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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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Duckweed in slow flowing water

Cover page

Duckweed in slow flowing water: Duckweed grows in lentic ecosystems. However, in slow flowing waters, behind barriers or between the vegetation near riverbanks, they have a chance to grow. The photo shows *Lemna* growing in the rivulet Uchte in the city of Stendal, Saxony-Anhalt, Germany. Adventitious roots stabilize the fronds on water surface behind some stones. (Photo: Klaus-J. Appenroth, Germany).

In this issue

The 6th International Steering Committee on Duckweed Research and Applications Members

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

Letter from the Editor:

January 30th, 2026

Dear Duckweed Community,

Greetings of the year ahead, 2026!!!

We wish 2026 to be a great year for duckweeds and for the duckweed researchers and application specialists. The followers of `Duckweed Forum' must have already realized a great boom in the research and applications of duckweed in the last year evidenced by a rise in the number and quality of duckweed publications, innovations and organization of several local workshops and symposia to cater to the immediate needs of the region for networking and collaborations. One of them which you will find in this issue is a report of a symposium held in Chengdu, China, by Prof. Shunong Bai detailing the strong networking of duckweed research groups in China. Some more goods news: Duckweed project from the Brno team which was introduced in the DF a couple of issues back has won the iGEM competition. Congratulations to team Brno!

A commentary on the article selected as a highlight in the previous issue focuses on understanding the structure and function of the duckweed microbiome using *Wolffia* as an example and decodes how this microbiome impacts the growth of the host. Further, an interesting science communication project led by one of the ISCDRA members, Prof. Eric Lam and his colleagues at the Rutgers University, USA is making efforts to take duckweeds to the common man through the videos made by students. The report presented here has links to some of them which are worth watching. And, the call to host the next ICDRA in 2028 is now open. We request the interested groups to take note of the requirements and to bid their applications for the same before the deadline. We are expecting as many as possible applications from various regions across the globe.

With an increasing focus on large scale production of duckweeds, there is a growing interest to learn about the pests and pathogens of duckweeds and to develop ways to counter them. One of the reports in the current issue on the insect pests of duckweeds and their control is by Mr. Sai Chand Sabbireddi who is also eagerly exploring an opportunity to work further in this field. We also present a student spotlight by Mr. Matteo Agastra, who is aspiring towards building duckweed biomass as biofuel. As always, we have the database collated by one of our members, Dr. Klaus Appenroth, which I am sure is a helpful resource for our community to keep up-to-date with the duckweed literature.

Happy to see the preparations of the 8th ICDRA coming along very well. Thanks to the organizers from Italy for all their efforts in hosting us. We recommend you to register as per the instructions in the announcement of this issue. We look forward to meeting you in Italy this year.

All best wishes from ISCDRA.

Enjoy reading !!!

Sincerely,
K. Sowjanya Sree
Chair, ISCDRA

Commentary: From water to frond - *Wolffia* shapes its microbiome to drive its growth




Yuparat Saimee[1], Hidehiro Ishizawa[2], Daisuke Inoue[1], Michihiko Ike[1]

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Plants and their associated microbiomes live together in various relationships–sometimes helping and sometimes harming each other. Their lives are deeply integrated and can be difficult to separate. Duckweeds are no exception. Symbiotic microbiomes have been proven to significantly influence the growth and physiological performance of rooted duckweeds such as *Lemna* and *Spirodela* (Ishizawa et al., 2017).

Meanwhile, *Wolffia*, the rootless and tiniest duckweed, is increasingly recognized as a promising bioresource for food and bioenergy production due to its biomass composition (Appenroth et al., 2018). However, we still know relatively little about the *Wolffia* microbiome and its effects on *Wolffia* growth, indicating the need for research on the *Wolffia* microbiome. Additionally, microbiome-based technologies have the potential to increase the yields and reduce the energy inputs in cultivation systems.

The structure and function of the *Wolffia* microbiome likely differ from those of rooted duckweed, given the differences in their morphology. Consequently, systematic characterization of the *Wolffia* microbiome is the missing key for developing advanced cultivation systems. We think that the *Wolffia*–microbiome interactions, specifically the structure and functional potential of the microbiome and its impact on host growth, must be understood to provide insights for the sustainable intensification of *Wolffia* biomass production to meet the growing global demand.

With this aim in mind, our recent paper (Saimee et al., 2025) systematically characterized the structural and functional properties of the *Wolffia* microbiome and its effects on host growth via the co-cultivation of *Wolffia globosa* #RDSC8152 with 18 bacterial communities of different origins. These communities were derived from ten freshwater pond (PW), four municipal secondary effluent (SE), and four activated sludge (AS) samples. The original microbial sources and surface-attached microbiomes on *Wolffia*, collected after removing ambient water, were characterized using 16S rRNA amplicon sequencing and diverse analyses to uncover the features of the microbiome associated with this rootless duckweed.

The 18 bacterial communities exhibited contrasting effects on the host over a 10-day co-cultivation period, with outcomes ranging from inhibiting to strongly promoting *Wolffia* growth. Municipal wastewater-derived bacterial communities (SEs and ASs) strongly enhanced *Wolffia* growth, increasing the frond area by 21–55.6% (Fig. 1). In contrast, PW-derived bacterial communities had neutral or inhibitory effects on growth,

decreasing the frond area by up to 12.2%. These findings suggest that PW-derived bacterial communities do not consistently provide the necessary microbial support for rapid *Wolffia* biomass production, challenging the assumption that natural habitats provide optimal or compatible beneficial microbiomes for *Wolffia* growth. Our findings suggest that wastewater environments serve as reservoirs for diverse or robust plant growth-promoting bacteria (PGPB) that promote *Wolffia* growth.

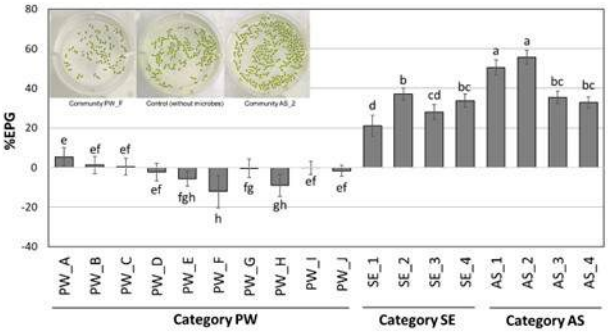

Fig. 1 Effect of bacterial communities on *Wolffia globosa* growth after a 10-day cultivation. Effect on plant growth (EPG) indicates an increase/decrease in frond area relative to the control. Error bars represent standard deviations (n=5) (modified from Saimee et al., 2025).

In addition, we found that although environmental sources provide a diverse pool of bacterial populations, *Wolffia* actively shaped its microbiome via the selective assembly and enrichment of specific taxa, forming a distinct community structure depending on the original microbial source (Fig. 2 a, b). Despite the variation in the dominant taxa among microbial sources, a core microbiome emerged, comprising six conserved families: *Beijerinckiaceae*, *Caulobacteraceae*, *Comamonadaceae*, *Methylophilaceae*, *Rhizobiaceae*, and *Sphingomonadaceae*. Although most of these families are also found as core taxa in the microbiomes of rooted duckweeds, our study highlights a key distinction: *Beijerinckiaceae*, belonging to Alphaproteobacteria, was identified as a core taxon in the *W. globosa* microbiome. *Beijerinckiaceae* have rarely been found in the microbiome of rooted duckweeds (Acosta et al., 2020; Bunyoo et al., 2022; Inoue et al., 2022). This finding suggests that *Beijerinckiaceae* plays a specialized role in the *W. globosa* microbiome and is integral to this holobiont. Furthermore, several genera were consistently identified as the core taxa in the *W. globosa* microbiome: *Allorhizobium-Neorhizobium-Rhizobium*, *Bosea*, *Brevundimonas*, *Caulobacter*, *Hydrogenophaga*, *Methylophilus*, and *Porphyrobacter*. Notably, we report *Bosea*, *Brevundimonas*, *Caulobacter*, and *Methylophilus* as the consistent core taxa in the *W. globosa* microbiome for the first time, which expands our understanding of the core microbiome of rootless duckweeds.

Rooted duckweeds are known to recruit microbes via distinct physical and chemical properties in both the rhizosphere and frond surface (Iwashita et al., 2020), like typical plants; however, frond surface colonization is likely key in shaping the *Wolffia* microbiome owing to its rootless morphology. Functional prediction based on 16S rRNA amplicon sequencing revealed considerable enrichment in common traits that facilitate colonization and plant interactions, including in genes related to motility (flagella assembly and chemotaxis), community behavior (quorum sensing and biofilm formation), secretion systems, and ABC transporters in the *W. globosa* microbiome. This finding suggests that active mobility and communication are critical for bacteria to effectively colonize this duckweed species. Additionally, we found that genes related to amino acid and energy metabolism as well as to xenobiotic biodegradation were enriched. These metabolic profiles likely reflect the response of the microbiome to metabolites secreted by the plant, which simultaneously provides functional advantages to the host.

Given the distinct growth-promoting/inhibiting effects observed for the co-cultivated microbes, we also attempted to identify the potential key players influencing *W. globosa* growth. We found that the abundances of 12 bacterial families positively correlated with plant growth, suggesting their direct or indirect contribution to host growth as PGPB. Conversely, the abundances of seven families (e.g., *Comamonadaceae*, *Pseudomonadaceae*, and *Burkholderiaceae*) negatively associated with plant growth (Fig. 2c), suggesting that members of these families act as potential plant growth-inhibiting bacteria that harm plant growth, which is an important concern for *W. globosa* cultivation systems.

Among the positively associated families, *Sphingomonadaceae* exhibited the strongest positive correlation with host growth. This family was not only part of the core microbiome and a central contributor to the microbiome structure but also significantly enriched in the wastewater-derived communities, promoting plant growth (SEs and ASs). Another group that deserves special attention is *Beijerinckiaceae*. In addition to being the core taxon unique to *W. globosa* as aforementioned, this family positively associated with host growth and enriched in growth-promoting communities. These findings collectively indicate that members of *Sphingomonadaceae* and *Beijerinckiaceae* may function as PGPB, which are compatible with and functionally important partners within the *Wolffia* holobiont. We also found that the abundance of *Bdellovibrionaceae* was enriched in the *Wolffia* microbiome and positively associated with *Wolffia* growth. *Bdellovibrionaceae* is a family consisting of obligate predatory bacteria; thus, the *Bdellovibrionaceae* may indirectly support *Wolffia*

growth and health by shaping community composition through predator–prey interactions as a population regulator (Inoue et al., 2023). The findings of this study suggest that the intentional use of predator–prey interactions among bacteria can be applied in future microbiome engineering strategies for *Wolffia*. However, the contributions of other families co-occurring in the microbiome cannot be excluded given the complexity of plant–microbiome interactions. We hope these insights will support future efforts to develop efficient biomass production systems to enhance *Wolffia* productivity.

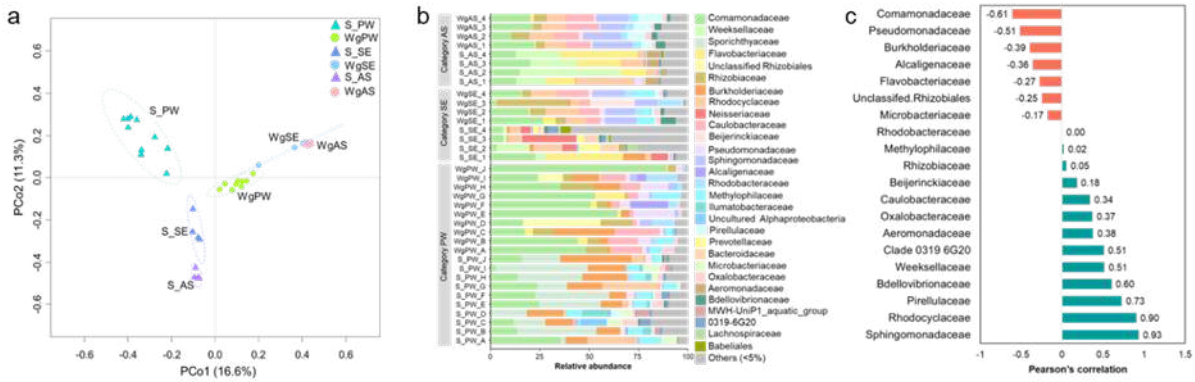

Fig. 2 Wolffia microbiome structure (a, b), and positive and negative key players influencing *Wolffia globosa* growth (c) (modified from Saimee et al., 2025).

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ICDRA-8: Announcement

8th International Conference on Duckweed Research and Applications
Model plant and novel crop, the thousand faces of duckweed

ICDRA 2026 | Portici (Naples), Italy | Sept 28 – Oct 2, 2026

Updates: Registration & Speakers

- **Registration Opening:** We are happy to confirm that registration will open in the **Beginning of April 2026**.
- **Speakers Published:** The list of invited speakers is now live on our website!

Don't miss the opening! Please pre-register to receive email alerts:

www.8thicdra.info/stay-informed

Invited Speakers

Robert A. Martienssen (Plenary)
Professor & HHMI Investigator, William J. Matheson Professor Cancer Center Member, Cold Spring Harbor Laboratory, USA

Arturo Marí-Ordóñez
Group Leader, Gregor Mendel Institute of Molecular Plant Biology (GMI) of the Austrian Academy of Sciences, Austria

Shuqing Xu
Institute of Organismic and Molecular Evolution, Johannes Gutenberg University of Mainz, Germany

Eric Lam
Distinguished Professor, Dept of Plant Biology, School of Env. and Bio. Sciences, Rutgers, The State University of New Jersey, USA

Anthony Bishopp
Associate Professor, Faculty of Science, School of Biosciences, University of Nottingham, UK

Metha Meetam
Assistant Professor Dr., Mahidol University, Nakhon Pathom, CEO Advanced Green Farm Co. Ltd., Thailand

Klaus J. Appenroth
Senior Guest Scientist, Matthias Schleiden Institute, Friedrich Schiller University Jena, Germany

K. Sowjanya Sree
Associate Professor, School of Biotechnology, Banaras Hindu University, Varanasi, India

Website: www.8thicdra.info | **Speakers:** View

Getting the public to know duckweed

Science communication with the help of FAME

Eric Lam[1] and Dena Seidel[2]

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Communicating science-related subject matters to the public is a challenging proposition. The messages that are relevant to the society-at-large and the passion for knowledge that are felt by the scientists involved can often be lost in translation between the two parallel worlds. Duckweed as an emergent crop that could address critical needs of humanity is a case in point. How could these tiny plants be the answer for grand challenges such as food security and sustainable biofuels? While there are many attributes of duckweed that support this view, effectively explaining our rationale and the science that supports our optimism to the public is not a trivial task. Gaining trust of the audience while delivering key messages in a simple and unambiguous manner are key ingredients for successful science communication. While methods and theories exist to implement effective public engagement efforts, most scientists are untrained in these skills. Over the past four months, we have collaborated to start a science communication effort designed specifically for the duckweed technology platform.

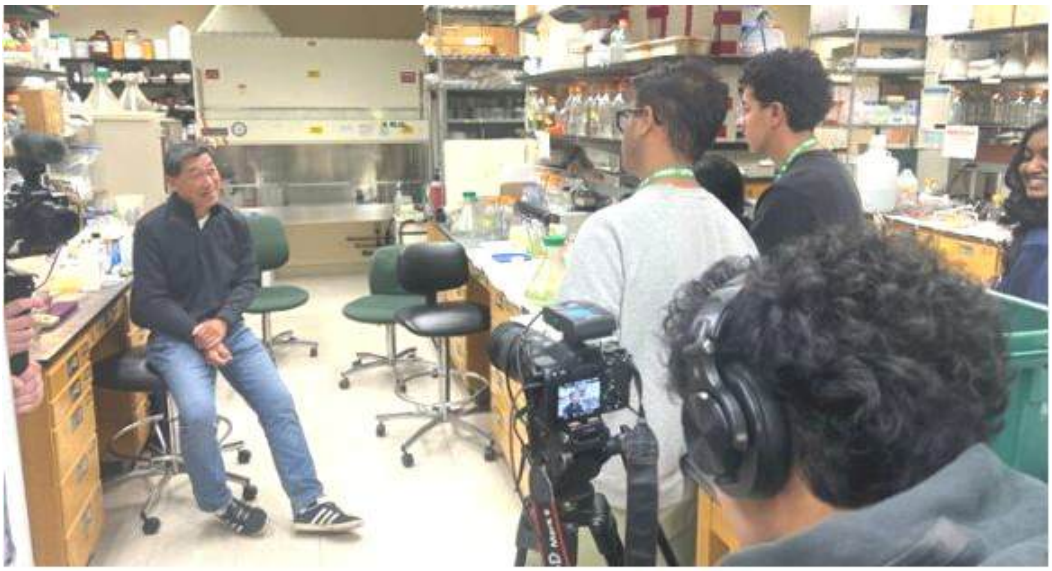
The FAME (Food, Agriculture, and Marine Ecosystems) afterschool 4-H program at Rutgers is transforming how high school students engage with science. This USDA-funded program supports youth to direct short video stories about authentic research in creative partnership with university scientists. FAME is an extension of an original science-in-action video storytelling model, designed by one of us (DS), with the purpose of engaging students in immersive STEM learning while producing story products that communicate the importance and value of research to the broader public. This science-in-action video storytelling model has produced more than a dozen high impact science film stories featuring scientists on journeys of discovery that have reached millions of people on multiple science communication platforms. The model's collaborative approach supports trusting partnerships between science storytellers and scientists to verify accuracy and create narratives that humanize scientists as complex, authentic people rather than stereotypes. Additionally, these science-in-action films often include members of the broader community who benefit from the featured science, such as farmers, fishers, teachers and business owners. Evidence of this model's effectiveness includes: 1) *Atlantic Crossing: A Robot's Daring Mission* airing more than 400 times on PBS stations reaching a potential audience of 180 million; 2) viewers (N=90) of the feature science documentary *Antarctic Edge: 70° South* showing increased understanding of climate change (71% to 91%); and 3) *Fields of Devotion* viewers (n=102) identifying scientists' passion (71.6%), desire to help others (70.6%), and commitment (69.6%) as key relatability factors—characteristics that research links to trustworthiness—while only 12.7% cited physical appearance, suggesting behavioral authenticity drives connection. The model's integration of documentary ethics, cognitive film theory, and collaborative storytelling creates "connectivity" between scientists and audiences, ultimately providing opportunities for audiences to relate to and trust in science.

Throughout the past Fall semester, students from the Edison High School in Edison township, New Jersey, create videos from their research, and interviews with one of us (EL), to mediate the messaging from his laboratory to the public or society-at-large. In addition to attending several lectures by EL, these students were welcomed into his laboratory to witness first-hand how duckweed can be grown under controlled environment. After they have done their follow-up research on their specific topics of interest, such as food security and

renewal fuel, the students then carried out in-person interviews with the scientist (see picture) and worked with their recordings to create the final video products. This collaborative storytelling fills a critical need for scientists to communicate their passion and vision so the impact of their work can be more readily conveyed and embraced by the public. The program is generously supported by a grant from the USDA-NIFA to Profs. James Simon (PI), Dena Seidel (Co-PI), Xenia Morin (Co-PI), and Oscar Schofield (Co-PI), with Marissa Staffen (co-PI) and Natalia Susana Hinds overseeing the high school youth recruiting and coordination activities. Additional support was also provided by the School of Environmental and Biological Sciences at Rutgers, as well as the Rutgers New Jersey Agriculture Experiment Station, New Brunswick, NJ.

After working with these talented and curious students over the past semester, we are very proud to share the fruits of their labor in these two short videos on duckweed's potential applications. We hope you will enjoy watching them and sharing them with your network and circles.

https://sites.rutgers.edu/fame/hungers-unexpected-hero-duckweed/
https://sites.rutgers.edu/fame/powering-our-future-with-duckweed/



FAME Fall 2025 cohort interviews Distinguished Professor Eric Lam about his research studying the food security and biofuel potential of duckweed.

Common insect pests on duckweed

A case study from Switzerland

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While exploring master's thesis topics on sustainable solutions, I became intrigued by duckweed after reading an article on the wide range of its applications. I was amazed that something often dismissed as mere green scum could serve as a key tool for building resilient ecosystems, advancing circular economies, and mitigating nutrient pollution. This sparked my strong interest in duckweed research, and currently I am seeking an opportunity to advance my work in this field.

Since 2015, the Research Institute of Organic Agriculture (FiBL) in Frick, Switzerland, has been investigating duckweed as a means to convert animal slurries into protein-rich feed. The project focuses on species including *Spirodela polyrhiza* (L.) Schleid., *Landoltia punctata* (G. Meyer), and *Lemna minor* (L.). During my tenure at FiBL, I worked with *L. minor* cultivated in four 7-m² outdoor pools and eight 1-m² experimental boxes (paloxes), fertilized with either liquid NPK fertilizer or diluted cow slurry. In the European regulatory framework, slurry is a defined product meaning "manure in liquid form, a mixture of excrements and urine of domestic animals, including possibly also water and/or a small amount of litter".

Our initial goal was to develop post-harvest processing techniques. However, severe and unexpected pest damage shifted my focus, I systematically documented the common insects infesting duckweed at the institute. Below, I outline the key pests, starting with the most destructive, and their impact on this miracle plant.

Small China-mark moth – *Cataclysta lemnata* Linnaeus, 1758 (Silent destroyers)

Two larval morphotypes were observed on the duckweed cultures in paloxes: surface-dwelling individuals and smaller, actively swimming instars (figure 1A and 1F, respectively). The latter caused the most damage. These tiny and translucent larvae (≃3mm) actively swim from the bottom of the water column to the top and vice versa, likely for feeding purposes. Despite initial lack of suspicion, the sampling trials revealed rapid population growth within two weeks, reaching 1.6 ± 0.15 larvae/cm² in palox 1 and 0.85 ± 0.1 larvae/cm² in palox 2, followed by decline and stabilization of the insect population (figure 2). This surge resulted in significant duckweed biomass loss. Mature larvae formed cocoons within fronds, with pupation sites often on dry margins. Adult moths, confined to pool edges, posed no threat.

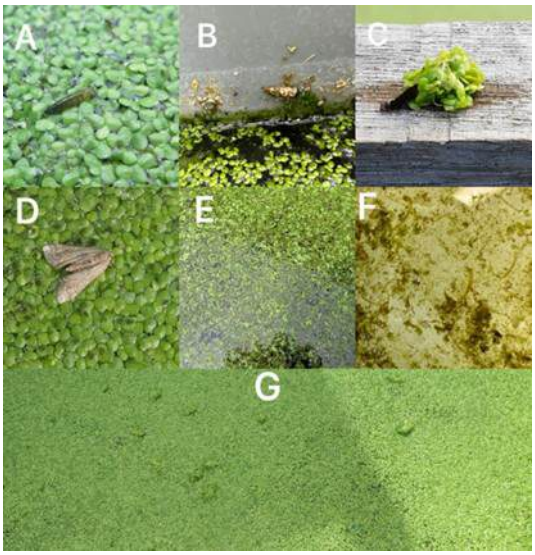

Figure 1: Life cycle of small China-mark moth, A- free living larva, B- pupa, C- larva inside cocoon, D- adult moth, E- heavily damaged duckweed population, F- tiny swimming larvae, G- cocoons on duckweed

Figure 2 Numbers of tiny swimming larvae of the small China mark moth on two culture boxes. Mean number of larvae per square centimetre – L/cm² on x-axis and sampling week on y-axis), values = mean ± SD/SE, N = 3

Water-lily aphids (*Rhopalosiphum nymphaeae*): Water-lily aphids were found in different developmental stages (assessed based on their size) residing on the surfaces of duckweed (figure 3A). These aphids did not appear to have a major impact on duckweed biomass, but are often found in large number.

Clubbed general soldier flies (*Stratiomys chamaeleon* Linnaeus, 1758): Clubbed general soldier flies (figure 3B) leave trails in duckweed, very active during warm and sunny conditions, did not find any apparent signs of damage even though these larvae were abundantly present.

Water beetles (*Helochares spp.*): A small number of water beetle larvae were found at the edges of the duckweed pools (figure 5). Microscopic observations revealed that these larvae possess mandibles near their mouth, which support their role as predators.

Figure 3 Water-lily aphids, A- aphid populations on edges of boxes, B- multiple stages of aphids, C- magnified image of aphids on duckweed

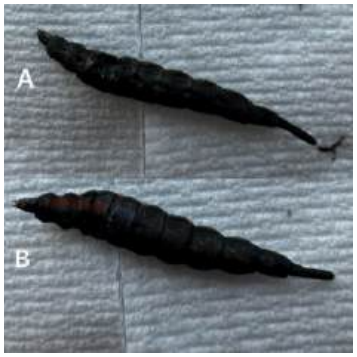

Figure 4 Clubbed general soldier fly larva, A- right later view, B- left lateral view

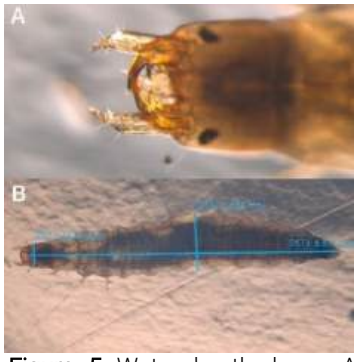

Figure 5 Water beetle larva, A- mouthparts in the head segment, B- measurements of the body

Overview

Among insects observed on duckweed at FiBL, only *C. lemnata* and *R. nymphaeae* were considered pests, with *C. lemnata* causing most of the damage. Similar problems have been reported in Vietnam, where rice caseworm larvae (*Parapoynx stagnalis*) had a substantial negative impact on a 4,000 m² *Spirodela polyrhiza* culture. Student on field work have reported similar issues in Ivory Coast. Mariani et al. (2021) suggested the use of the small China-mark moth as a biocontrol agent for invasive duckweed, however the same moth is a major management issue in production systems, especially larger setups. Apart from pests, diseases can also reduce yields: patches of discoloured and bleached *Lemna minor* spread quickly in hydroponic systems, and *Pythium myriotylum* is often identified as the cause. The first report of this pathogen refers to its presence on duckweed in Germany (Brand et al., 2021).

Control

To control duckweed pests, we tested Pyrethrum FS, an organic insecticide. Direct spraying on the plants had little effect on *C. lemnata* larvae because they hide under the fronds, avoiding exposure. Furthermore, the active compound breaks down quickly in sunlight, making dawn or dusk application preferable. It was discovered that it was more effective to harvest the duckweed, apply a 0.05% solution of Pyrethrum FS directly to the medium as instructed on the label, and then return the plants. This method effectively reached the concealed larvae. The same insecticide, when sprayed from above, worked well against surface-dwelling aphids, although these cause far less damage and have never led to duckweed culture collapse. Disease issues also need attention. Outbreaks caused by pathogens such as *Pythium myriotylum* can spread quickly in dense stands, and basic hygiene measures like cleaning tanks, removing infected material, and maintaining good water circulation are currently the most practical tools for prevention.

For future large-scale production, ranging from thousands of square meters to hectares, reliable and scalable strategies for both pest and disease control will be essential.

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Request for applications to host ICDRA-2028

In order to identify the best venue possible for the next International Conference on Duckweed Research and Applications (ICDRA-2028), applications from interested institutions/organizations are requested to be sent to one or more members of the ISCDRA members by 10th July, 2026.

The application should briefly introduce the proposed venue, its benefit/attractions, relevance to duckweed research and/or applications, and the responsible organizer's credentials as well as experience. The list of all applications will be sent out to the community in the July, 2026 issue of "*Duckweed Forum*" before the 8th ICDRA and decision by popular vote of the attendees will be made during the "General Assembly" at the end of the conference in Italy in October, 2026.

Duckweed's first iGEM Grand Prize

Making Duckweed "Sexy for biotech"

Matúš Grieš, **Matej Zámečník,** Jonáš Pospíchal, Pravoslav Žilka, Miroslav Rosputinský
Nitroduck (Email: miro.rosputinsky@gmail.com)

In the July 2025 issue of the Duckweed Forum, our team – iGEM Brno – was introduced through an interview conducted by Tsipi Shoham, the CEO of GreenOnyx. We participated in the world's largest synthetic biology competition with a goal of making genetic engineering of duckweed faster and simpler - essentially to make it "sexy for biotech" :). We believe that the time required and technical difficulty of genetic modification of duckweed species remain the main bottlenecks holding back the full potential of this remarkable plant.

Although our novel transposase-based genetic engineering system did not work in its entirety, we successfully achieved *in planta* genomic integration that remained stable across multiple generations. In parallel, we built a completely autonomous, app-controlled, modular cultivation unit and developed a high-accuracy computational model capable of predicting optimal harvesting ratios and frequencies.
At the iGEM competition in Paris, we presented our results alongside more than 400 teams from around the world. In iGEM's 21-year history, this was the first time duckweed made it to the Grand Stage. And sometimes, first time *is* the charm. After being selected as one of two finalist teams, we presented duckweed to an audience of roughly 5,000 attendees and answered questions from over 300 judges. Ultimately, our project was awarded the "Overgraduate Grand Prize". In addition, we received the Best Agriculture, the Best Plant Synthetic Biology and the Best Presentation awards and were nominated for the Best Hardware and the Best Wiki awards.
None of this would have been possible without the duckweed community. Throughout the competition, we were incredibly fortunate to receive generous support, advice, and shared expertise. We would like to especially thank Dr. Arturo Marí-Ordóñez for countless insights, materials, and knowledge; Prof. Asaph Aharoni and Julia-Eva Fortmueller for teaching us the basics and helping us lay the foundations; Dr. Laura Morello for providing duckweed lines; Dr. Tsipi Shoham and GreenOnyx for sponsoring the wet-lab part of our team - the Duckweed Hackers; and Prof. Marcel Jansen for cultivation insights. You are the giants on whose shoulders we stood.
iGEM may be over, but our work is only beginning. We are now transitioning to the commercial sector - we believe that another successful business case can help the improve the profile of duckweed worldwide. Still, we are continuing our efforts to simplify and accelerate genetic modification of duckweed. And because this community has given us so much, once we succeed, we will make the method freely available. We believe that realizing duckweed's potential is best done together - and that shared progress is the fastest way forward.

Weblink:
https://video.igem.org/w/ohNxoKMPBaENiaz3Ayxy9Z

Below: iGEM Brno Cultivation system

Right: iGEM Winners- Brno Team

Brief report on the Third Symposium on Duckweed Research and Development

Organized at Chengdu, China from 20-21 December, 2025

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The first Symposium on Duckweed Research and Development (1st SDRD) in China was initiated and co-organized in 2023 by Prof. Hai Zhao from the Chengdu Institute of Biology, Chinese Academy of Sciences (CAS), and Prof. Zheng-Biao Gu from Jiangnan University. Although duckweeds have been studied for decades, research had remained largely confined to academic circles as a niche subject until its recent renaissance. Prof. Hai Zhao chose to adopt the approach to focus on the application of duckweed as a bioreactor and also as a plant for ecological remediation. Since 2016, Prof. Zheng-Biao Gu is collaborating with Prof. Hai Zhao in duckweed research, demonstrating that duckweed starch quality is comparable to that of corn starch. This finding underscored the industrial value of duckweed as a bioreactor, at least for starch production. To promote duckweed research and development in China, they jointly organized the 1st SDRD.

Following the 2nd SDRD held in Wuxi in 2024 and hosted by Prof. Zheng-Biao Gu, the 3rd SDRD returned to Chengdu on December 20–21, 2025, hosted by Prof. Hai Zhao. Prof. Zhi-Hong Xu, former President of Peking University, delivered a written speech recalling the rise of duckweed R&D in China and emphasizing its importance for future agriculture. Prof. Yu-Long Yin, an Academician in swine husbandry, gave a keynote speech on the great potential of duckweeds in pig farming. Profs. Hai Zhao and Zheng-Biao Gu respectively reported on their work and progress. Prof. Lin Yang of Tianjin Normal University presented collaborative work with Prof. Dao-Xin Xie's laboratory at Tsinghua University on the effect of extracellular vesicles on tryptamine metabolism. Dr. Shuang Chen, a Research Associate in Hai Zhao's lab, reported improvements in duckweed genetic transformation. Prof. Yong-Gui Zhao of Yunnan University, a former student of Hai Zhao, shared his progress on using duckweed for treating livestock wastewater. Prof. Xin-Hui Wang from Chengdu University, also a former student of Prof. Hai Zhao, reported on duckweed application in remediating cadmium pollution in Sichuan Province. Dr. Yan-Lin Jin, a Research Associate in Hai Zhao's lab, presented findings on phosphate accumulation in duckweed. Prof. Wen-Wu Wu (Zhejiang Agriculture and Forest University, Lin'an, Zhejiang Province), reported his work on genomic changes during the transition of land angiosperms back to aquatic life, recently published in Current Biology. In addition to these presentations, five short talks covered a wide range of topics: a survey of duckweed distribution in China by Dr. Yao Xiao (Sichuan University of Science and Engineering, a former student of Prof. Hai Zhao); turion induction by Dr. Ya-Liang Xu (Institute of Urban Agriculture, CAAS, a former student of Prof. Hai Zhao); duckweed protein secretion by Prof. Qiong Zhao (East China Normal University); chloroplast development in duckweed by Dr. Yi-Chuan Wang (a Research Associate in Prof. Hong-Wei Guo's lab at the Southern University of Science and Technology, Shenzhen); and network analysis of duckweed starch metabolism by Qing-Yu Pang (a graduate student of Prof. Fang-Ting Li at Peking University). Finally, Prof. Lin Yang introduced a special issue on duckweeds published in Frontiers in Plant Science.

Group Photo of the attendees at the Third Symposium on Duckweed Research and Development. The organizers Prof. Hai Zhao and Prof. Zheng-Biao Gu are in the front row, 7th and 10th person from the right, respectively.

Beyond the regular presentations and short talks, the symposium featured two roundtable discussions. The first focused on the future and challenges of scaling duckweed production to an industrial level. Following an opening remark by Prof. Emeritus Shu-Nong Bai of Peking University—who elaborated on his rationale for calling the industrialization of duckweed production "the second agricultural revolution"—representatives from enterprises and government contributed their concerns and suggestions regarding duckweed R&D in China. Some of the topics discussed were: 1) where the initial investment may come from? 2) whether duckweed R&D could be put in the governmental agenda and which governmental sectors should coordinate? 3) how researchers and entrepreneurs may collaborate, were the key items explored. The second roundtable primarily addressed how researchers within the duckweed community in China could share resources, experiences, and interests.

A notable aspect of the symposium was the attendance—either in person or through representatives—of several well-established plant biologists. These included: Zhi-Yong Wang, an expert in brassinosteroid research from the Carnegie Institution for Plant Biology at Stanford University (CA, USA); Sodmergen, an expert in mitochondrial development from Peking University; Wei Wang, an expert in plant immunity from Peking University; Xiao-Dong Su, an expert in functional genomics from Peking University; Qiong Zhao, an expert in plant cell biology from East China Normal University; Fang-Ting Li, an expert in network analysis of biological processes from Peking University (represented); Le-Gong Li, an expert in ion channels from Capital Normal University (represented); Hong-Wei Guo, an expert in ethylene research from the Southern University of Science and Technology (represented); and Jia-Wei Wang, an expert in plant genome analysis from the CAS Center for Excellence in Molecular Plant Sciences, in Shanghai (represented). All newcomers expressed an optimistic outlook for the future of duckweed research and development.

The 4th SDRD would be held in Wuxi in 2026, hosted by Prof. Zheng-Biao Gu.

Acknowledgements

Author would like to thank Eric Lam for his invitation and critical reading of the report.

Student spotlight: Matteo Agastra

From New Providence High School to Cornell University: An Aspiring Biological Engineer's Interest in Duckweed Biofuels

(Email: matteo.agastra@gmail.com)

My journey with duckweed first began at the Waksman Student Scholars Program Summer Institute at Rutgers University, New Jersey, USA. At the Summer Institute, I learned the basic molecular biology techniques for working with DNA like polymerase chain reaction (PCR), restriction digestion (RD), plasmid isolation, and agarose gel electrophoresis so that I could teach these exact techniques back at my school. Coming into the Summer Institute, I was motivated by the premise of spreading my passion for molecular biology to underclassmen back at my high school. But as I continually jotted down notes from the lectures, I became fascinated by the overarching goal of the Waksman Student Scholars Program: duckweed.

During my time at the Summer Institute, I learned surprising facts about duckweed as a next-generation biofuel. Not only does it theoretically have triple the ethanol yield per acre compared to corn, but it's also soil-independent and acts as a bioremediator. This begged the question, why corn and not duckweed? With some more research, I learned a challenging aspect of duckweed: maintaining rapid biomass accumulation and consistently high starch content at commercial scale remains a major challenge (Faizal, 2021).

Currently, we are witnessing the transition from gas to electricity for powering cars. But I believe the near future will have a new electric–duckweed. I wish to be at the forefront of this emerging breakthrough field. And after reading through the Duckweed Forum, I've come to understand there is so much more to learn and optimize about duckweed and that breakthroughs are happening frequently. My career goal is to lead a successful duckweed biofuel startup, developing duckweed as the next biofuel and the new electric.

Once I complete my senior year of New Providence High School, New Jersey, U.S.A. in the spring of 2026, I will continue my education at Cornell University in Ithaca, New York, U.S.A. studying Biological Engineering. I'm hoping to connect with fellow duckweed researchers in the New York Metropolitan Area, and elsewhere, so that I can make my career aspiration of leading a major duckweed biofuel startup possible. If you would like to make any more inquiries or potentially connect with me, feel free to email me or connect with me on LinkedIn at Matteo Agastra.

Reference

Faizal A; Sembada AA; Priharto N (2021) Production of bioethanol from four species of duckweeds (*Landoltia punctata*, *Lemna aequinoctialis*, *Spirodela polyrrhiza*, and *Wolffia arrhiza*) through optimization of saccharification process and fermentation with *Saccharomyces cerevisiae*. Saudi Journal of Biological Sciences 28: 294-301.

From the Database

Highlights

Optimizing *Wolffia globosa* protein extraction by ultrasonic pretreatment and enhancing protein attributes through LAB fermentation

Taramark, N; Rice, D; Panya, A; Anal, AK. (2025) Sustainable Food Technology DOI10.1039/d5fb00550g

The rising global population is generating food security issues, particularly in protein demands and nutritional quality. Many plant-based foods have been explored to meet this demand, although they often lack total protein or protein quality. Therefore, underutilized water crops, such as *Wolffia globosa*, offer great potential for alternative protein production. This study investigated the parameters of ultrasonic-assisted alkaline extraction and probiotic fermentation to extract and enhance the qualities of *Wolffia* protein. Response surface methodology was utilized to optimize ultrasound-assisted alkaline extraction, resulting in a maximum soluble protein yield of 118.44 ±13.60 mg g-1 under conditions of 95% amplitude, 16 minutes extraction time, and a 1 : 20 g mL^{-1} solid-to-liquid ratio. This method increased total protein content by 127%. Subsequent fermentation with *Lactobacillus plantarum* 2075 then improved nutritional quality by raising digestibility from 55.82% to 70.45%, increasing essential amino acid content, and raising antioxidant activity by 15.24% and phenolic content by 98.89% relative to the raw sample. Fermentation also modified technofunctional properties of the extracted sample, reducing foaming capacity and emulsion activity but improving the foam and emulsion stability. These findings highlight that ultrasonic-assisted alkaline extraction improves protein yield, and sequential fermentation further modifies protein characteristics and significantly enhances nutritional quality. The dual processing strategy for *W. globosa* thus presents a promising sustainable alternative for quality plant protein in diverse food applications.

Comparative analysis of Araceae mitochondrial genomes: Implications for adaptation to ecological transitions in plants

Chen, YX; Gao, S; Wang, JQ; Cheng, X; Chen, Y; Chinta, V; Kan, SL. (2025) Genes 16: 1241.

Plant mitogenomes display remarkable variation in size, structure, and gene content, yet their evolutionary causes remain unclear. Araceae, the most significant family within Alismatales, encompasses both aquatic and terrestrial lineages, providing an excellent system for studying how ecological shifts influence mitogenome evolution. We assembled and annotated four new mitogenomes using both short- and long-read sequencing, including three aquatic taxa (*Pistia stratiotes* L., *Spirodela intermedia* W. Koch, *Wolffia australiana* (Benth.) Hartog & Plas) and one terrestrial species (*Amorphophallus konjac* K. Koch). Along with five previously published mitogenomes, we performed comparative analyses across nine Araceae species. These mitogenome sizes varied from similar to 178 kb to similar to 877 kb, consisting of one to 19 circular molecules, with aquatic species generally having smaller and simpler structures. Plastid-derived sequences (MTPTs) contributed 1.2-10.6% of genome content, peaking in *Zantedeschia aethiopica* (L.) Spreng. Despite significant structural heterogeneity, all species maintained core respiratory genes under strong purifying selection, while ribosomal protein-coding genes showed lineage-specific loss. RNA editing ranged from 363 to 772 sites per mitogenome, with the number of sites independent of mitogenome size. Overall, this study uncovers the dynamic evolutionary patterns of Araceae mitogenomes and offers a framework for understanding how habitat shifts between aquatic and terrestrial environments influence mitogenome diversity in plants.

DF Comment: Most duckweed researchers consider duckweed (Lemnaceae) as a plant family of its own, cf. Tippery et al. Lemnaceae and Orontiaceae are phylogenetically and morphologically distinct from Araceae. Plants (2021) 10: 2639. https://doi.org/10.3390/plants10122639.

Aquaculture/ Agriculture

Short-term effect of changing water regimes on the soil nematode community in rice-duckweed system under water-saving irrigation

Ahmed, Z; Xu, JZ; Liu, WX; Liu, XY; Li, YW; Guo, H; Chen, SY. (2026) European Journal of Soil Biology 128: 103791.

Rice agroecosystems have garnered global attention owing to a unique transition from traditional flooding to water-saving practices, such as alternate wetting and drying (AWD) irrigation, which features a dual-habitat ecosystem with high biodiversity throughout the growing period. Nematodes' taxonomic bioindication potential could reveal perturbations in the soil environment and changes in food web status under AWD. However, the influence of changing dual modes (dry and wet habitats) with duckweed (D) on nematodes was often overlooked. Therefore, a field experiment was conducted to quantify the impact of flooding irrigation, and alternate wetting and drying irrigation on nematode abundance across four treatments: flooding irrigation with (FI + D) and without duckweed (FI), alternate wetting and drying irrigation with (AWD + D) and without (AWD) duckweed. For flooding irrigation, soil samples were taken at tillering, pre-mid-season drainage (MD), and at flowering post-MD. Similarly, for alternate wetting and drying irrigation at tillering and flowering for respective dry and wet cycles. The first AWD dry-cycle with duckweed (AWD + D) significantly reduced nematode abundance compared to the wet cycle (AWD + D) ($P < 0.033$). In FI, MD significantly reduced nematode abundance ($P < 0.007$) compared to FI + D. Factor prediction analytics showed that pH in the FI post-MD and [NH4+-N] in flooded and AWD-integrated treatments were the most influential abiotic drivers governing nematode taxonomic and functional diversity. AWD dry-wet cycles with duckweed, fuel colonizer CP (1-2, r-strategists) nematodes, indicating better soil health and higher microbial turnover than without duckweed. The AWD drying cycle with duckweed produced more mature, fertile soils with balanced bacterivores/ fungivores and moderate C:N ratios, and demonstrated suppressive properties, which further seek an investigation into the link between soil biochemical enrichment from decomposing duckweed.

DF comment: The authors' method of collecting duckweed for this study was described as "Duckweed (*Lemna minor* L.) was gathered from a drainage channel near the experimental site". It should be pointed out that whether there is a mixture of various species or hybrids of different *Lemna* species would be difficult to know. How was this wild population identified to be *L. minor* should be described to enable others to replicate the findings or at least compare results.

Integrating alternate wetting and drying irrigation with duckweed for potential microplastic mitigation in rice ecosystems

Wang, Z; Hong, C; Qiu, R; Wang, Y; Shaghaleh, H; Hamoud, Y.A; Agathokleous, E. (2025) Journal of Hazardous Materials 502: 140973.

Water-saving irrigation in rice fields can reduce water input and enhance water productivity, while widespread microplastics (MPs) contamination introduces an environmental risk. Duckweed is a hydrophyte that commonly floats in rice fields and can capture MPs. Regular harvesting of these contaminated plants prior to decomposition could prevent secondary pollution and ensures the permanent removal of accumulated MPs from the paddy ecosystem. However, it remains unknown whether water-saving irrigation, such as alternate wetting and drying irrigation (AWD), can promote MPs capturing through duckweed physio-anatomical modifications. In this study, MPs-contaminated rice-duckweed system was exposed to two irrigation regimes, i.e. conventional continuous flooding irrigation (CF) and AWD. The results showed that AWD led to duckweed capturing up to 16.0-fold more MPs than under CF. We discovered that this increase was due to the considerably higher number of MPs particles per unit surface area (or per unit length) captured by duckweed, despite the reduction in duckweed coverage rate (42.4 %-48.8 %) and root length (23.2 %-67.7 %) during dry periods of AWD. We further revealed that the greatly increased MPs numbers per unit area on the frond of duckweed under AWD was primarily due to improved adhesion and physical interception, achieved through processes such as sticking, trapping, and entangling, facilitated by the rougher surface of duckweed. Notably, experimentally validated detachment curves of MPs, well fitted with linear with lower plateau models ($P < 0.01$), revealed that AWD resulted in greater capturing of MPs on abaxial fronds than CF. Our work uncovers a potential microplastic mitigation method in rice ecosystems by using duckweed combined with AWD in future.

DF comment: Authors stated that "The duckweed species (genus: Lemna; family: Lemnaceae) used in this study was obtained from the Shuangying Agricultural Supplies Store (Kunshan, China)." Additional information as to the species identity and heterogeneity of these biological samples are important to enable future replication of these results as well as to compare to other work using different or the same species/accession of duckweed.

Physiological and biochemical responses of lettuce to arbuscular mycorrhizal inoculation and *Landoltia punctata* extract applications

Patloková, K; Ferby, V; Slany, V; Oravec, M; Tríska, J; Masán, V; Burg, P; Pokluda, R. (2025) Horticultuae 11: 1310

The use of biostimulants offers a sustainable strategy to improve crop quality. This study assessed the effects of an arbuscular mycorrhizal fungi inoculum (consisting of species *Claroideoglomus claroideum, Claroideoglomus etunicatum, Funneliformis geosporum, Funneliformis mosseae* and *Rhizophagus irregularis*) and an 0.5% aqueous extract of *Landoltia punctata* on the growth and biochemical composition of lettuce (*Lactuca sativa* L. cv. Dubáček') under indoor conditions. Four variants were tested: control (C), mycorrhiza (M), *L. punctata* extract (L), and their combination (M + L), with biometric, physiological, and biochemical parameters evaluated. Simultaneously, the amino acid profile of *Landoltia* extract was determined, and the degree of plant colonization by mycorrhizal fungi was evaluated. While biostimulant treatments did not affect above-ground biomass, *L. punctata* extract (L and M + L) significantly raised chlorophyll a (by 15.9% and 16.0%) and chlorophyll b (by 55.5% and 42.8%) compared to the control. The combined treatment (M + L) achieved the highest total phenolic content (254.28 mg/kg). All treated variants significantly reduced leaf nitrate content, with M and M + L being most effective (-35.1% and -33.6%). Amino acid metabolomic analysis showed that the extract is rich in gamma-aminobutyric acid, valine, phenylalanine, tryptophan, and other proteinogenic amino acids that may drive its biostimulant effects. Microscopy confirmed successful root colonisation in mycorrhizal variants (58% in M, 42% in M + L). Although the biostimulants did not significantly affect growth, their application is recommended to improve lettuce quality by enhancing photosynthetic pigments and phenolic compounds while reducing nitrate content, indicating their potential for producing safe, higher-quality crops.

Nitrogen and phosphorus removal from anaerobic baffled reactor effluent using *Lemna minor* and fertiliser value of the biomass for ryegrass production

Muchaonyerwa, P; Oyawoye, AA; Odindo, AO. (2025) Environmental Monitoring and Assessment (2025) 197: 1188.

Effluents from decentralised wastewater systems, using an anaerobic baffled reactor (ABR), have high concentrations of nitrogen (N) unsuitable for safe disposal. The study investigated the effects of duckweed (*Lemna minor*) density and effluent dilution on N removal and biomass accumulation, over 14 days. The duckweed biomass was tested as a fertiliser for ryegrass (*Lolium perenne*) at (i) 200 kg N ha^{-1} (DWN), (ii) 80 kg P ha^{-1} (DWP), (iii) DWN with mineral P to 80 kg P ha^{-1} (DWN + P), compared with (iv) inorganic N fertiliser and two negative controls with (v) P and potassium (K), and (vi) K only. Biomass and N (11-56 mg L^{-1}) removal increased with effluent dilution and higher density. The 1:3 dilution (effluent: water) and chemical fertiliser (CF) had higher biomass than other treatments, except 1:1 at 800 g m^{-2}. The treatments had similar percentage N removal (> 79%), except the 3:1 with 400 g m^{-2} duckweed (73%). Duckweed treatments had higher ryegrass dry matter, and N and P uptake, than the controls without N. The DWP and inorganic fertiliser with N had similar ryegrass dry matter and N uptake, which were higher than for DWN and DWN + P. The findings showed that duckweed, cultured at 600-800 g m^{-2}, could efficiently remove N from diluted ABR effluent for safe disposal, and that duckweed biomass increases ryegrass N uptake and dry matter to similar levels as inorganic N fertiliser, especially when applied to meet P requirements.

DF comment: The duckweed used are described as "The duckweed (*L. minor*) was collected at Ashburton (29°40'S; 30°27'E) in Pietermaritzburg, KwaZulu-Natal". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be quite prevalent in nature.

Biotechnology

Extraction and characterization of functional duckweed proteins using alkaline, enzymatic, and ultrasound-assisted techniques

Akyüz, A; Ersus, S. (2026) Protein Expression and Purification 239: 106860.

The urgent need for sustainable, plant-based protein sources has brought aquatic crops like duckweed (Lemnaceae) into focus due to their high protein content, fast growth, and minimal resource requirements. This study aims to optimize and compare alkaline, enzyme-assisted, and ultrasound-assisted extraction techniques to produce high-yield, functional protein concentrates from *Lemna minor*. Key extraction parameters-pH, temperature, and solvent-to-solid ratio-were systematically optimized, identifying pH 9, 55°C, and 5 mL/g as ideal conditions. Ultrasound treatment (100 % amplitude, 10 min) and Alcalase L enzyme application significantly enhanced protein yields. Ultrasound-assisted extraction proved most effective, increasing protein yield by more than fivefold compared to conventional alkaline methods, reaching 60.09 % protein content on a dry basis. The resulting protein concentrates displayed desirable functional properties, including excellent solubility (92.19 % at pH 9), foaming capacity (92.62 %), and emulsion activity, all of which are critical for food applications. Comprehensive structural and compositional analyses confirmed the presence of essential amino acids, high mineral content (notably calcium and phosphorus), and acceptable techno-functional behavior. SEM and FTIR analyses supported the structural integrity of the extracted proteins, while flowability assessments suggested suitability for powder-based formulations. This research demonstrates that ultrasound-assisted protein isolation from duckweed offers a scalable and efficient strategy for producing high-quality protein concentrates. These findings position duckweed as a promising, sustainable protein source with strong potential for incorporation into functional foods and novel plant-based formulations.

DF comment: The duckweed used are described as "Duckweed (*Lemna minor*) samples were sourced from the Department of Botany at Ege University and from a local supplier in Izmir". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature.

Investigation of the radioprotective properties of lignin under conditions of acute gamma irradiation of duckweed *Lemna minor* L.

Bodnar, IS; Raskosha, OV; Karmanov, AP; Kocheva, LS. (2025) Russian Journal of Bioorganic Chemistry 51: 2948-2955.

The work is focused on protecting plant organisms from acute gamma radiation exposure. The study specifically investigates the use of lignin from wild rosemary (*Ledum palustre* L.) as a radioprotective agent for the aquatic plant *Lemna minor* L. *Lemna minor* L. was irradiated at doses up to 63 Gy, and the plant biomarkers were assessed. Water-soluble lignin from wild rosemary was used to cultivate plants in aqueous lignin-containing media to evaluate its potential radioprotective effects. The study found that water-soluble lignin from wild rosemary is not toxic to *Lemna minor* L. Pre-cultivating the plants in lignin-containing media resulted in a reduction of radiation-induced damage and an increase in root length, suggesting the adaptogenic and radioprotective properties of lignin. These findings were observed at both the organismal and populational levels. The research for the first time proposed a hypothesis about the radioprotective potential of lignin and experimentally demonstrated that exogenous lignin could protect plant organisms from the effects of acute exposure to high-dose gamma radiation.

Dual application of *Spirodela polyrhiza* and *Lemna minor* in sewage phytoremediation and feedstock production for black-soldier fly larvae cultivation: A novel bio-circular system

Pawaiya, A; Mishra, A; Suthar, S. (2025) Chemosphere 390: 144713.

This study investigates an integrated and sustainable approach to urban sewage phytoremediation using the duckweed (DW) species *Spirodela polyrhiza* and *Lemna minor*, as well as assessing the potential of harvested DW biomass as feedstock for black soldier fly larvae (BSFL) cultivation. Phytoremediation of sewage by DW caused significant removal (%) of NO_3-N (73.57-81.50), NH_4^+- N (42.13-63.89), PO_4^{-3} (70.33-74.44), SO_4^{-2} (57.01-63.32), and COD (75.17-77.87), indicating the suitability of such floating systems for sewage treatment. DW

biomass exhibited a high production rate of 128.15 ± 0.77 g-fresh weight/m^2/day (*S. polyrhiza*) and 73.26 ± 0.85 g-fresh weight/m^2/day (*L. minor*) in phytoremediation setups. Biochemical analysis revealed increased (dry-weight basis) content of total carbohydrates (13.68 %-19.80 %), crude protein (24.63 %-28.47 %), lipids (17.13 %-20.71 %), and starch (13.46 %-17.21 %) in harvested DW biomass, highlighting its potential as a feedstock for BSFL cultivation. However, using DW alone as BSFL feed may not be practical, but promising results can be achieved when combined with food waste (FW). Consequently, DW was mixed with FW at various ratios (30 %, 50 %, 70 %, and 100 %), and BSFL cultivation results indicated high feed conversion ratios, bioconversion rates, and BSFL growth in waste mixtures with 30 % DW. BSFL frass was also analyzed for potential use as manure, but was found unsuitable due to the low seed germination index (GI) (10.50-42.60 %). To address this, the frass was aerobically composted for 28 days, which significantly improved the GI (>70 %) and enriched the manure with valuable soil nutrients (N, P, and K), confirming its potential as a plant potting medium. In summary, integrated wastewater phytoremediation and plant biomass valorization in BSFL cultivation present a low-cost, sustainable solution for addressing water pollution and weed eradication, while enabling resource recovery through protein-rich animal feed and bio-manure production.

DF comment: The duckweed used are described as "*S. polyrhiza* and *L. minor* were employed for the cultivation of biomass in wastewater, originally collected from the plant nursery located near the Doon University campus in Dehradun". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature.

Bioactive potential of protein extracts derived from dried *Wolffia globosa* on in vitro antioxidant activities and pro-inflammatory cytokine production

Khonkarn, R; Daowtak, K; Kraseasintra, O; Luetragoon, T; Usuwanthim, K; Taynawa, K; Chanphong, K. (2025) Molecules 30: 4092.

This study investigated the contamination, composition, and functional properties of *Wolffia globosa* from northern Thailand. The results showed that the heavy metal content of dried *W. globosa* complied with Thai regulations, ensuring its safety. Its proximate analysis revealed high protein levels with lysine, leucine, and phenylalanine as the principal essential amino acids. The protein was effectively extracted using the alkaline extraction method, followed by precipitation induced by acid or heat. The precipitates and supernatants resulting from various acid- or heat-induced protein precipitation were obtained. The highest protein content was found in the pH 3 precipitate (51.15 ± 6.71%). In contrast, the pH 5 supernatant exhibited the most potent antioxidant activities (2.22 ± 0.05 mmol Trolox/mg and 4.55 ± 0.18 mmol Fe^{2+}/mg), as determined by ABTS and FRAP assays, respectively. Additionally, a strong correlation was observed between phenolic content and antioxidant activity. Both supernatant and precipitate protein extracts from *W. globosa* exhibited no cytotoxicity in THP-1 cells and displayed anti-inflammatory effects by decreasing the production of IL^{-1} beta and IL^{-6}. They also downregulated phospho-NF-kappa B, phospho-I kappa B-alpha, and COX-2, consistent with reduced NF-kappa B pathway activation. These findings position *W. globosa* as a promising, sustainable plant-based protein with bioactive and functional properties, making it a viable candidate for functional food formulations that enhance dietary health and add value to local agricultural resources.

Enhancement of the duckweed biomass and starch production utilizing biogenic MnO and ZnO microparticles

Anar, M; Shuvro, SK; Munis, MFH; Morikawa, M. (2025) Biotechnology Reports 47: e00907.

The effects of biologically synthesized MnO and ZnO microparticles (MPs), were evaluated on the growth of *Lemna minor*. Both MnO and ZnO MPs promoted the growth of *L. minor* at low concentrations of 1-8 mg/L. In contrast, 1000 mg/L MnO and ZnO MPs reduced the growth of *L. minor*. It was also found that the starch content of *L. minor* and *Spirodela polyrhiza* was enhanced upon growing in 1 mg/L of MnO MPs when compared to no MnO MPs. These effects were suggested to be due in part to the fact that biogenic MPs harbored small but significant amounts of inorganic phosphorus. Our findings indicate that biologically synthesized MnO and ZnO MPs can act as novel plant growth regulators to enhance the production of useful duckweed biomass with high starch content.

Exploring natural dyes from microalgae and plants for high-efficiency in TiO2-based dye-sensitized solar cells

Lana, GM; Adenigba, VO; Alamu, GA; Oyewole, OJ; A Ajayeoba, Y; Babalola, KK; Oyeshola, HO; Bello, IT; Adedokun, O. (2025) Journal of Materials Science-Materials in Electronics 36: 1713.

This study explored the potential of natural pigments extracted from *Chlamydomonas starii, Coelastrella* sp., *Sorghum bicolor* leaves, and *Spirodela polyrhiza* as sensitizers in dye-sensitized solar cells (DSSCs) using TiO2 as the photoanode. Characterization of TiO2 via XRD, SEM, FTIR, and DRS confirmed its suitability for efficient dye adsorption and electron transport. UV-Vis spectroscopy revealed distinct absorption spectra for each dye extract, with bandgaps ranging from 1.67 eV (*Coelastrella* sp.) to 2.87 eV (*Spirodela polyrhiza*). Photovoltaic performance evaluation demonstrated *Spirodela polyrhiza* as the most efficient sensitizer, achieving a power conversion efficiency (PCE) of 3.685%. Furthermore, a synergistic effect was observed with a combination of *Sorghum bicolor* and *Chlamydomonas starii*, yielding a PCE of 3.762%. These findings highlight the promising potential of natural dyes, particularly from aquatic sources, for developing low-cost and sustainable DSSCs. Further research is warranted to optimize dye extraction, enhance dye-TiO2 interactions, and improve long-term stability for real-world applications.

Towards higher energy conversion efficiency by bio-hydrogen and bio-methane co-production: Effect of enzyme loading and initial pH

Zhang, XT; Song, ZP; Jiang, DP; Xia, CX; Li, ZJ; Li, WZ; Zhang, QG. (2025) Fermentation 11: 503.

Bio-hydrogen and bio-methane co-production was a promising way to enhance the energy conversion efficiency, and enzyme loading and pH are key factors influencing anaerobic fermentation processes. Therefore, in this study, the co-production process of bio-hydrogen and bio-methane was evaluated based on the effect of enzyme loading (20%, 30%, and 40%) combined with initial pH (6.0, 7.0, 8.0, and 9.0). The results indicated that, compared with other conditions, 30% enzyme loading with an initial pH of 8.0 was more feasible for bio-hydrogen and bio-methane co-production from duckweed, achieving a bio-hydrogen yield of 114.56 mL/g total solid (TS) and a bio-methane yield of 260.32 mL/g TS. Under optimum condition, the energy conversion efficiency was 71.4%, which was 6-fold and 4.8-fold higher than that of the single bio-hydrogen production stage (pH 8, 40% and 10.2%) and single methane production stage (control group with 12.30%), respectively.

DF comment: The authors' source of duckweed powder was described as "The duckweed used in this experiment was purchased from Jinantang Traditional Chinese Medicine Co., Ltd. (Bozhou, China)". It should be pointed out that whether there is a mixture of various species or hybrids of different species would be difficult to know. What species is actually present in this material should be described to enable others to replicate the findings or at least compare results in the future.

Ecology

Water residence time and water depth influence on nutrient conditions, eutrophication endpoints and habitat quality in backwater lakes of a large floodplain river

Giblin, SM; Larson, JH; King, JD. (2025) River Research and Applications DOI10.1002/rra.70083

Many eutrophication studies focus on the external supply of critical nutrients like nitrogen and phosphorus, but hydrology and geomorphology can enhance or dampen the effects of excessive nutrient supply. We studied six backwater lakes in the Upper Mississippi River that varied in water residence time and water depth. Eutrophication in these systems is responsible for negative impacts such as cyanobacterial blooms and toxicity, and floating plant and algal mats that disrupt recreational water uses. Increasing backwater residence time was associated with more nitrate removal and a greater likelihood of nitrogen limitation, as well as greater accumulations of duckweed. Backwaters with greater depth and lower nitrogen concentration had less likelihood of filamentous algal accumulations. The median water residence time of backwaters with low duckweed (11.7 days) and no filamentous algae (16.9 days) approached the 12-day target to maintain overwintering conditions in backwaters for fisheries survival, supporting that water residence times in this range would likely improve both winter and summer water quality. Mean depth in backwaters with low duckweed and no filamentous algae was similar to 1.3 m, while shallower backwaters were more likely to produce duckweed and filamentous algae mats. This indicates that deeper backwaters might reduce the likelihood of eutrophication impacts. Natural resource management at the local level may not always be able

to answer global and regional threats, but habitat restoration of hydrology and geomorphology can possibly alleviate or reduce large-scale threats at the local level.

Feed & Food

Wolffia meat analogues produced by high-moisture extrusion: Physicochemical, microstructure, and textural characteristics

Phothiset, S; Sirijariyawat, A; Prommakool, A; Onsaard, W; Aunsri, N; Saenmuang, S. (2026) Food and Bioprocess Technology 19: 68.

This research investigates the use of *Wolffia*, a protein-rich aquatic plant, as a novel ingredient in the formulation of plant-based meat analogues produced via high-moisture extrusion (HME). Different substitution levels of *Wolffia* powder (0, 25, 50, 75, and 100%) were applied to replace pea protein, a conventional plant protein in the extrudate mixture, and the physicochemical properties, texture, and microstructure of the extrudates were investigated. The results indicated that increasing *Wolffia* content altered their composition, reducing protein and lipid levels while increasing carbohydrate, ash, and fiber. This led to a decline in total amino acids, though glutamic and aspartic acid remained predominant. The FTIR and SDS-PAGE analyses revealed that increasing *Wolffia* levels led to protein unfolding and aggregation, along with a darker green coloration. *Wolffia* enhanced anisotropic structure formation but had minimal effect on texturization degree. Although its addition reduced hardness and chewiness while increasing springiness, variations among different Wolffia levels were not statistically significant. The highest cooking yield was observed in the 100% *Wolffia* formulation, with no significant differences across the 0-75% range. Overall, *Wolffia* promoted the development of fibrous structures with acceptable texture, highlighting its potential as a sustainable, protein-rich component for the production of plant-based meat analogues through high-moisture extrusion.

DF comment: The authors' source of duckweed powder was described as "The *Wolffia* used in this study originated from a farm in Kalasin Province, Thailand". It should be pointed out that what species is actually present in this material should be described to enable others to replicate the findings or at least to compare with results in the future.

In vitro digestibility, peptide profile, and bioactivities of water lentil (duckweed) protein compared to commercial protein isolates

Muller, T; Aboubacar, H; Tourret, M; Thibodeau, J; Cudennec, B; Ravallec, R; Bazinet, L (2026) Food Research International 224: 117973.

Water lentils (duckweeds) are a promising protein source, however their digestibility and potential to release bioactive peptides remain underexplored. This study investigated, for the first time, the in vitro digestibility of proteins from water lentil protein concentrates (WLPCs) and their associated by-products obtained through chemical or electrochemical purification, in comparison with the initial native water lentil powder (IP) and commercial protein isolates (egg white, whey, and soy), using the INFOGEST protocol. Following the intestinal phase of the digestion, WLPCs exhibited moderate digestibility, likely due to protein denaturation during extraction, whereas the bioaccessible fraction (similar to 38 %) of IP showed high digestibility. Peptide profiling further revealed that IP produced a more diverse peptide pool than WLPCs and their by-products. Regarding bioactivity, intestinal digestate supernatants from IP, whey and soy protein isolate showed the strongest ACE inhibition, while WLPCs exhibited the highest DPP-IV inhibition. These findings indicate that water lentil protein purification does not necessarily improve digestibility, but they confirm the potential of water lentil proteins as a valuable source of bioactive peptides.

Effects of dried *Wolffia* powder on the quality of corn milk beverage mixed with dried *Wolffia* powder

Wirivutthikorn, W. (2025) Current Research in Nutrition and Food Science 13: 1268-1281.

DOI10.12944/CRNFSJ.13.3.18

Wolffia is a plant-based protein alternative that has recently gained significant much attention due to its nutritional value and its ability to be processed into a variety of food products. This research aimed to study

the effects of mixing different amounts of dried *Wolffia* powder with corn milk beverage, especially in terms of quality. This was done by analyzing important chemical components for application in the development of plant-protein beverage products with high nutritional value in order to meet the needs of today's health-conscious consumers. The development of a corn milk product mixed with dried *Wolffia* powder was divided into 4 treatments. The first step was to study the analysis of protein and water activity in fresh corn and dried *Wolffia* powder to use as basic information for research and development to improve the treatment of corn milk beverage mixed with dried *Wolffia* powder. It was found that the protein and water activity values were 2.71, 2.54%, 0.950 and 0.475, respectively. A physicochemical, microbiological and sensory analysis of the beverage product was conducted. The Completely Randomized Design experimental design was used for physicochemical product analysis, while for sensory evaluation analysis. The results showed that different amounts of dried *Wolffia* powder affected the physicochemical properties in all treatments, except for the total acidity percentage. The microbiological results showed that the microorganisms in all treatments were within the specified standards (Community Product Standard 124/2003). The sensory analysis results from 30 untrained panelists using the 9-point hedonic scale received the highest liking score in all aspects, including appearance, color, odor, taste and overall acceptability. The experimental results indicated that the corn milk beverage mixed with dried *Wolffia* powder had a high overall liking level among beverage products. Therefore, the study of *Wolffia* plant-based protein is beneficial as it is another intriguing alternative use in the development of beverage products, thus increasing nutritional value for health-conscious individuals. Further, it is suitable for development into health beverage products that meet consumer demand and expand production capacity in the food industry.

Effects of alkaline extraction and ultrasonication on digestibility, nutritional quality, and bioactive peptide release of duckweed (*Wolffia arrhiza*) protein extracts

Wongsasulak, S; Nitiwuttithorn, C; Vongsawasdi, P; Hongsthong, A; Yongsawatdigul, J. (2025) Journal of Functional Foods 135: 107110.

Duckweed is a promising sustainable protein source; however, its dense structure and anti-nutritional factors (e. g., saponins and tannins) may limit protein digestibility, nutritional, and biofunctional qualities. This study compared duckweed protein extracts (DPE) produced by alkaline extraction (AE), ultrasound-assisted alkaline extraction (UAAE), and ultrasound-assisted water extraction (UAWE) to native duckweed powder (DPw) regarding protein digestibility, antioxidant capacity, and bioactive peptide (BAP) release during in vitro gastrointestinal digestion. DPw exhibited the lowest digestibility, whereas DPEUAE showed the highest, with UAWE achieving a DIAAS of 0.75, meeting FAO criteria for good protein quality. Enhanced digestibility strongly correlated with increased BAP release; DPEUAE boosted long-chain BAPs by 1.4-fold and short-chain BAPs by 1.8fold versus DPw, without altering bioactivity distribution. Furthermore, DPEUAE demonstrated superior antioxidant capacity, with ACE and DPP-4 inhibitory activities as the top-two predominant bioactivities. UAE effectively produces DPE, demonstrating potential as a functional protein ingredient for food and dietary supplement applications.

DF comment: The authors' source of duckweed was described as "Fresh duckweed (*Wolffia arrhiza*) was purchased from Areeya's Duckweed Farm in Kalasin, Thailand.". It should be pointed out that *W. arrhiza* is not known to be native in Thailand. How this species is actually identified for this material should be described to enable others to replicate the findings or at least to compare results in the future.

Cultivation of *Wolffia globosa* and its application in functional food development

Sirison, J; Ruangsomboon, S; Jongput, B; Aue-umneoy, D; Tongsri, P. (2025) Scientific Reports 15: 38255.

This study assessed the biomass productivity, biochemical composition, and functional food potential of *Wolffia globosa* cultivated in four nutrient media: AB hydroponic solution, Chlorella medium, 16-16-16 fertilizer, and 16-16-16 supplemented with a vitamin B complex and $FeSO_4$ (16-16-16-B). Cultivation was conducted for eight weeks, with biomass harvested weekly. Among the treatments, the Chlorella medium produced the greatest biomass yield (133.82-236.00 g FW $tank^{-1}$) and daily productivity (23.90-42.14 g FW m^{-2} day^{-1}), significantly surpassing the other media ($p < 0.05$), followed by 16-16-16-B. Medium replenishment at four weeks initially enhanced growth but was later accompanied by a decline in biomass. Biochemical analysis indicated that the Chlorella medium yielded the highest protein content (46.10 ± 0.93% DW), while the AB medium supported the greatest chlorophyll-a (4.08 ± 0.00% DW) and carbohydrate levels (37.21 ± 1.12% DW);

all differences among treatments were statistically significant ($p < 0.05$). Considering cost-effectiveness and nutritional value, the 16-16-16-B medium was optimal for cultivating *W. globosa* for food applications. Dried biomass from this medium was incorporated into fresh pasta and fried sweet potato balls, significantly enhancing chlorophyll-a, carotenoids, protein, fiber, and calcium contents. Sensory evaluation using a 9-point hedonic scale showed strong consumer acceptance even at higher inclusion levels. These findings highlight the role of nutrient-enriched media, particularly 16-16-16-B, in enhancing biomass yield and nutritional quality of *W. globosa*, affirming its potential as a functional food ingredient. Beyond nutritional enhancement, *W. globosa*-based foods exhibit immunomodulatory and anticancer potential through the antioxidant activity of pigments and other bioactive compounds.

Liquid and encapsulated duckweed (*Lemna minor* L.) extracts differentially shape metabolomic fingerprints of packaged beef burgers during shelf-life

Rocchetti, G; Rebecchi, A; Dallolio, M; Del Buono, D; Freschi, G; Zengin, G; Lucini, L. (2026) Meat Science 231: 109975.

Oxidative deterioration and color loss are critical factors limiting the shelf-life and consumer acceptance of fresh beef burgers under modified atmosphere packaging (MAP). This study evaluated *Lemna minor* (duckweed) extracts, applied in liquid form (LLE) and encapsulated with gum Arabic (AGL) or maltodextrin (ML), as natural antioxidants in beef burgers stored at 4°C for 14 days. Extracts were tested at 0.1 %, 0.5 %, and 1 % (w/w), and their effects were assessed through physicochemical, microbiological, and untargeted metabolomics analyses. Encapsulation yields were high (similar to 80 %) for both carriers, with gum Arabic retaining higher antioxidant capacity. The liquid extract at 1 % and the encapsulated extracts at 0.1 % were the most effective treatments, preserving redness, reducing lipid oxidation below the sensory off-flavour threshold, and limiting oxygen consumption by day 7. Conversely, higher doses of encapsulated extracts (0.5-1 %) were less effective and in some cases induced pro-oxidant effects. Untargeted metabolomics revealed modulation of oxidative biomarkers, including glutathione, hemin, and tryptamine, supporting the antioxidant role of duckweed extracts in stabilising lipid and protein oxidation pathways. No antimicrobial effect was observed. These findings indicate that duckweed extracts can serve as sustainable, plant-based antioxidants for fresh beef burgers, with recommended application levels of 0.1 % (w/w) for encapsulated forms and 1 % (w/w) for liquid extracts. This represents the first demonstration of duckweed-based antioxidants in meat systems, offering a promising alternative to synthetic preservatives and supporting the transition toward clean-label strategies in the meat industry.

DF comment: The duckweed used here was earlier described as "Duckweed (*Lemna minor* L.) was collected from a freshwater basin located near the city of Perugia, Italy (43°05'56.1" N 12°27'29.5" E)". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature, in addition to other non-*Lemna* species.

Growth & Development

Optimization of hydrodynamics for enhanced co-cultivation in bubble column reactors: Investigating flow dynamics and biomass productivity

Singh, N; Chetty, M; Rathilal, S. (2025) Results in Engineering 28: 107841.

Hydrodynamic effects on algal cultivation have been studied, but limited work has addressed how bubble column diameter influences the co-cultivation of microalgae and duckweed in sewage wastewater systems and biofuel production. This is the first empirical study that fills the gap by experimentally evaluating the co-cultivation of *Scenedesmus* sp. and *Lemna* sp. (Duckweed) in wastewater fed reactors. This study evaluated how bubble column diameter and co-cultivation with Lemna sp. influence the biomass productivity and hydrodynamics of *Scenedesmus-Lemna* regimes. Three bubble columns with internal diameters of 5, 10, and 15 cm were operated under identical aeration (3 - 9 L/hr) and illumination conditions in both monoculture and co-culture configurations. The 10 cm column (C2) achieved the highest performance in co-culture, with a specific growth rate of 0,74 ± 0,05 mg d^{-1} and biomass productivity of 93,85 ± 4,12 mg L^{-1} d^{-1}, representing almost 90% increase compared with monoculture. Additionally, co-culturing *Scenedesmus* sp. with duckweed led to an 11 % increase in growth rates. Increased biomass suggests potential for increased biomolecules,

including lipids and hydrocarbons. Co-cultivation also stabilized pH and dissolved oxygen and promoted attached growth on *Lemna* roots, offering a low-cost and novel attached harvesting pathway. These results highlight that reactor geometry and cocultivation strategies are critical determinants of algal productivity and can substantially influence productivity and harvesting efficiency in large scale algal phycoremediation systems and enhance stability in wastewater treatment systems.

Caloric restriction-mediated reproductive lifespan extension across multiple strains of the clonal aquatic plant *Lemna turionifera*

Ketler, JA; Herman, NM; Chmilar, SL; Laird, RA. (2025) Oikos DOI10.1002/oik.11699

Lifespan extension due to caloric restriction (CR) is a well-established aspect of animal senescence that has been observed in many taxa. Contrastingly, there is much less evidence in plants, even though it is straightforward to manipulate CR by restricting photosynthesis through reduction in light intensity. One of the few studies to report CR-mediated plant lifespan extension investigated reproductive lifespan in a single strain of the duckweed *Lemna minor*, a tiny, floating, aquatic plant. Here, with an aim of beginning to test the generality of this phenomenon in plants, we considered a congeneric species, *L. turionifera*, and examined CR-mediated lifespan extension in eight strains collected from Alberta, Canada. We grew plants in the lab under axenic conditions, and manipulated light intensity (and hence, putatively, CR) with neutral density filters. Plants that grew under dimmer conditions had longer reproductive lifespans, on average, than those that grew under brighter conditions, consistent with CR-mediated lifespan extension. However, this came at the expense of a reduction in the capacity to contribute to population growth: plants in dimmer conditions produced about the same total offspring spread across their longer lifespans, leading to a reduced intrinsic rate of increase, measured at the level of the individual. Expanding the taxonomic scope of studies on CR-mediated lifespan extension - especially in plants - remains an important goal in senescence research.

Interaction with other organisms

Synergistic glyphosate degradation in a rice-duckweed-microbe system: Mechanistic insights and sustainable remediation potential

Song, YJ; Chen, QX; Hu, ZL; Yang, XW; Lu, YL. (2026) Journal of Hazardous Materials 501: 140803.

Glyphosate pollution in rice paddies poses serious environmental and food safety concerns. This study evaluated a rice-duckweed (*Oryza sativa-Spirodela polyrhiza*) system for glyphosate removal and residue reduction. Four treatments, duckweed alone (F), rice-duckweed with microbes (RP), rice-duckweed with sterilized soil (SRP), and rice alone (R), were examined using HPLC-MS/MS, enzyme assays, and 16S rRNA sequencing. The RP system achieved up to 90 % glyphosate removal and significantly reduced residues in rice tissues compared with R ($p < 0.05$). Lower antioxidant enzyme activities and malondialdehyde levels indicated alleviated oxidative stress in rice. Microbial analysis revealed enrichment of *Proteobacteria, Thiobacillus*, and *Desulfitispora*, indicating active glyphosate degradation. Four isolate, *Priestia megaterium, Bacillus proteolyticus, Paenarthrobacter ureafaciens*, and *Bacillus siamensis*, exhibited strong degradation ability, and their consortium (Syn-4) achieved 87 % removal with efficient aminomethylphosphonic acid (AMPA) turnover. The results demonstrate that rice-duckweed-microbe interactions synergistically enhance glyphosate degradation, providing a sustainable approach for herbicide remediation in paddy ecosystems.

Microbial consortium of *Streptomyces* spp. from mining environments enhances phytoremediation potential of *Lemna minor* L.

Djebaili, R; Farda, B; Gialdini, O; Vaccarelli, I; Danesh, YR; Pellegrini, M. (2025) Plants 14: 3467.

The presence of substantial amounts of heavy metals in the environment can result in various significant ecological issues and human health risks. Currently, bioremediation employing microorganisms is garnering significant interest due to its effectiveness. The present investigation aimed to isolate actinobacterial strains from an Italian mine and to characterise them for heavy metals resistance and plant growth-promoting characteristics. The different samples were processed for DNA extraction and 16S rRNA gene metabarcoding to investigate the bacteria and archaea communities. Cultivable microbiota were isolated and evaluated for heavy metals tolerance and different PGP traits. The most pertinent strains were tested for compatibility,

merged into a consortium, and tested on *Lemna minor* L. Metabarcoding analysis revealed that amplicon sequence variants (ASVs) at the phylum level were mostly assigned to proteobacteria and bacteroidota. Uncultured and unknown taxa were the most prevalent in the samples at the genus level. A total of ten strains were obtained from the culture-dependent approach exhibiting interesting heavy metals tolerance and plant growth-promoting traits. The best strains (MTW 1 and MTW 5) were selected and further characterised by 16S barcoding. These strains were identified as *Streptomyces atratus* (99.57% identity). An in planta experiment showed that the metal-tolerant consortium MTW 1-5 improved plant physiology by significantly optimising plant growth and tolerance to heavy metals. The experiment conducted provided evidence for the possibility of using actinobacteria as bioaugmentation agents to improve the phytoextraction abilities of *L. minor*.

DF comment: The duckweed used here is described as "Duckweed was sampled from different fountains in the Abruzzo region (Abruzzo, Italy)". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature, in addition to other non-*Lemna* species.

Microbial community restructuring and functional response in Giant Duckweed (*Spirodela polyrhiza*) fronds driven by cadmium stress

Liu, BL; Yang, C; Wan, X; Chen, SM; Tao, Y; Li, Q; Zhao, H; Wang, XH. (2025) Microorganisms 13: 2423.

As a typical heavy metal pollutant, cadmium (Cd) poses significant threats to ecosystems and human health. Giant duckweed (*Spirodela polyrhiza*), a small aquatic plant characterized by rapid growth and efficient heavy metal accumulation, holds great promise for phytoremediation. However, the mechanisms by which *S. polyrhiza* enriches Cd-particularly the contributions of its surface-associated microbiota-remain poorly understood. In this study, *S. polyrhiza* fronds were exposed to 0, 1, and 10 μM Cd, and we observed a concentration-dependent increase in the abundance of epiphytic microorganisms on the frond surfaces. High-throughput 16S rRNA gene sequencing revealed that Cd stress significantly altered the diversity of the frond-epiphytic bacterial community. Notably, the relative abundances of the genera *Herbaspirillum, Enterobacter*, and *Pantoea* increased significantly with rising Cd concentrations. Functional prediction using PICRUSt2 indicated enrichment under Cd stress of specific traits-such as the nitrate/nitrite transporter NarK, signal transduction mechanisms, and ion channel proteins-suggesting these taxa may actively participate in Cd uptake and tolerance. Together, our results reveal a synergistic *S. polyrhiza*-microbiome response to Cd and identify taxa/functions as targets and biomarkers for microbe-augmented remediation.

Diverse members of the phylum Armatimonadota promote the growth of aquatic plants, duckweeds

Iwashita, T; Makino, A; Nakai, R; Yoneda, Y; Kamagata, Y; Toyama, T; Mori, K; Tanaka, Y; Tamaki, H. (2025) International Journal of Molecular Sciences 26: 9824.

Duckweeds are small, fast-growing aquatic plants with high starch and protein content, making them promising candidates for next-generation plant biomass resources. Despite their importance, little is known about their interactions with microorganisms, particularly plant growth-promoting bacteria (PGPB), which play key roles in enhancing plant productivity. In this study, we report the plant growth-promoting effects of strain LA-C6, a member of the phylum Armatimonadota, isolated from duckweed fronds. Based on 16S rRNA gene analysis, this strain represents a novel genus-level lineage, and is referred to as *Fimbriimonadaceae bacterium* strain LA-C6. In axenic co-culture experiments, strain LA-C6 promoted duckweed growth, increasing the frond proliferation of four duckweed species (*Lemna minor, Lemna aequinoctialis, Spirodela polyrhiza*, and *Landoltia punctata*) by 1.8- to 4.0-fold compared with uninoculated controls. Importantly, three other phylogenetically distinct Armatimonadota species also exhibited significant plant growth-promoting effects on *L. minor*, increasing frond number by up to 2.3-fold and dry weight by up to 2.4-fold. This finding highlights the broader potential of diverse Armatimonadota members as PGP bacteria. A survey of the IMNGS database showed that strain LA-C6 and other Armatimonadota species are widely distributed across diverse plant-associated environments. Biochemical assays and gene prediction analyses revealed that strain LA-C6 produces indole-3-acetic acid (IAA) as a representative PGP trait, whereas no additional PGP-associated traits were detected. These results suggest that diverse bacterial lineages within the phylum Armatimonadota exert growth-promoting effects on aquatic plants, potentially through yet-to-be-identified mechanisms.

DF comment: To determine the presence of IAA that could be produced by bacteria, the author cite a 1991 publication for the use of a colorimetric assay, "IAA production was evaluated using the Salkowski reagent in R2A liquid medium supplemented with 0.5% (w/v) L-tryptophan". However, this assay has been shown to be

prone to false positives due to the reagent's cross-reactivity to indole-related compounds, many of which are not auxins.

Molecular Biology and Genomics

Glutathione-associated genes *SpGSH1* and *SpPCS1* differentially modulate cadmium accumulation and tolerance in duckweed under micronutrient deficient environments

Chen, Y; Hou, HW; Hu, JZ; Xiong, XQ; Ye, CN; Zhang, YH; Zhang, LY; Sun, ZL. (2026) Plant Physiology and Biohemistry 230: 110937.

Decontaminating cadmium (Cd) pollution, a severe threat to aquatic ecosystems, requires efficient phytoremediation solutions. Duckweed (*Spirodela polyrhiza*) exhibits Cd bioremediation potential, but the roles of glutathione (GSH)-associated genes under Cd and iron/manganese (Fe/Mn) deficiency stress remain unclear, limiting its practical applications. To address this, we generate transgenic lines overexpressing *SpGSH1* (G line), *SpPCS1* (P line), and both *SpGSH1/SpPCS1* (GP line) and investigate their roles in Cd accumulation and tolerance under these three stress conditions. We report elevated GSH levels and total antioxidant capacity in all transgenic lines, but only P and GP lines significantly enhance Cd accumulation under 20 μM Cd stress. Under combined Fe deficiency and 20 μM Cd stress, *SpGSH1* overexpression impairs growth, whereas *SpPCS1* overexpression causes severe photosynthetic damage. Only the GP line restores Cd accumulation capacity and effectively mitigates oxidative stress, indicating a synergistic detoxification mechanism. Under combined Mn deficiency and 20 μM Cd stress, nutrient-specific divergence emerges with the G line, with enhanced growth, improved ROS scavenging, and protected photosynthesis, whereas the P line accumulates O_2- levels. Simultaneously, the GP line maintains robust antioxidant protection while boosting Cd/Zn uptake. Importantly, *SpGSH1/SpPCS1* co-overexpression synergistically improves Cd accumulation, antioxidant capacity, and photosynthesis across stress conditions, overcoming limitations of single-gene overexpression. We pioneer a novel *SpGSH1/SpPCS1* co-overexpression strategy for robust Cd remediation in nutrient-depleted waters that represents a promising approach to enhance phytoremediation efficacy in challenging real-world environments.

Genome-wide characterization of WRKY family genes in four Araceae species and their expression analysis in *Amorphophallus konjac*

Shi, HL; Zou, Y; Yang, M; Qi, Y; Gao, PH; Zhao, YT; Huang, FY; Liu, JN; Zhao, JR; Li, LF. (2025) Frontiers in Plant Science 16: 1671100.

The Araceae family is a large family of angiosperms containing many economically valuable and ecologically important species, such as *Amorphophallus, Zantedeschia elliottiana*, and *Spirodela intermedia*. The *WRKY* family is one of the largest plant-specific transcription factor families and plays a crucial role in plant responses to biotic and abiotic stresses. In this study, *WRKY* family members were identified and characterized in four species-*Amorphophallus konjac*, *Amorphophallus albus*, *Zantedeschia elliottiana*, and *Spirodela intermedia*-using bioinformatics approaches. Characterization included analyses of physicochemical properties, gene structure, phylogenetic relationships, chromosomal distribution, collinearity, and cis-regulatory elements. Expressions were specifically performed in *A. konjac* using transcriptomics data to examine *AkWRKY* expression across various tissues and stages of corm development. These expression profiles were further validated by quantitative real-time PCR (qRT-PCR), including tissue types (leaf, petiole, corm, and root); hormone treatments (abscisic acid (ABA)); jasmonic acid (JA); salicylic acid (SA); biotic stress (infection by *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc)), and abiotic stresses (low temperature, drought, and salt). A total of 79, 57, 59, and 36 *WRKY* members were identified in *A. konjac, A. albus, Z. elliottiana*, and *S. intermedia*, respectively, with the majority predicted to be localized in the nucleus. Most *WRKY* members contained the conserved heptapeptide WRKYGQK domain within their motifs, and genes within the same subgroup shared similar gene structures and motif distributions. Phylogenetic analysis revealed that most Araceae *WRKY* members belong to Group II. Collinearity analysis indicated that segmental duplication was the primary driving force for the expansion of the *WRKY* gene family in these Araceae species (Ka/Ks < 1), suggesting the action of purifying selection. Cis-element analysis revealed that the promoter regions of *WRKY* genes contain numerous regulatory elements associated with plant growth and development, hormone regulation, stress responses, and light responses. Transcriptome analysis demonstrated that *AkWRKY*s exhibit

tissue-specific expression patterns in leaves, petioles, corms, and roots, with most genes revealing up-regulated expression during developmental stages 2 to 3 of the corm. To elucidate the expression patterns of *AkWRKY*s under biotic and abiotic stresses, qRT-PCR was used to analyze the expression profiles of 14 *AkWRKY*s in response to ABA, JA, SA treatments, Pcc infection, as well as low temperature, drought, and salt stress. These 14 *AkWRKY* members displayed significantly differential expression characteristics under hormone regulation, biotic stress, and abiotic stress, responding to various stress treatments to different degrees over time. Conclusion: Among the 79 identified *AkWRKY* members, *AkWRKY*38 and 53 exhibited high expression levels in *A. konjac* under hormone treatments, biotic stress (Pcc infection), and abiotic stresses (low temperature, drought, and salt stress). This study provided new insights into the roles of *WRKY*s in *A. konjac* responses to soft rot disease, low temperature, drought, and salt stress. Additionally, it laid a foundation for breeding stress-resistant *A. konjac* cultivars.

DF Comment: Most duckweed researchers consider duckweed (Lemnaceae) as a plant family of its own, cf. Tippery et al. Lemnaceae and Orontiaceae are phylogenetically and morphologically distinct from Araceae. Plants (2021) 10: 2639. https://doi.org/10.3390/plants10122639.

Post-transcriptional regulation dominates protein biosynthesis in *Landoltia punctata* under biogas slurry stress

Gong, XL; Li, JY; Li, JZ; Ran, C; Zhou, LL; Zhou, T; Su, HH; Lu, TT; Zhang, SL. (2025) Frontiers in Plant Science 16: 1694864.

Duckweed represents a promising alternative protein source, yet enhancing its protein content remains essential for large-scale applications. This study investigated how high nitrogen and phosphorus stress from biogas slurry affects protein accumulation in *Landoltia punctata* and explored the underlying molecular regulatory mechanisms. *L. punctata* were cultivated in 1/5 strength Hoagland medium supplemented with 0-5% pig farm biogas slurry. The experimental groups showing the highest (4%) and lowest (0%) protein content were selected for integrated transcriptomic and proteomic analyses. Differentially expressed genes (DEGs) and proteins (DEPs) were identified and functionally characterized. Biogas slurry treatments significantly increased crude protein content in a concentration-dependent manner, with the 4% treatment showing the highest value of 24.18% compared to 18.13% in controls. Multi-omics analysis revealed a low correlation between mRNA and protein expression (R=0.1387), indicating dominant post-transcriptional regulation. Ribosomal proteins were significantly upregulated at the protein level without corresponding transcriptional changes, suggesting enhanced translation efficiency. Concurrently, key enzymes in amino acid catabolism were downregulated, potentially conserving substrates for protein synthesis. The photosynthetic system showed coordinated downregulation at both transcriptional and protein levels, with suppression of light-responsive genes and carbon fixation pathway components, indicating redirected carbon and energy flows toward nitrogen assimilation. Biogas slurry enhances duckweed protein accumulation primarily through post-transcriptional regulation. Enhanced translation efficiency coupled with metabolic reallocation from photosynthesis to nitrogen assimilation optimizes protein synthesis. This first multi-omics perspective on post-transcriptional regulation under biogas slurry stress provides theoretical support for molecular breeding of high-protein duckweed.

Morphology

Neutrons reveal the dynamics of leaf thylakoids in living plants

Stingaciu, LR; O'Neill, H; Liu, CH; Evans, BR; Nagy, G. (2025) Scientific Reports 15: 38810.

The study is the first known exploration of photosynthetic membranes dynamics in living plants by high resolution quasielastic neutron scattering spectroscopy. We investigated the mobility and flexibility of thylakoid membranes in common duckweed (*Landoltia punctata*) and identified dynamics across various length scales corresponding to individual membranes and membranes stack. We employed classical models typically used to study lipid bilayers to characterize the undulation modes and rigidity of the membranes and reveal how structural variations influence the observed complex dynamics. Our findings show that the stacks of thylakoids in duckweed behave as rigid systems, exhibiting an effective bending coefficient in the lower range associated with surfactant membranes. In contrast, the single thylakoid leaflets display greater apparent flexibility and are well situated within the bi-continuous surfactant phase dynamics. While our observations enhance the understanding of the intricate architecture and mobility of photosynthetic cellular machinery, they

also highlight the limitations of applying ideal lipid membranes models to describe complex biological systems. This work opens more questions and the need for further investigations across extended length and time scales, as well as the importance of rigorous sample preparation and experimental control.

Physiology & Stress

Reduced graphene oxide modulates physiological responses of *Lemna minor* under environmental heavy metal stress

D'Eugenio, M; Casentini, B; Iannelli, MA. (2025) Environment 12: 407.

The expanding development of graphene-based materials (GBMs) requires immediate and balanced environmental assessment balancing two key areas: investigating the risk of graphene oxide toxicity to ecosystems and evaluating GBMs' potential to act as solutions for challenges like heavy metal stress mitigation. This study analyzed the effects of reduced graphene oxide (rGO) on copper (Cu) and nickel (Ni) toxicity in *Lemna minor*. Our findings reveal that rGO's protective effects are metal-specific. *L. minor* demonstrated significant sensitivity to nickel, but rGO offered no mitigation; growth parameters, pigment content, and nickel accumulation showed no significant improvements with rGO co-exposure compared to Ni-plants. This suggests that rGO does not enhance *L. minor*'s ability to tolerate or absorb nickel, especially after 14 days (T14). In contrast, rGO showed a partially protective effect against copper toxicity. At T14, the presence of rGO significantly improved plant performance under copper stress, resulting in a 17% increase in biomass, a 19% increase in relative growth rate, and enhanced pigment content, including a 40% increase in chlorophyll when compared to Cu-plants. The protective effect of rGO was directly tied to a 37% reduction in copper accumulation, providing strong evidence that rGO reduces copper's bioavailability, thereby limiting plant uptake. The divergent effects on Cu and Ni uptake suggest differing affinities of these metals for rGO. Future research, including large-scale experiments with various GBMs and *Lemna* clones, is crucial to fully assessing their phytoremediation potential.

Elevated light intensity and temperature enhance biomass, protein, and starch accumulation in duckweed

Islam, MF; Yang, JJ; Zhao, XY; Kazmi, A; Li, XZ; Hu, HK; Sun, ZL; Chen, Y; Heenatigala, PPM; Hou, HW. (2025) Industrial Crops and Products 237: 122118.

Duckweeds represent a promising non-food feedstock for sustainable bioenergy production, owing to their rapid growth, high starch accumulation, and adaptability to diverse environmental conditions. Light and temperature are significant environmental factors influencing the duckweed growth and biomass accumulation. This study investigates the effects of varying light intensities (3000, 6000, 9000, 12,000, 15,000, and 20,000 lux) and temperatures (18°C, 22°C, 25°C, 28°C, and 31°C) on *Lemna trisulca*, focusing on biomass, starch, and protein yield. The results showed that increasing light intensity and temperature enhanced the growth and biomass production of duckweed, peaking at 20,000 lux (796.7 g m^{-2}) and 28°C (792.5 g m^{-2}). Under an optimal light regime (12,000 lux), starch yield reached 348.8 g m^{-2}, with starch content reaching 51.8 % of dry biomass and total biomass output of 724.7 g m^{-2}. Protein accumulation was maximized at 15,000 lux and 25°C (88.5 g m^{-2}). Our findings uniquely demonstrate that optimizing light intensity and temperature enhances starch and protein accumulation in submerged duckweed, providing a scalable framework for industrial cultivation. This study offers practical insights for the development of energy-efficient systems to produce starch-rich biomass of *L. trisulca* without compromising overall growth performance.

DF Comment: Lux is a light unit connected with human vision. In plant science we need µmol m^{-2} s^{-1} as unit.

GLR channels are involved in the mechanism of chloroplast avoidance response in *Lemna trisulca*

Krzeszowiec, W; Gabrys, H (2025) Plants 14: 2990.

The complete signalling pathway leading to light-induced chloroplast movement in plant cells is not yet fully understood. The process may involve GLR channels, which have previously been shown to participate in light signalling in plants. Therefore, using in vivo photometry we analysed chloroplast movements in the water plant *Lemna trisulca* treated with GLR channel inhibitors, MK-801 and CNQX. MK-801, a non-competitive antagonist

that blocks NMDA channels was found to inhibit the avoidance response of chloroplasts controlled by phototropin2. This inhibition depends on pH and requires alkaline conditions. On the contrary, CNQX, a competitive receptor antagonist that blocks AMPA channels did not change the parameters of chloroplast movements in either mild alkaline or acidic conditions. Our study reveals that GLR NMDA channels play a role in chloroplast movements and provides new insights into the phot2 signalling pathway. The results also suggest that the activity of these channels depends on pH, similar to NMDA receptors present in animal cells.

Phytomedicine

Selenopeptide with antioxidant and anticancer activities from Se-enriched *Wolffia globosa*: fractionation, isolation and identification

Pakdeebamrung, P; Phongthai, S; Kingwascharapong, P; Issara, U; Tian, JH; Jung, YH; Rachtanapun, P; Rawdkuen, S; Tangjaidee, P. (2025) Applied Food Research 5: 101372.

Bioactive selenopeptides derived from plant materials represent promising candidates for development as plant derived bioactive agents for applications in health and agriculture. *Wolffia globosa*, a protein-rich, rootless aquatic plant, has the ability to absorb selenium (Se) from water and synthesize selenopeptide. The study aimed to extract selenoprotein using Osborne fractionation and enzymatic hydrolyzed to produce seleopeptide from Se-enriched *W. globosa*. Subsequently, the antioxidant and anticancer activities of these selenopeptides were evaluated, and their amino acid sequences were identified. The results indicated that seleno-albumin (SePA) and seleno-glutelin (SePG) are the major fractions derived from selenoproteins in Se-enriched *W. globosa*, with Se contents of 181.40 and 112.64 µg Se/g protein, respectively. The selenopeptides showed significant antioxidant properties through DPPH (55.90 and 50.75 %), ABTS (54.70 and 44.59 %), FRAP (6.61 and 4.42 mg TE/g) and ORAC (251.21 and 259.39 mg TE/g) radical scavenging assay. Additionally, they exhibited notable anticancer effects against cervical cancer cells (C33A, SiHa) and lung cancer cells (A549), reducing cell viability to below 60 % at 500 µg/mL concentration. Importantly, the selenium-enriched peptides from *W. globosa* exhibited superior biological activities compared to Se-free peptides, suggesting their potential applications as functional ingredients in the food and pharmaceutical industries.

Phytoremediation

Evaluating *Lemna minor* for bioethanol production and bioremediation of petrochemical wastewater

Shafiq, F; Din, IU; Khattak, AA; Afzal, M. (2026) Biomass & Bioenergy 208: 108826.

The increasing cost and scarcity of fossil fuels necessitate exploring alternative energy sources, and bioethanol has emerged as a significant transportation fuel on a global scale. However, its reliance on food crops has sparked controversy. *Lemna minor* presents a novel solution that provides a sustainable feedstock for bioethanol production without competing with food crops. In this study, *L. minor* samples were collected and cultivated in a Steinberg growth medium. Rich in starch, *L. minor* was used as a bioethanol feedstock and optimized through alpha-amyloglucosidase pretreatment to enhance sugar release and ethanol production. Nutritional analysis revealed high carbohydrate (43.65 ± 0.56 %), protein content (27 ± 1.2 %), and low moisture (8.35 ± 0.86 %), positioning *L. minor* as a promising biofuel resource that can adapt to harsh conditions. Its mineral-rich ash (26.2 ± 1.32 %) suggests phytoremediation potential, and crude lipids (4.80 ± 0.38 %) hinted at its potential use in biodiesel production. Pretreatment methods, including alkali and Acid assisted by enzymes, showed improved sugar yields. Saccharomyces cerevisiae fermentation highlighted the effectiveness of enzyme-assisted methods in achieving higher bioethanol conversion rates. FTIR analysis and SEM images confirmed structural changes induced by pretreatments, supporting their efficacy. This study uniquely investigates *L. minor* cultivated in petrochemical refinery wastewater from Makorhi and Gurguri, demonstrating its dual capacity for bioethanol production and wastewater bioremediation.

DF comment: The moisture content of 8.53 % was reached after sun drying, not after cultivation. In addition, the duckweed used here is described as “Plant sampling was conducted using sewage from a nearby garden. All plant samples were gathered and transported to the laboratory, where they were subsequently transferred to a stock culture tank“. The method of species identification as *Lemna minor* should be described, since

multiple types of hybrids from *L. minor* are now known to be prevalent in nature, in addition to other non-*Lemna* species.

Ecotoxicological evaluation of textile effluent and its treatment using aquatic plants: A case study

Shrivastava, R; Singh, NK; Singh, RP. (2025) Water and Soil Polluion 237: 252

In present study, pollution load, ecotoxicological impact, and treatability potential of aquatic plants, viz. *Eichhornia crassipes*, *Pistia stratiotes*, and *Spirodela polyrhiza* evaluated for textile effluent in Jaipur region, Rajasthan, India. Wastewater quality of textile effluent exhibits high pollution load (mg/L) in terms of total suspended solids (TSS, 1986), biological oxygen demand (BOD, 72), chemical oxygen demand (COD, 2851), and metal contamination with concentration of Zn (6.41) > Cu (4.66) > Fe (4.61), Pb (4.58) > Cr (3.64) > Ni (3.53) > Cd (1.89). Evaluation of the Water Quality Index (WQI), Heavy Metal Pollution Index (HMPI), and Heavy Metal Evaluation Index (HMEI) reveals that textile effluent has detrimental effects on both water and soil quality. Additionally, evaluation with using geographical information systems (GIS) showed differences in water quality at different locations, and principal component analysis (PCA) indicated that soil quality varied significantly, with changes of 79.74% and 87.48% ($p < 0.05$). Luxuriant and fast growth of the selected aquatic plants, viz. *E. crassipes, P. stratiotes*, and *S. polyrhiza* in diluted textile effluent and their potential for reducing pollution load demonstrate their feasibility to be use in developing low cost and sustainable technique for wastewater treatment. However, maximum reduction in pollution load observed in textile effluent treated with *E. crassipes* in terms of removal of TSS (31%), BOD (38%), and COD (41%). Present study provides new insights in integrating advanced techniques for ecotoxicological assessment using GIS and PCA and developing sustainable methods for treatment of textile effluent by exploring efficient aquatic plants.

Monitoring and optimization of heavy metal phytoextraction in constructed wetlands amended with organic chelators

Farid, M; Mussarat, A; Zubair, M; Asam, ZU; Siddiqua, A; Sarfraz, W; Murtaza, R; Alghanem, SMS; Alhaithloul, HAAS. (2025) Environmental Monitoring and Assessment 197: 1363.

The present study was conducted in a lab-scale constructed wetland to investigate the extraction potential of *Typha latifolia* and *Lemna minor* for heavy metals (HMs) from tannery wastewater (TWW), supplemented with citric acid (CA) as a chelator. After 6 weeks of treatment, harvesting was followed by a recording of agronomical trait data. The data indicated a significant ($p < 0.05$) decline in the morpho-physiological and biochemical attributes of the plants, which correlated increase in TWW concentration. Maximum accumulation of selected HMs including chromium (Cr), cadmium (Cd), and lead (Pb), in L. minor was observed at 100% TWW, with respective values of 148.4%, 129.4%, and 85.6% as compared to 25% TWW. In *T. latifolia*, a significant increase in Cr (143.8%), Cd (148.3%), and Pb (126.8%) in the roots was also observed at 100% TWW as compared to 25% TWW. CA amendment under TWW conditions significantly enhanced Cr uptake in *L. minor* by 61% and accumulation by 94%. Similarly, in the roots of *T. latifolia*, Cr uptake was elevated by up to 11%, with accumulation increasing by 117%. These findings indicate that phytoextraction of heavy metals can serve as an efficient strategy for the remediation of tannery wastewater using *Lemna minor* and *Typha latifolia* in constructed wetlands.

DF comment: The duckweed used here is described as "Fresh plants of *T. latifolia* and *L. minor* were collected from their natural habitat in the catchment area of University of Gujrat in Gujrat, Pakistan". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature, in addition to other non-*Lemna* species.

Effects of fish farm effluents on contaminant-stimulated enzyme activity in *Lemna valdiviana* and *Hyalella azteca* under field and laboratory conditions

Alvarado-Flores, CO; Nimptsch, J; Woelfl, S; Soto, C; Barrera, M; Vega, R; Oberti, C; Aguilera-San Martín, Y; Anabalón, L; Encina-Montoya, F; Esse, C. (2025) Aquaculture Environment Interactions 17: 201-216. DOI10.3354/aei00503

Fish farm effluents can significantly impact water quality and aquatic ecosystems, by inducing oxidative stress and sublethal biochemical alterations in organisms. This study assessed the pro-oxidant potential of fish farm

effluent by measuring the enzymatic activities of catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), and the levels of reduced glutathione (GSH) and oxidised glutathione (GSSG) in *Lemna valdiviana* and *Hyalella azteca*. Both species were exposed to different concentrations (100 to 3.125% v/v) and to ambient waters collected from sampling stations downstream of a fish farm. *L. valdiviana* was tested under controlled laboratory conditions, while *H. azteca* was evaluated both in situ and in laboratory bioassays. Increased conductivity, nutrient concentration, dissolved organic carbon (DOC), and dissolved organic matter (DOM) were recorded downstream of the discharge point, reflecting the influence of fish farming on water quality. Significant changes in antioxidant enzyme activity including CAT, GST, GR, and GPx were observed in *L. valdiviana* across all sampling sites and different effluent concentrations, alongside variations in GSH and GSSG levels after 120 h of exposure. In *H. azteca*, significant changes in CAT, GST, GR, and GPx activities were recorded at the sampling sites after 48 h of exposure and at different effluent concentrations after 48 and 72 h. These findings indicate that fish farm effluents induce oxidative stress in aquatic species across different trophic levels. Our results highlight the relevance of antioxidant biomarkers for the early detection of oxidative stress on aquatic ecosystems and support the need for improving environmental management strategies to mitigate these effects.

Integrated biochar and *Lemna minor* system for sustainable remediation of Benzophenone-3 from wastewater

Sawant, SS; Bharti, VS; Shukla, SP; Verma, AK; Yadav, VK; Shinde, S. (2025) Scientific Reports 15: 40329.

Benzophenone-3 is an Emerging Pollutant having significant ecotoxicological effects on aquatic organisms, owing to its widespread use as a UV filter and stabilizer to prevent photodegradation of commercial products, and has a ubiquitous presence. The present study entails an investigation of the bioremediation potential of an integrated system of biochar and *Lemna minor* against Benzophenone-3 from aqueous solution through batch studies. This integration in spiked distilled and municipal wastewater yielded a total removal of 73.82% and 80.46% of Benzophenone-3, respectively. Quantitative analysis of the FTIR spectra showed Benzophenone-3 adsorption onto the sugarcane bagasse biochar in a similar trend, comparable with its experimental removal efficiency. The reactions followed pseudo-second-order and intraparticle diffusion kinetics and the Freundlich isotherm modelling. Metabolites of Benzophenone-3, namely 2,4-Dihydroxybenzophenone and the first report of 2,3,4-Trihydroxybenzophenone in plants, were observed in tissues of *L. minor*. Cation exchange and pore-filling in the case of biochar and plant uptake and metabolism in the case of *L. minor* were the major removal mechanisms. Physicochemical analysis of the municipal wastewater pre- and post-treatment revealed an improvement in its overall quality, rendering the water suitable for reuse. The study provides baseline data about the potential of biochar and *L. minor* in an integrated system for the remediation of Benzophenone-3. It finds potential application in constructed wetlands for the efficient, cost-effective and eco-friendly remediation of Emerging Contaminants upon further research.

Investigation of nutrient removal capacity and growth rate of duckweed (*Lemna minor*) under different harvesting protocols in aquaponics

Molnár, PI; Bényi, BC; Bársony, P; Posta, J; Fehér, M (2025) Water 17: 3203.

In aquaculture systems, a high proportion of nutrients end up in the water as a by-product of metabolic processes. These must be neutralized through filtration, but to increase efficiency, the integration of some aquatic plants is advisable. Through the nutrient uptake capacity of these plants, the environmental impact of aquaculture systems can be decreased, so they become more sustainable. In this experiment, common duckweed (*Lemna minor*) was used under different harvesting protocols (control, and 25% and 50% of surface area harvested) to examine the nutrient uptake capacity of the plant and the effects on fish (common carp-*Cyprinus carpio*) production parameters. It can be concluded that the treatments used did not have a significant effect on fish production parameters. However regular duckweed harvesting had a positive effect on the plant's biomass production and daily growth rate. By the end of the experimental period, the harvested groups had accumulated more biomass than the control group, though there was no difference between the 25% and 50% harvest rates. In our experiment, the control group achieved a yield of 17.9 t/ha/year, while the regularly harvested (25% and 50%) treatments achieved yields of 23.4-24 t/ha/year (based on extrapolated data). Regular harvesting of duckweed resulted in lower ammonia levels, as the free water surface available to the plants after harvesting allowed for more intensive growth, enabling them to absorb more organic matter. The dynamics of nitrite, nitrate and orthophosphate concentrations are primarily determined by the internal biochemical processes of the system and temporal development, while treatments such as duckweed harvesting had no direct effect on these parameters.

DF comment: The duckweed used here is described as "The common duckweed (*Lemna minor*) and common carp (*Cyprinus carpio*) used in the experiment came from the Aquaculture Laboratory's own stock ". The method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature, in addition to other non-*Lemna* species.

CO_2 emission avoidance in organic waste management: Co-digestion of brewery's spent grain pulp with *Lemna minor*

Gambelli, AM; Di Mario, J; Priolo, D; Del Buono, D; Gigliotti, G. (2025) Sustainability 17: 9985.

This study focuses on biogas production within lab-scale semi-batch bioreactors using agro-industrial wastes and dry biomass of an invasive aquatic species. In particular, the primary objective is to increase the yield of anaerobic digestion processes, with a specific focus on reducing CO_2 emissions associated with the degradation of biomass, by co-digesting different raw biomasses and agro-industrial wastes. In detail, the experiments concerned the pulp of Brewery's Spent Grain (BSGp), consisting of the residual of Brewery's Spent Grain after fiber deconstruction with ionic liquids-based treatment, and *Lemna minor* L. (LM). The two biomasses were studied separately and then co-digested. Co-digestion was carried out using a 1:1 (VS basis) mixture of *L. minor* and Brewery's Spent Grain pulp. Due to the lack of organic nitrogen, BSGp showed low biogas production if compared with untreated BSG (1.14×10^{-3} vs. 1.71×10^{-3} Nm^3/gVS). Differently, LM has a high nitrogen content and, when digested alone, produced 9.79×10^{-4} Nm^3/gVS. The co-digestion tests allowed us to reach the highest performance: 2.94×10^{-3} Nm^3/gVS. In terms of bioenergy production, the two biomasses showed high synergy when used in co-digestion. The amount of energy produced was calculated using a lower heating value (LHV) of CH_4 equal to 52 MJ. The results showed that co-digestion yielded 64.9 ± 0.6 MJ/kgVS, followed by BSG (43.3 ± 5.3 MJ/kgVS), BSGp (25.6 ± 0.3 MJ/kgVS), and LM (19.3 ± 1.0 MJ/kgVS). In addition, in terms of CO_2 avoided, the following results were achieved: 0.38-0.40 g CO_2/gVS with BSGp, 0.73-0.8 g CO_2/gVS with LM. Conversely, co-digestion tests allowed for the avoidance of 1.68-1.91 g CO_2/gVS. In conclusion, co-digesting BSGp with *L. minor* yields more methane and less CO_2 per unit processed, providing an effective way to convert readily available waste and biomass into bioenergy.

DF comment: The duckweed used here is described as "*Lemna minor* was cultivated at the Department of Agricultural, Food and Environmental Sciences of the University of Perugia". The origin of the duckweed and is method of species identification as *Lemna minor* should be described, since multiple types of hybrids from *L. minor* are now known to be prevalent in nature, in addition to other non-*Lemna* species that may be morphologically similar.

Effects of tetracycline on growth and nutrient removal by *Lemna aoukikusa* and *Spirodela polyrhiza* under short-term cultivation

Dinh, UTT; Nakagawa, S; Shimizu, T; Soda, S. (2025) Applied Sciences 15: 11621.

This study assessed the effects of tetracycline (TC) on growth of *Lemna aoukikusa* and *Spirodela polyrhiza* under batch conditions. The duckweeds were exposed to a range of 0.0-5.0 mg L^{-1} of TC for 7 days in a medium containing 10 mg L^{-1} total nitrogen (TN) and 1 mg L^{-1} total phosphorus (TP). The relative growth rate (RGR) of each species was determined from the frond area measurement using image analysis. The EC_{50} values as the TC concentrations causing a 50% reduction in RGR, were 4.4 mg L^{-1} for *L. aoukikusa* and 0.65 mg L^{-1} for *S. polyrhiza*. At 5.0 mg L^{-1} TC, TP removal decreased to 60% in the *L. aoukikusa* culture and 77% in the *S. polyrhiza* culture, compared to 85-91% and 96%, respectively, under lower TC exposure. Nevertheless, TN and TP removals were not significantly impaired at TC concentrations found in swine wastewater. The TC removals were 76-94% for the *L. aoukikusa* culture and 68-91% for the *S. polyrhiza* culture, which were attributed to adsorption and plant uptake. These findings highlight the feasibility of duckweed-based stabilization ponds for simultaneous antibiotic attenuation and nutrient removal.

DF comment: For the origin of the duckweed accessions used in this work, the author's previous referenced work cited "*L. aoukikusa* and *W. globosa* were purchased from Charm Co., Ltd. (Ora, Gunma, Japan). Also, *S. polyrhiza* was purchased from Tojyaku Engei Co. Ltd. (Joyo, Kyoto, Japan)". The assignment of the species *L. aoukikusa* should be taken with caution due to its recent determination as a natural hybrid between *L. perpusilla* and *L. aequinoctialis*, the latter being quite prevalent in Asia and can often be confused with its hybrids.

Meat processing wastewater; the use of *Lemna minor* L. to convert an environmental burden into a new resource

Kislioglu, MS; Katsara, A; Redmond, C; Drenckhan, W; Jansen, MAK. (2025.) Frontiers in Environmental Science 13: 1622266.

The meat processing industry generates a considerable amount of meat processing wastewater (MPW) that is potentially harmful when released in the natural environment. Therefore, current industry practices involve extensive MPW remediation before release of effluent into local waters. Here, it was investigated whether aquatic duckweed (*Lemna minor* L.) can be used to remediate and retain nitrogen and phosphorus present in MPW that had undergone primary and secondary treatment. Physicochemical analyses, as well as laboratory and glasshouse growth trials, show the suitability of MPW as a growth medium for duckweed. Quantitative analysis revealed that duckweed growth on MPW is associated with rapid removal of nitrogen and phosphorus with calculated uptake rates similar to those reported in the literature. Longer term cultivation on MPW (>6 days) led to increased salinity problems, however, short-term (3 days) remediation of MPW was found to be sufficient to achieve wastewater discharge requirements. Thus, a duckweed-based system can be used to remediate MPW. The suitability of duckweed biomass as a source of protein, bioenergy and/or fertiliser will facilitate retention of plant nutrients within the agri-feed sector in line with the principles of the circular economy and constitute a promising avenue towards more sustainable meat processing. Future work needs to focus on upscaling duckweed remediation under realistic industry conditions, while exploring technical (salinity and seasonality), economic (cost-benefit), social, regulatory and sanitary aspects.

Comparison of the phytoremediation potential and biomass yield of *Wolffia brasiliensis* and *Landoltia punctata* grown in animal wastewater

Mignoni, DSB; Pereira,; Michelon, W; Nunes, ED; Luchessi, AD. (2025) Chemistry and Ecology 42: 18-30.

The intensification of swine production has introduced substantial ecological pressures, primarily through the discharge of nutrient-enriched effluents containing high loads organic matter, heavy metals, and microbial pathogens. This study investigates the phytoremediation capacity of *Wolffia brasiliensis* and *Landoltia punctata* swine wastewater treatment across a gradient of TAN (ammoniacal nitrogen TAN) concentrations. Both macrophytes demonstrated high functional efficiency in pollutant removal, with reductions in chemical oxygen demand (COD) by up to 77.5%, phosphate (PO_4^{3-}) by 69%, and complete removal (100%) TAN and zinc (Zn). *L. punctata* exhibited greater efficacy in heavy metal sequestration, particularly copper (Cu), achieving 92% removal under high nitrogen conditions (90 mg L^{-1} TAN), whereas *W. brasiliensis* was more effective in microbial load reduction, attaining a 100% decrease in coliform populations. Estimated annual fresh biomass productivity reached 49.7 and 33.2 t ha^{-1} $year^{-1}$ for *W. brasiliensis* and *L. punctata*, respectively, with considerable nutrient assimilation (50 g kg^{-1} total nitrogen and 15 g kg^{-1} total phosphorus). The results indicate that *W. brasiliensis* is well-suited for wastewater with moderate nitrogen contamination, whereas *L. punctata* is more effective in high-strength wastewater. The findings support that the duckweed species promoting scalable and ecologically sound approaches for integrated wastewater treatment and nutrient recovery.

Influence of duckweed (Lemnaceae) on the characteristics of dissolved organic matter (DOM) in urban ponds

Zhang, XY; Zhu, SH; Lan, X; Tian, SY; Lu, J; Tang, JH; Chen, XL; Gao, L; Lv, JT; Yin, YG; Yang, GL; Jiang, T; Tang, ZW; Wang, DY. (2025) Hydrobiologia DOI10.1007/s10750-025-06007-9.

Dissolved organic matter (DOM) is a vital component of the carbon cycle in freshwater ecosystems, including small water bodies such as urban ponds. This study investigated the effects of duckweed on DOM in such ponds. Field observations revealed that duckweed-covered ponds had reduced light penetration and oxygen availability, inhibiting DOM photodegradation and microbial degradation. Duckweed may also contribute to persistent DOM. A multi-property persistence index (PI) confirmed greater DOM persistence in duckweed-covered ponds, consistent with the optical property analysis. Laboratory experiments in which duckweed was removed or added showed that duckweed-covered water had higher dissolved organic carbon, chromophoric DOM, protein- and humic-like substances, and higher PI. These findings mirrored field results. Overall, reduced sunlight and oxygen in duckweed-covered ponds hinder DOM degradation; however, duckweed itself may also contribute to persistent DOM. These findings enhance the current understanding of DOM dynamics and biogeochemical processes in small inland waters with hydrophytes, especially in artificial freshwaters, such as urban ponds.

Phytotoxicity

Combined toxicity of chloride-based and eco-friendly deicers with nanoplastics on *Lemna minor* and *Salvinia natans*

Song, Y; Kim, D; An, YJ. (2026) Marine Pollution Bulletin 224: 119093

With the increasing frequency of heavy snow events projected under climate change, the use of deicers for winter road maintenance is expected to rise, leading to their discharge into freshwaters where they may co-occur with nanoplastics (NP). This study evaluated the combined toxicity of four deicers, sodium chloride (NaCl), calcium chloride ($CaCl_2$), a NaCl-$CaCl_2$ mixture, and the eco-friendly calcium magnesium acetate (CMA) with polystyrene NP to the aquatic plants *Lemna minor* and *Salvinia natans*. Endpoints included growth inhibition, root cell viability, and photosynthetic performance. Co-exposure outcomes were de-icer-specific and generally increased with NP concentration; however, interaction analysis indicated no clear synergistic toxicity. Notably, CMA produced lower EC_{50} values than the chloride salts and elicited a distinct response pattern, potentially related to oxygen demand during CMA degradation. Species-specific physiological differences were also observed between *L. minor* and *S. natans*. These results indicate that nanoplastics can modify deicer toxicity and that both traditional and eco-friendly deicers may pose ecological risks, underscoring the need for careful regulation and evaluation of alternative deicing agents.

Is the aquatic macrophyte *Landoltia punctata* tolerant to high concentrations of polystyrene nanoplastics?

Lalau, CM; Simioni, C; de Oliveira Franco Rossetto, AL; Puerari, RC; de Carvalho, CR; Ouriques, LC; Matias, WG (2025) The Science of the Total Environment 1010: 181165.

Nanoplastics (NPs), arising from the degradation of plastic waste, are emerging environmental contaminants with high adsorptive potential for hydrophobic pollutants. Despite their recognized risks, knowledge of their effects on living organisms under natural environments remains limited. This study aims to evaluate the adverse effects and toxicological mechanisms of polystyrene nanoplastics (PSNPs) in the aquatic macrophyte *Landoltia punctata*. To this end, PSNPs were synthesized and characterized, followed by in-depth toxicological assessments. *L. punctata* was exposed to six concentrations of PSNPs (50, 100, 200, 400, 800 and 1600 mgL^{-1}), and a control, under standardized conditions (ISO DIS 20079, 2005), followed by a second exposure phase using EC_{50} (682.5 mgL^{-1}), LOEC (50 mgL^{-1}), and the highest concentration at which growth was still observed (800 mgL^{-1}). This exposure aimed to identify the factors underlying the observed effects on plant growth and to enhance understanding of plant response mechanisms. Morphological, functional, and ultrastructural modifications, alongside alterations in photosynthetic pigment profiles and oxidative stress biomarkers were analyzed, to elucidate the mechanisms underlying nanoplastic toxicity. The results demonstrated alterations in the plant's growth rate, enzymatic activity, and tissue structure, alongside evidence of PSNP adsorption and cellular internalization. Nevertheless, metabolic activity remained unaffected, allowing sustained plant development even at elevated concentrations. These findings indicate that, despite physiological and structural impacts, the organism displays resilience to PSNP exposure. Consequently, it presents promising potential for use in bioremediation strategies targeting environments contaminated by this pollutant, underscoring its significance as a biological agent for environmental restoration.

Investigating the phytotoxic effects of binary mixtures of diclofenac and paracetamol on duckweed - synergistic or antagonistic interaction?

Zezulka, S; Kummerová, M; Oravec, M; Babula, P. (2025) Environmental Pollution 387: 127295.

Mixtures of emerging aquatic contaminants, like diclofenac (DCF) and paracetamol (PCT) commonly occurring in the environment, can exhibit combination effects. Accumulation of drugs in duckweed plants exposed for seven days to DCF + PCT mixtures was similar to that under individual drugs (both 0.2, 2, and 20 mg/L). But the nature of drug interaction in mixtures was ambiguous, even though the toxic impact of DCF was more substantial than that of PCT. Mixtures with a DCF-to-PCT ratio of 1:1 exhibited a synergistic interaction manifested by a decrease (up to 90 %) in plant number, especially in mixtures DCF 2 + PCT 2 and DCF 20 + PCT 20 mg/L. In mixtures with unequal ratios (1:10 or 1:100 with a predominance of DCF or PCT), their interaction attenuated with time to additive or even antagonistic. This finding (shift from synergistic to additive interaction) was supported even by changes in the plants' dry weight, leaf area, or photosynthetic pigment

content. Similarly, a negligible decrease in photosynthetic performance in plants exposed to the mixtures with unequal ratios supported the mitigation of the drug mixture effect. Both individual drugs and their mixtures of 2 and 20 mg/L elevated the production of reactive oxygen species. The antioxidant defence mechanism activity was elevated already by low drug doses (0.2 and 2 mg/L), and the highest contamination (20 mg/L) led to its decrease. Utilizing the "number of plants" parameter showed that the nature of drug interaction in the mixture is fluctuating, and the causal factors include the ratio of mixture component concentrations and the length of the exposure period.

DF Comment: In the full text the authors explain that they obtained *Lemna minor* from the German Environmental Agency

Diclofenac uptake does not affect the growth and photosynthetic performance of *Spirodela polyrhiza* (L.) Schleid. fronds under laboratory conditions: Implications for the pharmaceutical compound phytoremoval

Pietrini, F; Urbaniak, M; Passatore, L; Marzi, D; Zacchini, M; Donati, E. (2025) Aquatic Toxicology 289: 107598.

The growing presence of pharmaceutical residues in freshwater ecosystem requires to expand our knowledge of the impact of these pollutants on biota. In this regard, studies on the effects of diclofenac (DCF), one of the most detected pharmaceutical compounds worldwide, on freshwater biota are needed to better understand the mechanisms associated to its toxicity, especially in plants. As model plants for toxicity assay in freshwater environment, duckweeds can be targeted for this scope. In this study, *Spirodela polyrhiza* L. Schleid. fronds were exposed to 4, 20 and 100 μg L^{-1} DCF for 7 days under laboratory conditions. At the end of the assay, biometric, spectral reflectance and chlorophyll fluorescence parameters were analysed to investigate the effects of DCF on the growth, pigment content and photosystem performance, revealing a lack of toxic symptoms at any concentration tested. The analysis of DCF concentration in the medium alone, exposed to light, evidenced the photosensitivity of DCF (removal of 22 % and 62 % of the initial DCF content at 4 and 100 μg L^{-1} DCF, respectively) even in lab conditions. The removal rate of DCF further increased when plants were grown in the medium, reaching the 81 % of the initial DCF content at 4 μg L^{-1} DCF. Diclofenac was slightly accumulated by *S. polyrhiza* fronds without a linear relation to its concentration in the medium. The ability of *S. polyrhiza* plants to remove DCF is discussed, considering the significant reduction in the DCF content of the medium in which the plants were grown, the low DCF accumulation in the plant tissues, the physico-chemical characteristics of the molecule and the experimental conditions used.

Taxonomy & Geobotany

Genetically verified mass material of *Lemna* in temperate East Europe: frequent *L. minor* - *L. x japonica* co-occurrence and new records of *L. gibba* and *L. turionifera*

Volkova, PA; Ivanova, MO; Matieva, LG; Bobrov, AA .(2026) Aquatic Botany 202: 103950.

Native representatives of the economically important genus *Lemna* in Europe belong to the taxonomically problematic *L. minor* complex (in addition to well-distinguishable *L. trisulca*). Distribution of the taxa from this complex in East Europe and their diagnostic characters are still unclear. We explored 41 waterbodies in the model area of ca. 30 km^2 in Yaroslavl Region (Russia). We genetically verified (second intron length polymorphism in beta-tubulin nuclear gene) taxonomic identity of 175 plants (2-10 plants per site). *Lemna minor* was the most frequent; either alone (20 sites), or together with *L. x japonica* (17 sites). In one site we found only *L. x japonica*. We have not found difference of the type, pH and mineralization of waterbodies with or without *L. x japonica*. Our quantitative data on mass live material of *L. minor* and *L. x japonica* did not support the existence of differences between them on the main diagnostic characters (color and shape of the fronds and sizes of papules on their upper side). We provided the first genetic documentation of *L. gibba* and *L. turionifera* occurrence in the region, extending their verified areas. We found flowering *Lemna* spp. with fully fertile pollen in 12 (29 %) waterbodies of all available types. For the first time we managed to verify genetically taxonomic affiliation of plants, flowering in nature (*L. minor* and *L. x japonica*). All genetically tested fruiting plants appeared to be *L. minor*.

Hybridity of mainly asexually propagating duckweeds in genus *Lemna* - dead end or breakthrough?

Lee, Y; Braglia, L; Stepanenko, A; Fuchs, J; Schubert, V; Gianì, S; Romano, LE; Aronne, G; Forti, C; Schubert, I; Morello, L. (2025) New Phytologist DOI10.1111/nph.70748

The cosmopolitan, mainly vegetatively propagating, organ-reduced monocotyledonous aquatic duckweeds are the smallest and fastest growing angiosperms, distributed world-wide and flower rarely in nature. Recently, we reported intra- and interspecific hybrids and ploidy variants in the genus *Lemna*. Thus, contrary to the expectation, sexual propagation may occasionally occur within and between *Lemna* species. Our main goal was to uncover whether the ecologically successful hybrids are evolutionary dead ends or initiate further speciation and novel sexual recombination. We investigated flower development, pollen viability, seed set, and seed germination in hybrids and their parental species and characterized genome size and genetic markers in the progenies. Intraspecific crosses yielded fertile progeny, but all diploid and triploid interspecific hybrids were male sterile. Only an established allotetraploid hybrid reproduced sexually, while colchicine-induced allotetraploids from allodiploids did not regain sexual competence so far. We concluded that only established allotetraploid hybrids represent an evolutionary breakthrough in duckweeds. Our results regarding sexual traits within the duckweed genus Lemna and the sexual competence of diverse hybrids pave the way for further investigation in this understudied field, provide fundamental data regarding the evolutionary potential of duckweed hybrids and are important for future breeding efforts on this emerging crop.

Do interspecific hybrids lead to new evolutionary avenues in the plant family Lemnaceae? (Commentary)

Sree, KS; Appenroth KJ (2026) New Phytologist https://doi.org/10.1111/nph.70904

Hybridization in plants has been researched for a considerable amount of time and in several directions in terms of its role in evolution. However, some of the plants that predominantly propagate by vegetative means have not been in the limelight, in this context, probably due to the scarce availability of sexually propagating lab-based bioresources. One such group of plants is the family Lemnaceae, which is commonly termed as duckweeds or water lentils. Although these are angiosperms capable of generative propagation, they majorly propagate by the budding of daughter fronds from mother fronds, that is by vegetative propagation (Landolt, 1986). The article recently published in New Phytologist, by Lee et al. (2025; doi: 10.1111/nph.70748) entitled 'Hybridity of mainly asexually propagating duckweeds in genus Lemna – dead end or breakthrough?' has investigated inter- and intraspecific hybrids of different ploidy levels in two of the sections of the genus *Lemna*, *Alatae* and *Lemna*, of the family Lemnaceae, for their role in evolution of this group of aquatic monocots.

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).

References:

All references cited in the text should be listed at the end of the article. They must be arranged alphabetically. References should be cited in the text as author name and year in braces (Maheshwari, 1954; Appenroth and Lam, 2019; Smart et al., 1993). Link to a webpage should be provided only in case it is not a regular publication.

- Each reference should be listed as in the following examples:

Khurana JP; Tamot BK; Mahshwari SC (1986) Induction of flowering in a duckweed, *Wolffia microscopica*, under non-inductive long days, by 8-hydroxyquinoline. Plant & Cell Physiology 27: 373–376.

- Books or other non-serial publications which are quoted in the references must be cited as follows:

Landolt E. (1986) The family of Lemnaceae – a monographic study. Vol. 1, Biosystematic Investigations in the Family of Duckweeds (Lemnaceae). Veroeffentlichungen des Geobotanischen Institutes der ETH, Stiftung Ruebel, Zurich, Switzerland.

Bog M et al. (2020) Genotyping-by-sequencing for species delimitation in *Lemna* section *Uninerves* Hegelm. (Lemnaceae). – In: Cao XH et al. (eds) The Duckweed Genomes. Springer, pp 115–123.

Authors:

- All authors are responsible for the content of the manuscript.
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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://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 request our readers to provide us with the new or updated data about their stock collection in order to update the existing list.

Note to the Reader

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