations with 5 ppb U also had a detrimental effect on frond number. Results for chlorophyll content and antioxidant capacity were less meaningful, suggesting that environmentally relevant concentrations of PFOA and U have only subtle effects on *L. minor* growth and health status.

Effects of microplastics and cadmium on growth rate, photosynthetic pigment content and antioxidant enzymes of duckweed (*Lemna minor*)

Yang, X; Liao, HM; Tan, AJ; Gan, SX; Yang, GL. Environmental Science and Pollution Research (2023) 30: 96181-96190.

Cadmium (Cd) and polyethylene (PE) seriously contaminate the aquatic environment and threaten human health. Many studies have reported the toxic effects of Cd and PE on plants, whereas few have reported the combined contamination of these two pollutants. In this study, duckweed (*Lemna minor*) was used as an indicator to explore the effect of PE microplastics (PE-MPs) at concentrations of 10, 50, 100, 200, and 500 mg/L on tolerance to 1 mg/L Cd. The results showed that different concentrations of PE-MPs inhibited the growth rate and chlorophyll content of duckweed to different degrees, both of which were minimal at 50 mg/L PE-MPs, 0.11 g/d, and 0.32 mg/g, respectively. The highest Cd enrichment (7.77 mg/kg) and bioaccumulation factors (94.22) of duckweed were detected when Cd was co-exposed with 50 mg/L of PE-MPs. Catalase and peroxidase activity first decreased and then increased with increasing PE-MPs concentrations, showing "hormesis effects", with minimum values of 11.47 U/g and 196.00 U/g, respectively. With increasing concentrations of PE-MPs, the effect on superoxide dismutase activity increased and then declined, peaking at 162.05 U/g, and displaying an "inverted V" trend. The amount of malondialdehyde rose with different PE-MPs concentrations. This research lay a foundation for using duckweed to purify water contaminated with MPs and heavy metals.

Ecotoxicity of diazinon and atrazine mixtures after ozonation catalyzed by Na^+ and Fe^{2+} exchanged montmorillonites on *Lemna minor*

Benghaffour, A; Azzouz, A; Dewez, D. Molecules (2023) 28: 6108.

The toxicity of two pesticides, diazinon (DAZ) and atrazine (ATR), before and after montmorillonite-catalyzed ozonation was comparatively investigated on the duckweed *Lemna minor*. The results allowed demonstrating the role of clay-containing media in the evolution in time of pesticide negative impact on *L. minor* plants. Pesticides conversion exceeded 94% after 30 min of ozonation in the presence of both Na^+ and Fe^{2+} exchanged montmorillonites. Toxicity testing using *L. minor* permitted us to evaluate the change in pesticide ecotoxicity. The plant growth inhibition involved excessive oxidative stress depending on the pesticide concentration, molecular structure, and degradation degree. Pesticide adsorption and/or conversion by ozonation on clay surfaces significantly reduced the toxicity towards *L. minor* plants, more particularly in the presence of Fe(II)-exchanged montmorillonite. The results showed a strong correlation between the pesticide toxicity towards *L. minor* and the level of reactive oxygen species, which was found to depend on the catalytic activity of the clay minerals, pesticide exposure time to ozone, and formation of harmful derivatives. These findings open promising prospects for developing a method to monitor pesticide ecotoxicity according to clay-containing host-media and exposure time to ambient factors.

Deep learning-based automatic duckweed counting using StarDist and its application on measuring growth inhibition potential of rare earth elements as contaminants of emerging concerns

Kurnia, KA; Lin, YT; Farhan, A; Malhotra, N; Luong, CT; Hung, CH; Roldan, MJM; Tsao, CC; Cheng, TS; Hsiao, CD. Toxics (2023) 11: 680.

In recent years, there have been efforts to utilize surface water as a power source, material, and food. However, these efforts are impeded due to the vast amounts of contaminants and emerging contaminants introduced by anthropogenic activities. Herbicides such as glyphosate and glufosinate are commonly known to contaminate surface water through agricultural industries. In contrast, some emerging contaminants, such as rare earth elements, have started to enter the surface water from the production and waste of electronic products. Duckweeds are angiosperms from the Lemnaceae family and have been used for toxicity tests in aquatic environments, mainly those from the genus *Lemna*, and have been approved by OECD. In this study, we used duckweed from the genus *Wolffia*, which is smaller and considered a good indicator of metal pollutants in the aquatic environment. The growth rate of duckweed is the most common endpoint in observing pollutant toxicity. In order to observe and mark the fronds automatically, we used StarDist, a machine learning-based tool. StarDist is available as a plugin in ImageJ, simplifying and assisting the counting process. Python also helps arrange, manage, and calculate the inhibition percentage after duckweeds are exposed to contaminants. The toxicity test results showed dysprosium to be the most toxic, with an IC_{50} value of 14.6 ppm, and samarium as the least toxic, with an IC_{50} value of 279.4 ppm. In summary, we can provide a workflow for automatic frond counting using StarDist integrated with ImageJ and Python to simplify the detection, counting, data management, and calculation process.

Transcriptomic and functional analyses of two cadmium hyper-enriched duckweed strains reveal putative cadmium tolerance mechanisms

Yang, GL; Huang, L; Yang, X; Li, Z; Liao, HM; Mao, K; Liu, ZJ; Geng, HY; Cao, Q; Tan, AJ. International Journal of Molecular Sciences (2023) 24: 12157.

Cadmium (Cd) is one of the most toxic metals in the environment and exerts deleterious effects on plant growth and production. Duckweed has been reported as a promising candidate for Cd phytoremediation. In this study, the growth, Cd enrichment, and antioxidant enzyme activity of duckweed were investigated. We found that both high-Cd-tolerance duckweed (HCD) and low-Cd-tolerance duckweed (LCD) strains exposed to Cd were hyper-enriched with Cd. To further explore the underlying molecular mechanisms, a genome-wide transcriptome analysis was performed. The results showed that the growth rate, chlorophyll content, and antioxidant enzyme activities of duckweed were significantly affected by Cd stress and differed between the two strains. In the genome-wide transcriptome analysis, the RNA-seq library generated 544,347,670 clean reads, and 1608 and 2045 differentially expressed genes were identified between HCD and LCD, respectively. The antioxidant system was significantly expressed during ribosomal biosynthesis in HCD but not in LCD. Fatty acid metabolism and ethanol production were significantly increased in LCD. Alpha-linolenic acid metabolism likely plays an important role in Cd detoxification in duckweed. These findings contribute to the understanding of Cd tolerance mechanisms in hyperaccumulator plants and lay the foundation for future phytoremediation studies.

DF Comment: The authors used *Lemna minor* 0009 and 0010 without giving the method of their identification.

Impact of mercury on photosynthetic performance of *Lemna minor*: a chlorophyll fluorescence analysis

Singh, H; Kumar, D; Soni, V. Scientific Reports (2023) 13: 12181.

The purpose of this study was to evaluate the effectiveness of chlorophyll fluorescence analysis in detecting the effects of mercury (Hg) treatment in duckweed species *Lemna minor*. The results showed that Hg treatment (ranging from 0.0 to 0.4 µM) significantly impacted the plant's photosynthetic ability, with a decrease in variable chlorophyll fluorescence, energy fluxes, density of reaction centres, and performance index. Complete inhibition of electron transport was observed in plants treated with high Hg concentrations, and the quantum yield of primary photochemistry and the ratio of dissipated energy to absorption both decreased with increasing Hg concentrations. Performance Index (PI) was significantly affected by the Hg concentrations, reaching zero in plants treated with the highest Hg concentration. Overall, JIP analysis was found to be an effective tool for detecting deleterious effects of Hg in plants.

Carbon allocation of *Spirodela polyrhiza* under boron toxicity

Pagliuso, D; Pereira, JPD; Ulrich, JC; Cotrim, MEB; Buckeridge, MS; Grandis, A. Frontiers in Plant Science (2023) 14: 1208888.

Pectic polysaccharides containing apiose, xylose, and uronic acids are excellent candidates for boron fixation. Duckweeds are the fastest-growing angiosperms that can absorb diverse metals and contaminants from water and have high pectin content in their cell walls. Therefore, these plants can be considered excellent boron (B) accumulators. This work aimed to investigate the relationship between B assimilation capacity with apiose content in the cell wall of *Spirodela polyrhiza* subjected to different boric acid concentrations. Plants were grown for 7 and 10 days in 1/2 Schenck-Hildebrandt media supplemented with 0 to 56 mg B L^{-1}, the non-structural and structural carbohydrates, and related genes were evaluated. The results showed that B altered the morphology and carbohydrate composition of this species during plant development. The optimum B concentration (1.8 mg B L^{-1}) led to the highest relative growth and biomass accumulation, reduced starch, and high pectin and apiose contents, together with increased expression of UDP-apiose/UDP-xylose synthase (AXS) and 1,4-α-galacturonosyltransferase (GAUT). The toxic state (28 and 56 mg B L^{-1}) increased the hexose contents in the cell wall with a concomitant reduction of pectins, apiose, and growth. The pectin content of *S. polyrhiza* was strongly associated with its growth capacity and regulation of B content within the cells, which have AXS as an important regulator. These findings suggest that duckweeds are suitable for B remediation, and their biomass can be used for bioenergy production.

Taxonomy & Geobotany

Population genetic structure of two cryptic duckweed species (*Lemna minor* & *L. turionifera*) in Alberta using a genotyping-by-sequencing approach

Senevirathna, KM; Crisfield, VE; Gazeley, I; Laird, RA; Burg, TM. Aquatic Botany (2023) 189: 103687.

Identifying population genetic structure is important for the development of species-specific management plans. Investigating the population genetics of cryptic species is even more critical. Here we focus on two cryptic duckweed species easily mistaken for one another, *Lemna minor* L. and *L. turionifera* Landolt, which have overlapping ranges in our study region of Alberta, Canada, and elsewhere. We used genotyping-by-sequencing to determine the population genetic structure of both duckweed species. A total of 192 samples was sequenced. After filtering, 16,007 single nucleotide polymorphisms were used to examine patterns of genetic diversity between and within *L. minor* and *L. turionifera*. The two species showed clear differentiation. When examining L. minor singly, we discovered at least three genetically distinct populations among the 30 samples from eight sites, even though these were from a small geographic area. In contrast, when examining *L. turionifera* singly, we found no evidence of genetically distinct populations among 67 samples from 43 sites. We also examined the relationship between surface water quality variables and the distribution of the two *Lemna* species. The sites containing *L. turionifera* had a wider range of water chemistry variables suggesting they are more tolerant of different environmental conditions. In contrast, each of the three genetically distinct *L. minor* groups had different water chemistry profiles. Large differences between *L. minor* and *L. turionifera* in their regional distributions and degrees of genetic differentiation highlight the importance of documentation and careful monitoring of *Lemna* species within Alberta, and in other regions where they co-occur.

Elemental composition of the Lemnaceae family in urbanized territories of the Russian Federation

Baranovskaya, NV; Baranovskaya, AY; Sudyko, AF. Geochemistry International (2023) 61: 658-667.

The paper presents the first ever data on the contents of 28 elements in aquatic plants of the duckweed family (Lemnaceae) in the territory of 65 population centres of the Russian Federation. Data are presented on the elemental composition of duckweed in the urbanized regions of Russia and on the spatial distribution of the studied elements and their calculated concentration coefficients relative to the estimated mean values in the macrophytes. The elemental composition of duckweed is shown to provide significant information about the

current ecological and geochemical situation in the study area and can serve as an unbiased indicator of the impact of anthropogenic and natural factors on the environment.

Pre-prints

The “Duckweed Dip”: Aquatic *Spirodela polyrhiza* plants can efficiently uptake dissolved, DNA-wrapped carbon nanotubes from their environment for transient gene expression

Islam, T; Kalkar, S; Tinker-Kulberg, R; Ignatova, T; Josephs, EA. bioRxiv (2023) DOI10.1101/2023.08.21.554121

Duckweeds (Lemnaceae) are aquatic non-grass monocots that are the smallest and fastest-growing flowering plants in the world. While having simplified morphologies, relatively small genomes, and many other ideal traits for emerging applications in plant biotechnology, duckweeds have been largely overlooked in this era of synthetic biology. Here, we report that Greater Duckweed (*Spirodela polyrhiza*), when simply incubated in a solution containing plasmid-wrapped carbon nanotubes (DNA-CNTs), can directly up-take the DNA-CNTs from their growth media with high efficiency and that transgenes encoded within the plasmids are expressed by the plants - without the usual need for large doses of nanomaterials or agrobacterium to be directly infiltrated into plant tissue. This process, called the "duckweed dip", represents a streamlined, hands-off tool for transgene delivery to a higher plant that we expect will enhance the throughput of duckweed engineering and help to realize duckweeds potential as a powerhouse for plant synthetic biology.

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). 3,000 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