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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Group photos of the 8th ICDRA in Italy

Cover page

Group photos of the 8th ICDRA in Italy: The 8th International Conference on Duckweed Research and Applications was held at University of Naples Federico II, Naples, Italy from 28th September to 2nd October, 2026. Photos courtesy: 8th ICDRA organizers.

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The 7th International Steering Committee on Duckweed Research and Applications Members

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- **Prof. Eric Lam**, Rutgers, The state University of NJ, New Brunswick, USA; ericL89@hotmail.com
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All prior Duckweed Forum issues: http://www.ruduckweed.org/

Letter from the Editor:

October 30, 2026

Dear Duckweed Community,

It was great meeting several of you at our 8th International Conference on Duckweed Research and Applications (ICDRA-8) held recently in Naples, Italy from 28 September to 2 October, 2026. The meeting was a vibrant mixture of young minds and expert professionals in both research and applications of duckweeds. Kudos to the organizers, Leone Ermes Romano and Giovana Aronne from University of Naples Federico II and Laura Morello and Luca Braglia from Institute of Agricultural Biology and Biotechnology (IBBA-CNR) from Italy for all their hard work in organizing this great academic duckweed event and for their wonderful hospitality. More than 100 participants from across the globe deliberated on diverse topics of duckweed research and applications which were bundled into the sections: Cell Biology, Physiology and Metabolisms; Genes, Genomes and Evolution; Microbiome and Interactions; Ecology and Diversity; Applications — Food, Feed & Nutrition; Wastewater Remediation; Biotechnology; Round Table Discussion (challenges and opportunities of duckweed as novel crops). The coverpage of this issue highlights this event with the group photos of the participants of the 8th ICDRA. For those who could not attend the meeting, the abstracts presented at the meeting can be accessed from the website of the 8th ICDRA.

The ISCDRA thanking the organizers for the successful conduct of 8th ICDRA

The conference talks started with an honorary talk entitled "Elias Landolt (1926 – 2013), father of modern duckweed research" in order to celebrate the 100th birth anniversary of Late Elias Landolt. Klaus-J. Appenroth from Friedrich Schiller University, Jena, Germany who delivered this talk was honoured for his lifetime achievements in the field of duckweed research and applications at the closing ceremony of this conference.

Honoring the achievements of Klaus-J. Appenroth by Marcel Jansen (Left) and by Eric Lam (Right) presenting a plaque to Klaus Appenroth, University of Jena, Germany

Student participation through both oral and poster presentations was phenomenal. The Best oral presentation award sponsored by the *New Phytologist* was won by Julia Eva Fortmueller from Weizmann Institute of Science, Israel (Topic: Recombinant Expression of Silk Sericin in Duckweed (*Lemna japonica*) as a Sustainable Platform for Functional Biomaterial Production) and the Best Poster presentation awards sponsored by *Plants* MDPI were won by Alberto Giuliano from University of Naples Federico II, Italy; Rebecca Fairburn from The University of Nottingham, UK; Anna Kolehmainen from Johannes Gutenberg University Mainz, Germany; Cristian Perna from Institute of Agricultural Biology and Biotechnology-NRC, Italy and Dalia Rishmawi Avraham Levy from Ben Gurion University of the Negev, Israel. Congratulations to all of you.

Best Oral and Poster Presentation Awards presented to the winners

At this meeting the 7th International Steering Committee on Duckweed Research and Applications was constituted with the following elected members: Klaus-J. Appenroth (Friedrich Schiller University, Jena, Germany), Eric Lam (Rutgers University, New Brunswick, USA), Ingrid van der Meer (Wageningen University & Research, The Netherlands), Hongwei Hou (Shenzhen University of Advanced Technology and Institute of Hydrobiology, Chinese Academy of Sciences, China) and K. Sowjanya Sree (Banaras Hindu University, Varanasi, India). All the members unanimously elected K. Sowjanya Sree as the Chair of the 7th ISCDRA. It is our pleasure to introduce in this issue of DF, the two newly elected members to the ISCDRA, Ingrid van der Meer and Hongwei Hou. The ISCDRA thanks the members of the previous terms, Marcel Jansen, Tsipi Shoham and Asaph Aharoni for their services to the duckweed community and their active participation in the activities of the ISCDRA.

The 7th ISCDRA (2026-28). From Left to Right: Eric Lam, Ingrid van der Meer, K. Sowjanya Sree, Klaus-J. Appenroth and Hongwei Hou

The participants of the 8th ICDRA voted for the host venue of the 9th ICDRA to be held in 2028. Out of the four places that contested for the same from Australia, Belgium, China and Vietnam, Shenzhen University of Advanced Technology, China won the election. You are invited with a welcome note for

the 9th ICDRA to be held in 2028 from the President of the Shenzhen University of Advanced Technology, China in this issue. We are already looking forward to it. In the next several issues of Duckweed Forum, updates of the conference arrangements of the 9th ICDRA will be announced.

Along with these highlights, this DF issue presents two commentaries, one on the cooperative cobamide biosynthesis in a clone of *Wolffia globosa* based on the highlight article from the database section of the previous issue by Dutta et al., 2026 and another based on Baghini et al., 2026 on engineering of duckweeds for molecular farming; an interview of Dr. Krachang Bhanthumnavin who highlighted the potential of duckweed as a protein source already decades back, student spotlight by Hu Huangkai from Institute of Hydrobiology, Chinese Academy of Sciences, China, an opinion article that states that growth rate of duckweed per cultivation area cannot be defined consistently and the Database by our ISCDRA member, Klaus Appenroth. Also, a summary of the open discussion on *in vitro* cultivation of *Lemna trisulca* as facilitated by the DF is presented in this issue.

Enjoy reading!

Wish you a good year-end time ahead.

Sincerely,

K. Sowjanya Sree

Chair, 7th ISCDRA

Introduction of new members elected to the 7th ISCDRA

The 7th International Steering Committee on Duckweed Research and Applications was constituted on 2nd October, 2026 during the General Assembly of the 8th ICDRA in Italy. Along with the re-elected members, Eric Lam, Klaus-J. Appenroth and K. Sowjanya Sree, two members were newly elected to the committee, Ingrid van der Meer and Hongwei Hou.

Dr. Ingrid van der Meer

Wageningen University & Research, The Netherlands

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It is a great pleasure for me being elected as a member of the next steering committee of International Duckweed Research and Application (ISCDRA). My specific task within the ISCDRA will be to present the more applied part of duckweed research and focus on the various topics of interest relevant to application as well as commercialization of duckweeds.

A bit about myself. I am a senior-researcher at Wageningen University and Research, with over 100 peer-reviewed scientific publications with h-index of 45, and for 12.5 years heading the department of Bioscience at the Plant Sciences group. I gained my PhD in Plant Molecular Genetics at the Free University in Amsterdam on genetic regulation of flavonoid biosynthesis in Petunia. Studying the regulation and organization of plant biochemical pathways remained an important research topic in my scientific career. The research at my department is focused on gaining a better understanding of plant processes. An important target is the biosynthesis and health aspects of plant metabolites and proteins for food and nutrition.

My first introduction to duckweed, two decades ago, was in a research project in which we wanted to use duckweed as a 'factory' for the production of human monoclonal antibodies (mAbs). Especially mAbs for which the different glycosylation pattern is not important. To use duckweed for molecular farming was not new at that time. However, it was still a challenge to set up an efficient transformation and regeneration system for *Lemna minor*, but we succeeded and could successfully produce the human protein.

Figure (Above): GFP positive frond regenerant from *Lemna minor* nodules (left), and Western blot analysis of 10 transgenic *Lemna* lines expressing mAb (right panel).

Having worked with this little plant that possess so many interesting features, planted the little (duckweed) seed in me to start researching if it could also be used for human nutrition. And indeed, all our next molecular analyses on metabolite and protein composition, nutritional value, but also growth capacity and putative consumer acceptance, led me to my new goal to get the approval from the European Food Safety Authority as being safe for human nutrition as vegetable. This was my new quest, and I was very lucky to obtain the financial support for all the research that would be necessary via charitable foundations. At that time, growers and food industry did not want to spend money on building such an expensive Novel Food dossier.

Next to all the technological and scientific research that was needed for the Novel Food dossier of 'water lentils', we also worked on many more duckweed research projects, ranging from the use of duckweed as animal feed grown on bioslurry produced by a biodigestor at small holding farms in Indonesia, to protein extraction and (functional) food product development. I might be the specialist on EFSA Novel Food legislation, but also a generalist on many more duckweed research topics.

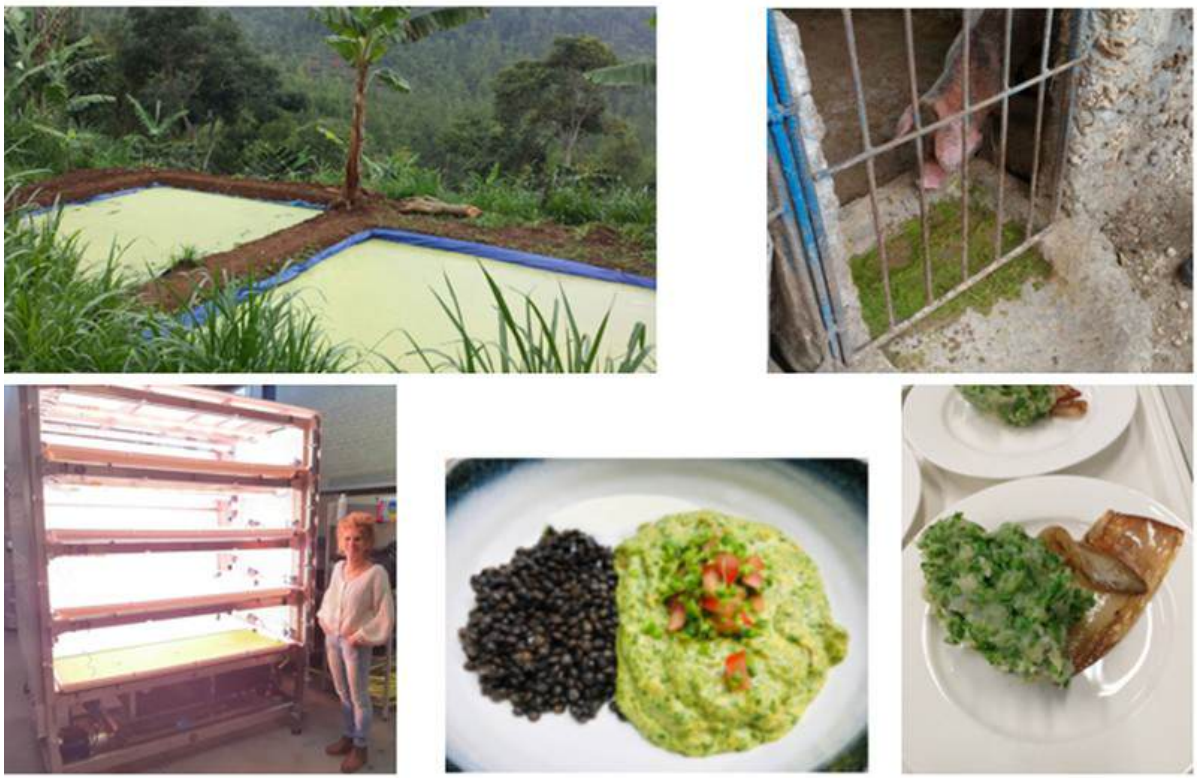

Figure (Above): A variety of research projects: from cultivation of duckweed for feed in Indonesia (upper panels), to indoor vertical farming of water lentils for human consumption (lower panels)

Since 2019 I have been attending the ICDRA conferences, my first one was in Rehovot, Israel. And I enjoyed each conference since then. It is also a pleasure to see the number of attendees growing and the broadness and quality of the research themes intensifying. It might be because of my broad interest in research topics, but there were many presentations in Portici that made my inner science geek very happy and got my full attention. On top of that, it was nice talking to research colleagues, companies, and old friends about our common interest, duckweed, during the coffee breaks/ lunch/ dinner events.

I am looking forward to 'serve' this, for me special, community of people working on duckweed research and applications.

Dr. Hongwei Hou

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I am a professor affiliated with Shenzhen University of Advanced Technology and the Institute of Hydrobiology, Chinese Academy of Sciences. I received my PhD from the School of Life Sciences, Peking University in 2004. Afterwards, I pursued research in plant development, physiology and ecology at the University of Lethbridge, the University of Toronto, Agriculture and Agri-Food Canada research centers, and the Institute of Hydrobiology, Chinese Academy of Sciences.

I studied how auxin shapes leaf vein development through developmental canalization in the model plant *Arabidopsis thaliana*, and elucidated the functional mechanism of the cytokinin receptor family underlying nitrogen-fixing nodule formation in the model legume *Lotus japonicus*. I have also established a set of aquatic plant research models, including duckweeds and *Hygrophila difformis*. In this work, I systematically uncovered the unique adaptive mechanisms of aquatic plants in morphology, photosynthetic regulation and carbon assimilation, and demonstrated their promising potential for biomass production, plant protein substitution and aquatic ecological restoration.

To date, I have published more than 100 SCI-indexed papers in high-impact journals including *Plant Cell, Plant Physiology, New Phytologist, Plant Journal, Current Biology, Advanced Science, Plant, Cell & Environment, Journal of Experimental Botany, Plant Molecular Biology, PNAS Nexus, Bioresource Technology,* and *Environmental Research*, among others. My publications have received over 3000 citations.

My current research centers on duckweed, focusing on biomass accumulation, photosynthetic carbon assimilation, metabolic dissection, vertical farming, and the construction of synthetic biology plant chassis. Using multi-omics approaches to dissect photosynthetic, metabolic and developmental pathways, I identify key genetic resources enabling rapid duckweed growth and efficient protein accumulation. I engineer elite high-yield, high-protein duckweed germplasm and investigate the biosynthesis of pharmaceutical flavonoids and isoflavonoids using this plant chassis, advancing research and applications of duckweed-derived high-protein feed. I also characterize the effects of light, temperature, carbon sources and microorganisms on duckweed productivity, and translate improved germplasm, technologies and equipment to develop a low-cost, high-yield duckweed production system.

Duckweed is widely used as a model plant for metabolic studies. With rapid growth, strong biosynthesis, short life cycle, simple structure, space-saving and factory-scalable cultivation, it represents a highly valuable plant chassis for biomanufacturing production.

As a committee member of the International Steering Committee on Duckweed Research and Application, I will foster international collaboration in duckweed research and application, advance the duckweed industry, and mentor early-career scientists.

Commentary: Sharing the vitamin B_{12} burden: cooperative cobamide biosynthesis in *Wolffia globosa* Mankai

Rhishika Dutta

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What is the origin of vitamin B_{12} that are found in the Mankai strain of *W. globosa*?

Vitamin B_{12} is not abundant in the plant world. Plants apparently neither synthesize nor require this vitamin (Acosta *et al*.,2024), and its production is apparently restricted to certain bacteria and archaea. Yet the edible duckweed *Wolffia globosa* Mankai strain is known to contain bioavailable B_{12}, at about 2.8 µg per 100 g dry weight, and the B_{12} from Mankai has been shown to be bioavailable to humans (Sela *et al*., 2020). This raises the question of the origin for this vitamin B_{12}?
Previous work suggested that bacteria living on the plant surface do not fully explain B_{12} accumulation in duckweed (Acosta *et al*., 2024). We therefore looked inside the plant and asked whether B_{12} production might instead be a function of the endophytic microbial community. In our recent study (Dutta *et al*., 2026), we found that the answer could be more interesting than identifying a single B_{12} producing bacterium. The Mankai endosphere appears to contain a *cooperative cobamide biosynthesis guild*, in which a small number of potential producers coexist with bacteria that can salvage, remodel, or use cobamide intermediates.

From the cultivation water to the inside of the plant

In our recent work, Mankai duckweed was sampled from a controlled greenhouse cultivation system in Hinoman company at Kibbutz Be'eri, Israel. We examined bacteria from three compartments: the cultivation medium, washed fronds representing the plant associated fraction, and surface sterilized fronds representing the endosphere.
The first result confirmed that Mankai strongly filters its microbiome. Bacterial richness and diversity decreased from the cultivation medium to the plant associated community and then to the endosphere. Shannon diversity declined from 4.2 in the medium to 3.3 in the washed fronds and 3.1 in the endosphere. The three communities were also clearly separated by their bacterial composition, and only 18 amplicon sequence variants were shared across all three compartments. In other words, the bacteria living inside Mankai were not simply a random subset of those found in the surrounding water. We have found that the endosphere was distinct, relatively low in diversity, and enriched in taxa with potential cobamide biosynthetic capacity. Therefore, we focused metagenomic analysis on this compartment.

A few producers, many salvagers

Genome resolved metagenomics revealed an uneven distribution of B_{12} biosynthesis genes across the endophytic community. Several *Alphaproteobacteria* carried near complete sets of genes for *de novo* cobamide biosynthesis. Many other members of the community lacked several steps of the pathway but

retained genes for cobamide salvage and remodeling. This suggested a division of labour, rather than every bacterium carrying the expensive machinery required to synthesize a complete cobamide from scratch, some community members may depend on intermediates or completed cobamides supplied by others. To examine this scenario, we constructed a functional complementarity network based on the cobamide biosynthesis, salvage and remodeling genes found in the reconstructed genomes. The network identified bacteria with near complete pathways as highly connected hubs. These links represent predicted metabolic compatibility, not direct measurements of metabolite exchange. Nevertheless, the pattern is consistent with a producer salvager community in which the B_{12} biosynthetic burden is distributed among its members.

What happens when bacteria live as a community?

We compared four Mankai endosphere species genomes with genomes of the same species obtained from culture collections. The endophytic genomes generally contained more fragmented cobamide pathways, whereas their cultured counterparts retained more complete pathways. Because these cultured bacteria are related strains rather than the exact strains living inside Mankai, we cannot conclude that pathway loss occurred after colonization of the plant. Still, the pattern is consistent with metabolic streamlining: in a stable microbial community, individual bacteria may no longer need to maintain every costly biosynthetic step if essential metabolites or intermediates are available from their neighbours. Such division of labour is common for cobamides: most bacteria use them, but only about a third can synthesize them de novo (Shelton et al., 2019), and interspecies corrinoid exchange has been demonstrated in co-cultures (Yan et al., 2012), consistent with the Black Queen hypothesis.

A microbial economy rather than a plant-microbe trade

This changes how we think about B_{12} origin in duckweed. In the classic algal model, B_{12} producing bacteria provide the vitamin to B_{12}-dependent algae, often in exchange for photosynthetically fixed carbon. That explanation does not readily apply to Mankai because angiosperms have no known requirement for B_{12}. Instead, our results suggest a microbe to microbe economy occurring inside a plant host. Mankai appears to act as a strong ecological filter that brings together a specialized endophytic community. Within that community, some bacteria retain much of the machinery required for cobamide production, while others rely more heavily on salvage and remodeling. B_{12} production may therefore be better understood as a community level microbial function than as a trait of a single B_{12} producing bacterium.

Where do we go from here?

The next step is to move from genomic potential to experimental demonstration. Transcriptomics, metabolite tracing, and controlled combinations of producer and salvager bacteria could test whether the predicted exchange of cobamide intermediates actually occurs. Such experiments could also tell us how stable this metabolic partnership is under changing cultivation conditions and whether it can be managed to maintain B_{12} production. For the duckweed community, Mankai provides an unusually tractable system: unlike other B_{12}-containing duckweeds (Klamann et al., 2023; Acosta et al., 2024), its B_{12} is proven bioavailable to humans (Sela et al., 2020), and it is grown as a single rootless clone under controlled conditions, holding host genotype and environment largely constant. This allows us to ask a broader question: when we measure a useful trait in a plant, are we sometimes measuring the function of the plant and its microbiome as a unit? In the case of vitamin B_{12}, our results suggest that the microbial community, not any single resident bacterium, may be the more meaningful functional unit.

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9th ICDRA- 2028 in China

Welcome note

Dear Colleagues and Friends,

On behalf of Shenzhen University of Advanced Technology (SUAT), it is my great honor and sincere pleasure to welcome you to the 9th ICDRA (International Conference on Duckweed Research and Applications). SUAT will host this conference in Shenzhen, China, in 2028. The event window spans September to December, with specific dates to be announced at a later time.

SUAT is a new research university established by the Shenzhen Municipal Government and approved by the Ministry of Education. We are defined by our high starting point, research-focused mission, compact scale, and global openness. The university prioritizes fundamental and frontier scientific research and is dedicated to cultivating top innovative talent — exceptional scientists, outstanding engineers, and creative entrepreneurs. Though recently founded, SUAT has already assembled an impressive faculty team: over 90% hold doctoral degrees, 72% have overseas study or work experience. Our community includes 14 full-time academicians, 33 scientists listed among the world's top 2%, and four recipients of the Chinese Government Friendship Award.

Our campus sits at the heart of Guangming Science City, home to nine national major research platforms. These include our cutting-edge Synthetic Biology Research Infrastructure and the world's only 10,000-square-meter P3 laboratory. During the conference, I invite you to experience these one-of-a-kind large-scale facilities firsthand and explore new opportunities for research collaboration.

This conference is co-organized by SUAT's Institute of Emerging Agricultural Technology (iEAT). Led by internationally acclaimed plant biologist Professor Zhenbiao Yang, the institute deploys synthetic biology principles and advanced manufacturing strategies across staple crops, economic plants, agricultural microorganisms, and green biomanufacturing, seeking to set a global benchmark for future agriculture research. Duckweed research stands as a core pillar of iEAT, spanning germplasm resources, molecular breeding, synthetic biology, and scalable cultivation technologies.

As the founding president of this young, open and international university, situated within one of the world's most dynamic innovation hubs, I strongly believe that science advances through openness and exchange. My colleagues and I look forward to welcoming you to Shenzhen in 2028. We will share our facilities, introduce our researchers, and extend our friendship to make this an inspiring and memorable gathering.

Warm regards,

Jianping Fan, Ph.D.

Professor and President

Shenzhen University of Advanced Technology

China

DUCKWEED FORUM
ISCDRA International Steering Committee on Duckweed Research and Applications

Commentary: Tiny Plants, Big Potential: Engineering *Lemna minor* for the Future of Molecular Farming

Sadegh Shojaei Baghini, Nima Rad, Elham Taghipour, Ali Hatef Salmanian

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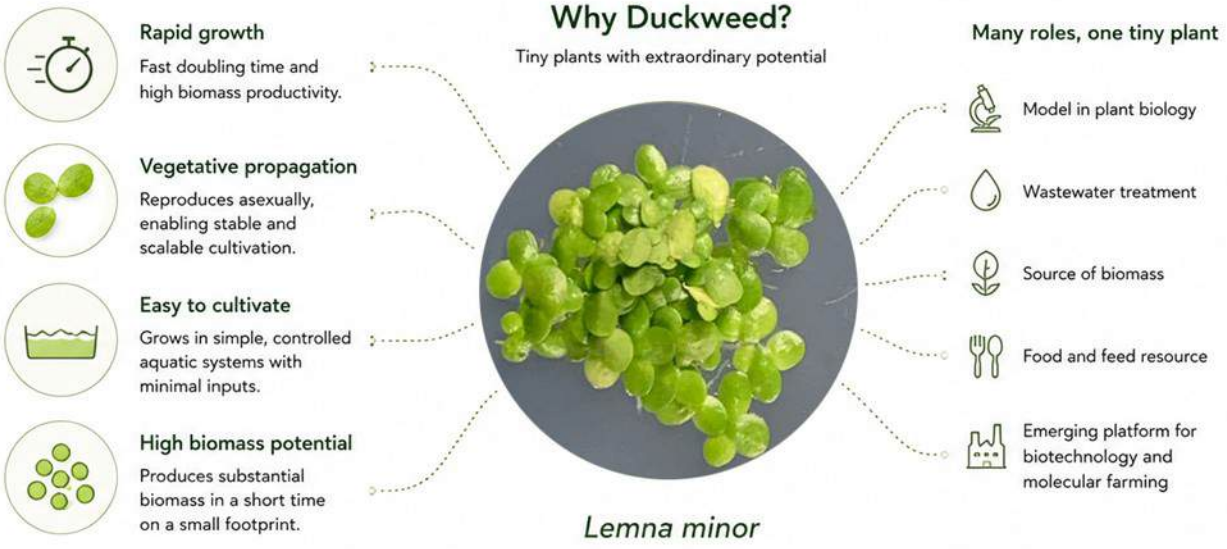
Why Duckweed?

Duckweeds are among the smallest flowering plants on Earth, but their biological potential is far greater than their size might suggest. These tiny aquatic plants can grow rapidly, reproduce predominantly through vegetative propagation, and produce substantial amounts of biomass within a short period. They can also be cultivated in relatively simple and controlled systems, making them attractive not only for basic research but also for a wide range of biotechnological applications. For decades, duckweeds have been studied as models of plant biology, wastewater treatment systems, sources of biomass, as well as potential food and feed resources. More recently, another possibility has attracted increasing attention: using duckweed as a biological factory for producing valuable proteins (Taghipour et al., 2022).



Schematic overview of duckweed applications

This concept is part of a broader field known as molecular farming, in which plants are engineered to produce recombinant proteins, including pharmaceutical proteins, antibodies, enzymes, and other high-value biological products (Heenatigala and Hou, 2024).

The appeal is straight forward. Plants can potentially provide scalable and cost-effective production platforms while avoiding some of the limitations associated with conventional microbial or animal-cell systems.

But if duckweed is to become a reliable molecular farming platform, an important question must be addressed: Can we engineer the plant itself to produce proteins with properties better suited to human applications?

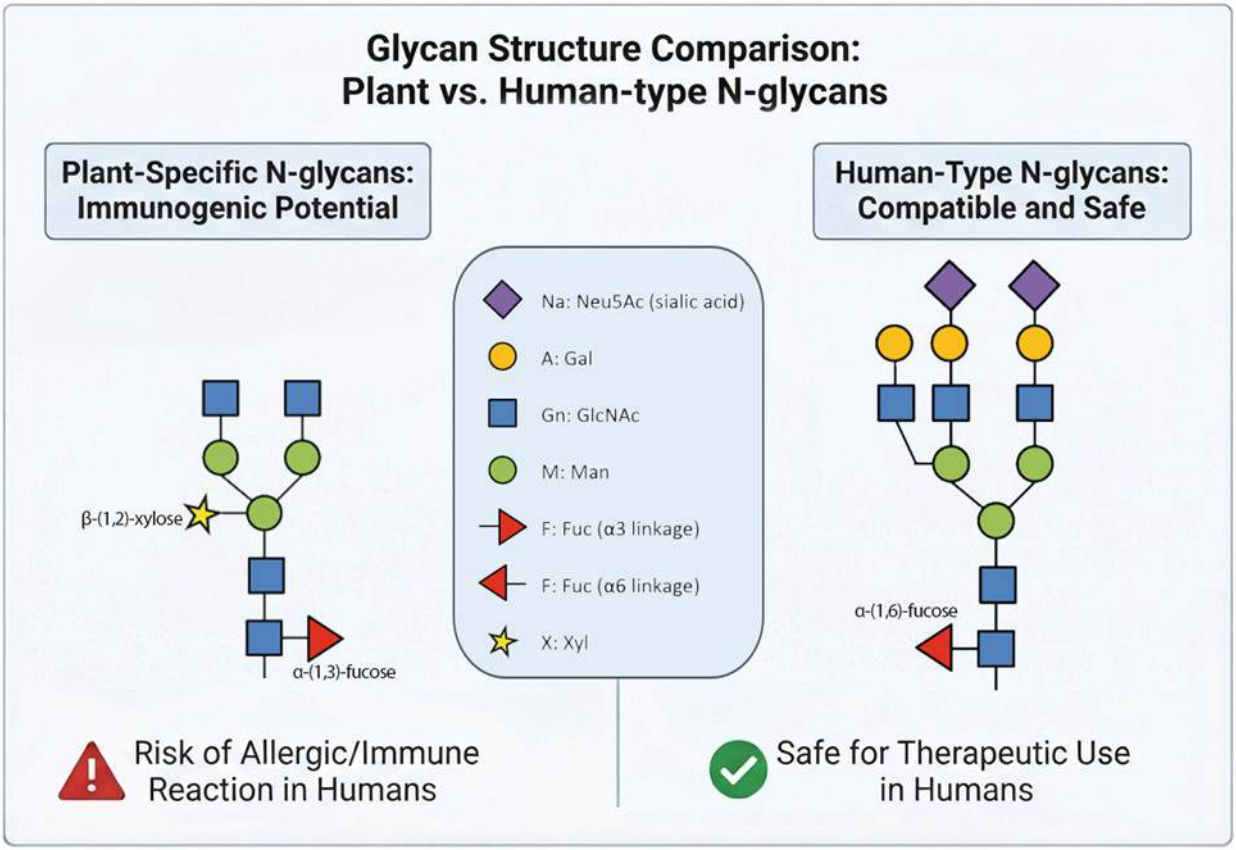
The Challenge Is Not Only Protein Production

Producing a protein is only part of the story. Proteins produced inside living cells often undergo a series of biochemical modifications after they are synthesized. One of the most important of these modifications is glycosylation—the attachment of specific sugar structures to proteins.

Glycosylation can influence protein folding, stability, biological activity, and protection from degradation. However, plants and mammals do not always produce identical glycan structures (Esmail et al., 2021).

Among the characteristic features of plant N-glycans are β-(1,2)-xylose and α-(1,3)-fucose residues. These plant-specific structures are different from those commonly found in mammalian glycoproteins and have therefore received considerable attention in the field of molecular farming (Strasser, 2022).

This difference does not mean that plants cannot produce useful proteins. Rather, it highlights an important opportunity: What if we could modify the plant's own glycosylation machinery?



Schematic illustration of the differences in N-glycosylation patterns between plant and human (Fischer et al., 2018)

Editing the Plant Instead of Accepting Its Limitations

Modern genome-editing technologies provide a way to address this challenge at its source. Two enzymes, β-(1,2)-xylosyltransferase (XylT) and core α-(1,3)-fucosyltransferase (FucT), play important roles in adding plant-specific sugar residues to N-glycans.

Therefore, modifying the genes encoding these enzymes could potentially alter the glycosylation profile of proteins produced by the plant. This was the central idea behind our study. Rather than simply asking whether *Lemna minor* can produce recombinant proteins, we asked a more ambitious question: Can we re-engineer the molecular machinery of *L. minor* to make it a more suitable platform for recombinant protein production?

CRISPR/Cas9: A Precise Tool for Genome Engineering

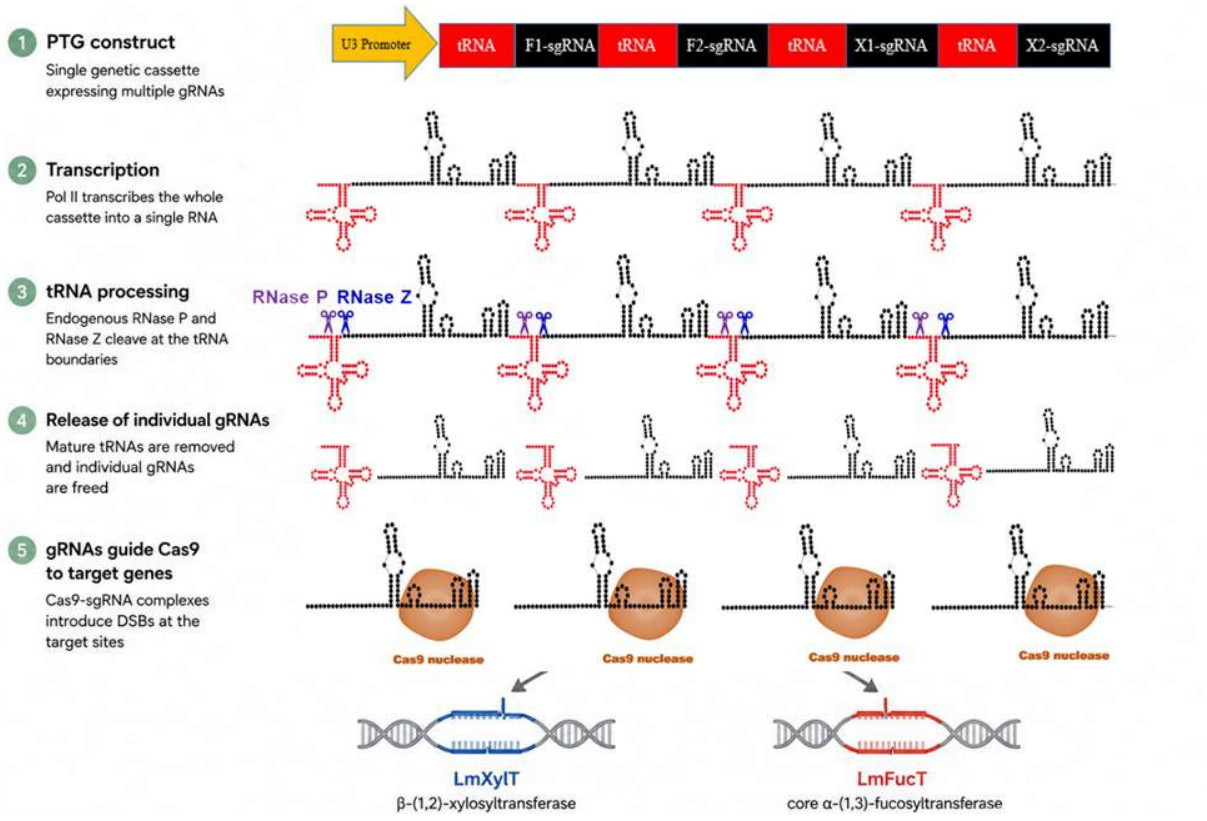
CRISPR/Cas9 has transformed the way scientists think about genome engineering. Its ability to target specific DNA sequences makes it possible to modify genes that control important biological traits. An additional advantage is that several targets can be addressed simultaneously, opening the door to multiplex genome editing. For duckweed research, this possibility is particularly interesting.

Duckweed biology involves many interconnected pathways, and improving a complex trait may require modification of more than one gene. In our study, we therefore focused on two genes associated with plant-specific N-glycosylation: *LmXylT* and *LmFucT*.

But targeting two genes simultaneously also creates a technical challenge: how can multiple CRISPR guide RNAs be efficiently delivered and expressed in the same plant? This is where the PTG strategy becomes particularly useful.

One CRISPR Construct, Multiple Targets

The polycistronic tRNA–gRNA, or PTG, strategy allows several guide RNAs to be assembled into a single genetic cassette. Instead of designing a separate expression system for every guide RNA, multiple guides can be organized within one construct. The plant's own tRNA-processing machinery helps release the individual guide RNAs, which can then work with Cas9 to target their respective DNA sequences (Xie et al., 2015).



Schematic illustration of polycistronic tRNA-gRNA (PTG) processing in the host cell

In practical terms, PTG provides a way to turn one CRISPR construct into a multi-target genome-editing system. For our study, we used this strategy to simultaneously target *LmXylT* and *LmFucT* in *L. minor*.

What Happened When We Edited the Two Genes?

Following transformation and plant regeneration, we analyzed the target regions using DNA sequencing. The results showed that both target genes could be successfully edited.

At the *LmXylT* locus, we identified homozygous, biallelic, and mosaic mutation patterns among the regenerated lines. At the *LmFucT* locus, homozygous and mosaic mutation patterns were also detected.

We also examined the corresponding transcripts and proteins. Sequencing of RT-PCR products confirmed that the mutations identified in the genome were reflected in the transcripts of the homozygous lines. Protein analysis provided further evidence that these genetic changes could influence the corresponding proteins. These results demonstrated that the PTG-based CRISPR/Cas9 system was capable of simultaneously targeting both genes in *L. minor*. The *L. minor* clone used was as described by Taghipour et al. (2022). For the design and bioinformatic analyses *L. minor* diploid reference genome (7210) was used.

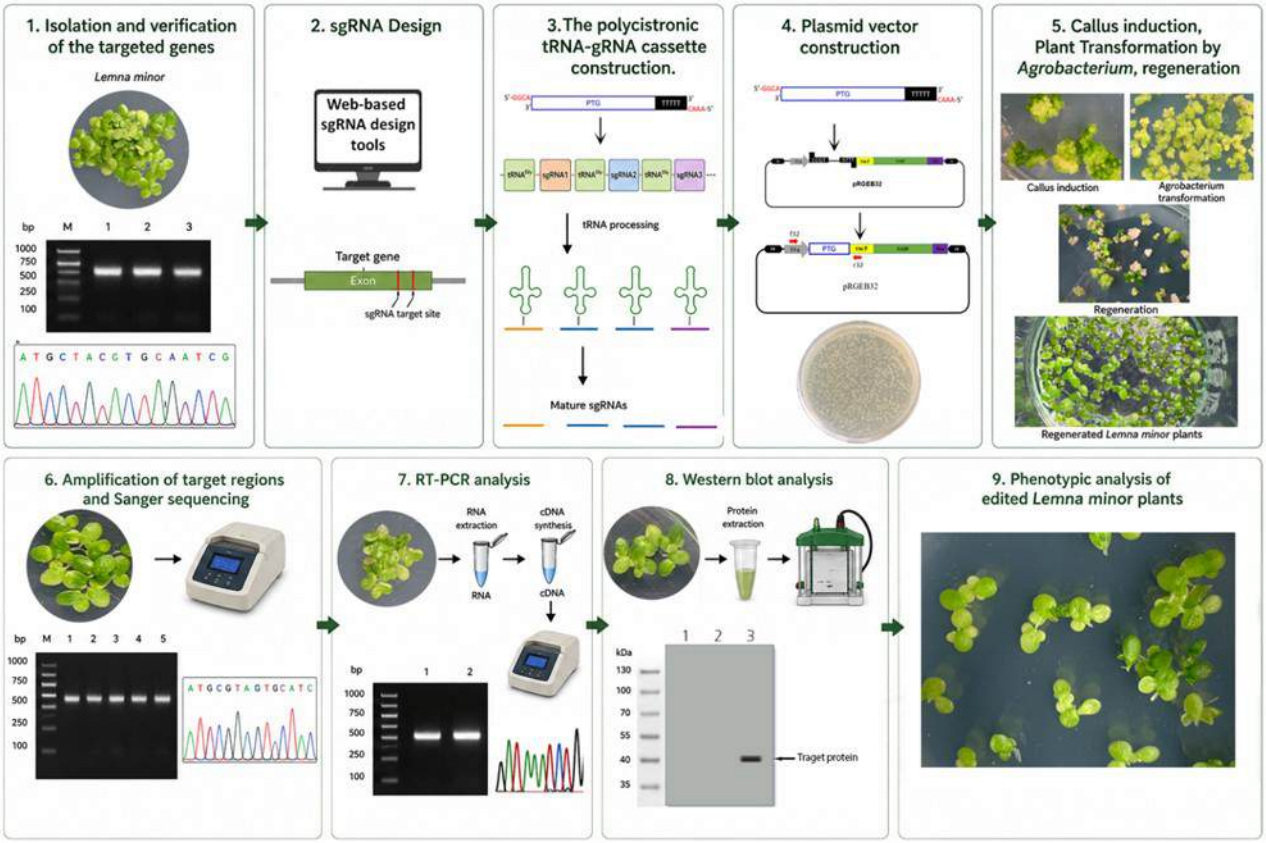
Why Does This Matter for Duckweed Research?

Duckweed research has expanded far beyond its traditional roles in plant biology. Today, duckweeds are being investigated for food, feed, environmental applications, biomass production, bioproducts, and molecular farming.

As these applications develop, the ability to precisely engineer duckweed biology becomes increasingly important. Our study adds another piece to this emerging toolbox.

By combining *L. minor* with CRISPR/Cas9 and the PTG strategy, we demonstrate a route for simultaneously modifying genes involved in an important post-translational modification pathway.

From Genome Editing to Real-World Applications



Schematic overview of the CRISPR/Cas9-mediated genome-editing workflow in *Lemna minor*

The ultimate goal of genome editing is not simply to create a mutant plant. The real value lies in what the engineered plant can do. If the glycosylation profile of recombinant proteins produced by *L. minor* can be predictably modified, engineered duckweed could become an increasingly attractive platform for producing proteins with applications in biotechnology, nutrition, diagnostics, and potentially pharmaceutical research. There is still work to be done. The next steps include detailed characterization of N-glycan structures, evaluation of recombinant protein quality and biological activity, and assessment of how consistently engineered lines maintain their desired characteristics. These studies will be essential for moving from genetic modification to functional application.

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Opinion: Growth rate of duckweed per cultivation area cannot be defined consistently

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In this paper we describe the calculation of relative growth rates (RGR) of duckweed and, on the basis of the same experimental data, the calculation of doubling time (DT) and relative yield (RY) (Ziegler et al., 2015). For experimental details see Appenroth (2015). In several publications, growth rates were given as increase of dry weight per time and cultivation area (Basnet et al., 2024; Guo et al., 2020; Islam et al., 2025; Xiao et al., 2013). This method is popular because it is assumed that this way quantitative data can be obtained about possible harvest yields. We are showing here that this is not the case.

1. Relative Growth rate and derived measures

Exponential increase of a population size can be described by the following homogeneous, first order differential equation:

$$dx/dt - k\,x = 0, \text{ or}$$
$$dx/dt = k\,x$$

with x the measured size (e.g. fresh weight, dry weight, frond number, two-dimensional plant area), t the time (usually in days, d) and k a constant coefficient (k > 0), which described the rate of exponential increase of the population.
By integrating between the limits of integration (t_0 = 0 d and t_7 = 7 d) and k = RGR, the following equation can be obtained:

$$RGR = (\ln x_7 - \ln x_0) / (t_7 - t_0) \quad (1)$$

with x_7 the value of the measured parameter after 7 days of growth and x_0 the initial value at start of the experiment.
For the RGRs of several duckweed clones from all five genera, the values were between 0.153 d^{-1} (*Lemna obscura* 7325) and 0.519 d^{-1} (*Wolffiella hyalina* 9525). (Ziegler et al., 2015; see also Sree et al., 2015).

This is not a very evident term, and therefore often used to calculate the doubling time (DT, usually given in d) or the relative yield (RY) (Ziegler et al., 2015).
The DT means the time of growth at which the measured parameter (e.g. fresh weight, dry weight, frond number, two-dimensional frond area) doubled the size. With $x_7 = 2x_0$, equation 1 can be transformed into equation 2:

$$DT\,(d) = \ln 2 / RGR \quad (2)$$

The DT is typically between 4.54 and 1.34 d (Ziegler et al., 2015).

The RY means the size of the used parameter after 7 days of plant cultivation starting with one unit, i.e. 1 g fresh matter,1 g dry matter, 1 frond or 1 cm^2 frond area). It is given by x_7 in equation 1 and can be calculated by the following equation 3:

$$\ln x_7 = 7RGR + \ln x_0, \text{ with } \ln x_0 = 0 \text{ and}$$
$$RY = x_7 = e^{(\ln x_7)} \quad (3)$$

RY values are typically between 2.57 and 37.8 g g^{-1} (Ziegler e al., 2015; see also Sree et al., 2015). When the plants are adapted to the growth conditions used between t_0 and t_7, there is practically no difference between the results obtained on the basis of FM, DM, frond number or two-dimensional frond area. However, in toxicity tests the effect of a toxicant, applied at t_0, is investigated and then the investigation of different parameters of growth is useful.

2. Relative growth rate per cultivation area

This kind of growth rate is defined by the difference of dry matter (DM) after 7 d / 1 week of cultivation and the initial dry weight at t = 0 (i.e. per 7 days) and square meter cultivation area (Basnet et al., 2024; Guo et al., 2020; Islam et al., 2025; Xiao et al., 2013):

$$GR = (DM_7 - DM_0)\ m^{-2}\ week^{-1} \quad (4)$$

Let´s assume the RGR = 0.3 d^{-1} and the dry matter content of 5%.
In a first virtual experiment, the initial fresh weight should be 100 g (equals to 5 g dry matter) on a cultivation area of 1 m^2. With the given RGR, the fresh matter after 7 days of cultivation is 816 g (equals to 40.8 g dry matter). The increase of dry matter is consequently 35.8 g. With equation 4 we get a GR of 35.8 g m^{-2} $week^{-1}$.

In a second experiment, the conditions should be almost the same, except that the cultivation area should be 0.25 m^2. The increase of dry matter is the same (35.8 g), however, per square meter we get the GR of 143 g m^{-2} $week^{-1}$.

In a third experiment, the initial fresh matter should be 1000 g, the cultivation area 1 m^2. All other parameters should be the same as in the two previous virtual experiments. The difference between the final and the initial dry matter would be 358 g, the growth rate consequently 358 g m^{-2} $week^{-1}$.

Sometimes, instead of DM, fresh matter (FM) was used for calculation of GR (Sun et al., 2020). Using the data set from experiment 1, initial FM was 100 g, the final FM after 7 days of cultivation was 816 g. As 1 m^2 cultivation area was assumed, GR calculates to 716 g m^{-2} $week^{-1}$. The GR defined this way is approximately twenty times higher than that calculated on the basis of DM.

It should be mentioned that in all four cases the RGRs are assumed to be identically with 0.3 d^{-1}.

3. Conclusions

Using the parameter GR as difference between final and initial biomass per square meter and week is very popular because it is assumed to give information about the biomass harvest. However, as shown above, the results vary strongly with the initial values and the experimental cultivation area. It would be possible to standardise the initial values by using the same initial fresh matter and the same cultivation area for all experiments. Considering both growth rate equations it becomes clear that in case of GR the initial dry matter (x_0) was just subtracted from the final dry matter (x_7 = RY if one unit was used as starting point). This is very artificial and is related to the linear growth model. Relating the grow rate to the cultivation area by dividing by any size of the cultivation area results in even more confusing “growth rates”. Moreover, even then the results would have no correlation with a possible harvest of biomass in the frame of biotechnology. To get the harvest under given conditions, the amount of practical total harvest of a season (biomass) must be related to the size of a pond or other cultivation area. Even then the data would strongly depend on the initial fresh weight

available at the beginning of the growth season. Nevertheless, these results could be of high ecological interest.

In summary, if the growth of species or clones should be characterised, the RGR according to equation 1 should be measured and might be converted in DT or RY to make the results more demonstrative. The term GR as difference between final and initial dry matter per 7 days and cultivation area should not be used anymore.

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Fifty-Five Years Later: Revisiting the Prospects for "*Wolffia* as an Inexpensive Protein Source"

- An Exclusive Interview with Dr. Krachang Bhanthumnavin

Interviewer: Metha Meetam

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Dr. Krachang Bhanthumnavin

In 1971, Krachang Bhanthumnavin and Michael McGarry published a pioneering paper in Nature[1], documenting traditional Wolffia cultivation in Thailand and highlighting its potential as an inexpensive protein source. Fifty-five years later, as duckweed gains renewed attention worldwide as a nutritious and sustainable food, I had the privilege of interviewing Dr. Bhanthumnavin, now 88, to share his recollections of his early research, the history of Wolffia cultivation in Thailand, and his reflections on the opportunities and challenges that lie ahead.

1. Can you tell us briefly about your background? How did you become interested in *Wolffia* research?

Having returned from my studies for B.Sc. (Hons.) in Agricultural Botany (1962) and Ph.D. in Plant Breeding (1965) at University of Reading, UK, I was assigned to work in the Department of Science and the Department of Plant Science at Chiang Mai University, a centre of higher education in northern Thailand--where I spent 5 years teaching.

During the 1950s to 1970s, the world was preoccupied with widespread malnutrition in every continent, among other politico-military crises. Thailand, then an underdeveloped nation, was also facing a serious malnutrition problem, due to both general poverty and lack of proper dietary knowledge among the rural population. The government's promotion of modern livestock farming and the westernization of food tastes and eating habits drew the well-to-do class of people towards meat foods, whereas the lower-income class and most of the rural population, who could not afford such luxurious habits, were left with obvious malnutrition, especially protein deficiency.

To the eye of a trained cytologist, economic botanist and plant breeder, the northern region of Thailand (in that period) had a vast diversity of domesticated, partially domesticated and wild food

plants. So, I spent my off-duty time walking through local traditional markets and visiting rural communities, observing their crops, cropping techniques and their food sources.

My scientific curiosity led me to find many (over 10) natural plant-foods, leaves, tubers, fruits, and among them was "Pham" or *Wolffia* sp. The widespread availability, ease of cultivation, (seeming) popularity and simple ways of cooking and eating Pham captured my attention.

2. What happened next? Can you tell us more about your research approach and the context of *Wolffia* farming back then?

I bought several kilograms of Pham and had the sample analysed for basic components, including carbohydrate, fat, protein and fibre. And the high ratio of protein content captured my imagination, thus leading to the term "an inexpensive protein source" later on in the title of my publication. I also visited several farms in the northern region to collect information regarding the growth conditions and productivity. In fact, back then, there was quite an organized effort to cultivate Pham in the villages I visited. Pham was cultivated in natural ponds using clean, still water. The ground was raised higher than the paddy fields, to avoid water discharged from the fields which might contain agrochemicals. The ponds were around 100-300 m^2 in size. To sell their products, there was a group of people who bought the harvested biomass from the villagers and sold it in the markets – so it was a pretty organized business.

After I left Chiang Mai University, I moved to Mahidol University in the Bangkok area. I then contacted a friend, Michael McGarry, who was an engineer at the Asian Institute of Technology (AIT), Thailand. We continued some *Wolffia* culture experiments in small tubs on the rooftop of a building at AIT, and together submitted the report for publication in the journal Nature. Unfortunately, due to my other obligations, I was not able to carry on the *Wolffia* experiments that we started. The popularity of Pham also declined over the generations until recently.

3. In the publication, you referred to the species as *Wolffia arrhiza*. Since the presence of *W. arrhiza* has not yet been confirmed in the region, can you elaborate on the taxonomic technique that you used in the study for species identification? Could it be possible that the species was *W. globosa*?

As I have mentioned about my activities, it was more of practical research, and it was conducted in a knowledge-poor, resource-limited environment in Thailand and its scientific community from 1950 to 1970. My species identification as *Wolffia arrhiza* was not confirmed by a specialized taxonomic technique and was rather limited to whatever references were at my disposal. To confess, honestly, I did not know of any other species of this genus at the time.

My fellow scientists who are experts in the area of duckweed taxonomy and diversity are welcome to explore more on this subject. And I shall be grateful if one or many of them wish to come to survey the *Wolffia* species in Thailand. I will gladly accompany them even though I am 88 years old now.

4. Pham is a traditional Thai food. However, relatively few people of the younger generations recognize it. Can you tell us more about its popularity from the 1950s to the 1970s?

As aforementioned, my studies of the rural natural foods for common people were conducted in my off-duty times and alone, so my information about the history of this food plant was limited to verbal accounts of growers in about 20 villages in the provinces of Chiang Mai, Lamphun, Chiang Rai and Khon Kaen (in northeast Thailand), and, sellers and consumers in towns. I am certain that Thai people have historically known and consumed Pham for a long time (to my estimate, over a span of 10 previous generations). In my survey conducted in later years, I found Pham sold in market places of smaller towns and rural communities and community-based Pham-growing villages in many provinces in all regions of Thailand.

Therefore, it can be safely stated that Pham is a traditional Thai rural food commodity since historical times.

5. In recent years, duckweed has been regarded around the world as a nutritious food and a sustainable source of protein. You have also witnessed the comeback of Pham in Thailand. Can you comment on this recent trend, and the future of duckweed? How can we further raise the acceptance of duckweed or Pham not only in Thailand, but globally?

As for the case of Pham as fresh vegetable consumption, I shall divide the consumption behaviors into two groups: 1) traditional consumption by low-income rural residents; and 2) trendy consumption by urban residents in larger cities.

As recently as 2025, I have visited about 10 villages growing Pham for markets in Phitsanulok, Khon Kaen and Chiang Rai provinces of Thailand. I found that fresh Pham is in good demand in local markets, but the consumption is largely confined to the older generation (aged 50 and above). Younger generations seem to be unaware of this peculiar minute foodstuff. Many well-to-do people in cities often do not recognize Pham since they never ate it before, and many of those who have tried are not impressed with the taste and (faint) odour associated with Pham. I believe significant marketing and education about Pham and its associated health benefits are clearly needed before it can become a mainstream food. In general, I think wider acceptance of Pham, and perhaps duckweed, could be achieved through:

1) Extend the use of Pham beyond "superfood." In the current global economic downturn, "cheap good food" will be more attractive to the wider populations.

2) Reduce cost of production, e.g. increasing yield and using less expensive growth facilities.

3) Find food categories in which an acceptable grade of commodity Pham or duckweed can be incorporated.

In my view, I still adhere to my original vision of *Wolffia* as an "inexpensive protein source," and I hope that one day the scientific community will make it happen.

Interview with Dr. Krachang Bhanthumnavin (Left) by Metha Meetam (Right)

Reference: [1]Bhanthumnavin K, McGarry MG. *Wolffia arrhiza* as a possible source of inexpensive protein. Nature. 1971 Aug 13;232(5311):495

Exchange of experience in cultivating *Lemna trisulca* L. (Lemnaceae) under *in vitro* conditions

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Summary

Growth of *Lemna trisulca* on inorganic medium is very slow but suitable for stock cultivation. Cultivation on agar is recommended. Increased growth rates were obtained by adding organic nutrients like glucose under axenic conditions. Alternatively, cultivation with higher growth rates may also be obtained under elevated CO_2 just by blowing filtered air or CO_2 in the nutrient medium. This effect of CO_2 is also known from other duckweed species.

1. Introduction

In the previous issue of the Duckweed Forum 14: 106 (2026) we invited to exchange experience in cultivating *Lemna trisulca* – a species that has been observed to be problematic in many stock collections. The following feedback were received in response to our request in the *Duckweed Forum*: "How to cultivate *Lemna trisulca* L.". Beside short feedbacks collated in the next section, we did some additional literature search to find additional published experiences. These references are organised chronologically at the end of this article.

2. Short feedbacks

Eric Lam, Rutgers University, NJ, USA

"Anecdotally, I have tried to sterilize this species with bleach and it is quite sensitive to it, so one needs to use lower concentrations to not kill the whole plant. But it could be done. I also noted that this species may not like Hoagland's medium, which works quite well for other duckweed species that I have tried. They could grow alright though on 0.5X SH agar plates, but show some evidence for strain/clone dependence. At the moment, the strain that looks decent for growth is 7431 in our collection at Rutgers."

Comment: 0.5X SH on agar plates seems good for stock cultivation.

Viktor Olah, University of Debrecen, Hungary

Kucala M; Sladyga M, Kamisnki A.: Phytoremediation of CYN, MC-LR and ANTX-a from water by the submerged macrophyte *Lemna trisulca*. *MDPI Cells* 2021, 10: 699.

"The axenic macrophyte *Lemna trisulca* (L., laboratory culture) were cultivated separately in BG11 medium (Stanier *et al*., 1979) in a phytotron at 22 ± 1°C with 80 ± 5% humidity and 25 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) under a 12-h photoperiod (lamps AQUAEL 18 W PLANT). …All cultures were shaken daily."

Relative growth rate was approximately 0.02 d^{-1}.

Ref.; Stanier RY; Deruelles J; Rippka R; Herdman M; Waterbury JB: Generic assignments, strain histories and properties of pure cultures of Cyanobacteria. *Microbiology* 1979, 111: 1–61.
Comment: This growth rate is extremely low as it is the light intensity, just large enough to keep the clone alive.

Halina Gabrys, Jagiellonian University, Krakow, Poland
The growth conditions that we used recently for *L. trisulca* are given in the publication:
Krzeszowiec W, Gabryś H. GLR Channels Are Involved in the Mechanism of Chloroplast Avoidance Response in *Lemna trisulca*. Plants. 2025; 14(19):2990. https://doi.org/10.3390/plants14192990
They are the following:
We used *Lemna trisulca* L. from the collection of the Jagiellonian University Botanical Garden in Kraków, Poland. The duckweed was cultured in daylight in Erlenmeyer flasks kept in a north-facing window. A twice-diluted liquid medium proposed by Appenroth et al. [Biologia Plantarum 1996; DOI10.1007/BF02879642] was used, i.e., 30 µM KH_2PO_4, 0.5 mM $Ca(NO_3)_2$, 4 mM KNO_3, 0.5 mM $MgSO_4$, 2.5 µM H_3BO_3, 7.5 µM $MnCl_2$, 0.2 µM Na_2MoO_4, 12.5 µM FeEDTA.
According to Weronika's observations *Lemna* grows poorly in the full medium. Another comment: pH of the nutrient medium changes strongly following the plant passage and needs adjustment for a few days.
Earlier experience: Formerly, for at least 10 years, I was growing *L. trisulca* in an ordinary aquarium standing by a west window, with fish and no aeration. One week before the experiment, plants were "standardized" in a growth chamber with an illumination cycle of 12 h L/ 12 h D (fluorescence lamps L6W/25, Osram) at 20^{0}C, and kept in the Hoagland's medium (Bonner, J., Galston, A.W. (1952) Principles of plant physiology. Freeman, San Francisco). The plants grown in this way had very uniform fronds and were very well suited for chloroplast movement experiments.
We also tried agar medium but without much success: mixotrophic conditions cannot be used because sucrose used for a long time inhibits chloroplast movements; the duckweed grown autotrophically on agar has small fronds.
Comment: Low nutrient concentrations seem useful for at least short term cultivation.

3. Literature search

Holst, RW; Yopp JH: Comparative utilization of inorganic and organic compounds as sole nitrogen-sources by the submergent duckweed, *Lemna trisulca*. *Biologia Plantarum* 1979, 21: 245-252.
"When doubling time for frond production was measured, the N sources in order of effectiveness were urea, aspartic acid, nitrate, glutamic acid, arginine, ammonium and casein of hydrolysate." 24°C day temperature/ 15°C night temperature; 16 h L/ 8 h D; light intensity 8000 lux" (KJA: ca. 120 µmol m^{-2} s^{-1} PAR).
Relative growth rate in optimised inorganic medium was ca. 0.04 d^{-1}, after addition of 55 mM glucose and peat extract it increased to 0.20 d^{-1} (Figs. 1 and 2).
Comment: Inorganic medium can be used only for stock cultivation, supplementation by glucose are required for normal growth rates. The role of peat extract is not clear.

Huebert DB; Mcilraith AL; Shay JM; Robinson, GGC: Axenic culture of *Lemna trisulca* L. *Aquatic Botany* 1990, 38: 295-301.
"Successful growth and a doubling time of 1.9–2.0 days occurred using inorganic carbon at pH 7.8 as the sole carbon source under 400 µmol $m^{-2}s^{-1}$ cool white fluorescent light at 28 ± 2°C. Cultures were aerated at a rate of 125 ml min^{-1} with sterile, humidified, room air. The growth medium was unbuffered and replaced once daily at an exponential rate based on the growth of the plant culture."
Comment: This is a remarkable publication correcting the conclusion published by Landolt (1957) and Bower et al. (1980) who stated that organic supplements, acidic pH and low light conditions were required for *L. trisulca* culture. More constant CO_2 levels maintained by aeration the liquid medium is necessary and sufficient in the absence of organic nutrients for normal growth rates.

Huebert DB; Shay JM: The effect of external phosphorus, nitrogen and calcium on growth of *Lemna trisulca*. *Aquatic Botany* 1991, 40: 175-183.
"The multiplication rate averaged 143 ± 6 (KJA: RGR = 0.33 d^{-1}) between 27.5 and 220 µM external calcium. Above 220 µM the growth rate was reduced but below 27.5 µM the plants either became deformed or did not survive."
Comment: This are important results as the molar concentration of Ca in most nutrient media is much higher than 0.22 mM.

Sender, J; Grzywna, A: Influence of temperature and photoperiod on the growth and development of *Lemna trisulca* and *Lemna minor* in controlled conditions. *Journal of Ecological Engineering* 2025, 26: 56–72.
Lemna trisulca was cultivated in the inorganic N medium (Appenroth et al., 1996) under 262 µmol m^{-2} s^{-1} light for 24 h or 12 h per day and either 15°C or 25°C for one week. The growth rates were higher with shorter photoperiods and lower temperature.
Comment: Under optimal conditions the RGR was 0.07 d^{-1}, which is very low compared to other species but in agreement with the results of other authors cultivating on inorganic media.

Wang, X; Zhao, X; Kazmi, A; Xia, M; Sun, Z; Yang, J; Li, G; Li, X: Hou, H: Supplementation of carbon dioxide ameliorated the physicochemical attributes of duckweed (*Lemna trisulca*). *Bioresource Technology Reports* 2025, 31: 102195.
"This study investigates the effects of varying carbon dioxide (CO_2) concentrations (400 ppm, 10,000 ppm and 50,000 ppm) on the relative growth rate, biomass accumulation and enzyme activity of L. *trisulca*. The results indicated that CO_2 supplementation significantly enhanced biomass production, photosynthetic pigment concentration, activity of photosynthetic enzymes, and starch/protein accumulation. Compared to ambient CO_2 concentration, under the 50,000 ppm CO_2 treatment, fresh weight, starch content and protein levels increased by 11.6 times, 3.3 times and 1.6 times, respectively." "cultivated in liquid B5 medium (1 % sucrose) at pH 5.8 for two weeks in an artificial climate chamber. …temperature of 22°C and a 16/8 photoperiod"
Comment: Even in the presence of sucrose, the growth rates are very low (ca. 0.02 d^{-1}) but increased strongly (ca. 0.18 d^{-1}) under elevated CO_2.

Special Issue Information

Dear Colleagues,

Wolffia globosa (Roxb.) Hartog & Plas, one of the most frequently used water lentil species (Lemnaceae Martinov). Photo credit: Viktor Oláh.

Duckweeds or water lentils are the smallest and fastest growing angiosperms belonging to the family Lemnaceae. In the last decade we learned that these plants offer a suitable model for ecologists, physiologists, geneticists, molecular biologists, and researchers of many other fields (cf. Plant Cell 2021, 33: 3207–3234; doi:10.1093/plcell/koab189). Moreover, from a practical point of view, they can help in coping with various aspects of the current humanitarian and environmental crisis, serve as indicators for environmental monitoring (biomonitoring), help in removing water pollutants (phytoremediation), and provide valuable biomass for easing worldwide protein shortages or the hunger for renewable energy. In cooperation with the journal Plants, we edited already two Special Issues under the title "Duckweed: Research Meets Applications" in order to update the dynamic research status in this field.

The first Special Issue was very well received by the duckweed community and, in total, 39 papers were published in MDPI's Plants journal and comprehensively published in an e-book (ISBN978-3-0365-9068-4). https://www.mdpi.com/journal/plants/special_issues/duckweed

In the second edition, open along the 7th International Conference on Duckweed Research and Applications in Bangkok, Thailand in 2024, 15 papers were published. An e-book is in preparation. Link to editorial: https://doi.org/10.3390/plants15121846

We have now launched the third volume of the Special Issue "Duckweed: Research meets Applications III" (https://www.mdpi.com/journal/plants/special_issues/59d5to5925), along the lines of the 8th International Conference on Duckweed Research and Applications which was held in Portici (Naples), Italy from 28th September to 2nd October 2026, allowing participants of the conference to contribute to the special issue. Also, all other Duckweed researchers are welcome to contribute. Contributions in the form of both original research papers and reviews from a broad scope of disciplines related to duckweed research and applications (e.g., morphology, taxonomy, and ecology), including ecological interactions, ecotoxicology, environmental monitoring and remediation, physiology, biochemistry, genetics, omics, biotechnology, as well as biomass production and its uses, are welcome. We encourage reports on large-scale cultivation and applications as well.

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Student spotlight: Hu Huangkai

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To many people, duckweed may seem like little more than an inconspicuous plant floating on the surface of a pond. To me, however, it represents a promising bridge between fundamental plant science, sustainable protein production, and agricultural resilience.

I am Hu Huangkai, a master's student in the Biological and Pharmaceutical Sciences program at the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, working under the supervision of Professor Hongwei Hou. My research focuses on nutritional regulation in duckweed, particularly the mechanisms underlying nutrient accumulation and protein production. My interest in this field began during an undergraduate internship at the Institute of Hydrobiology. Through my laboratory experience, I came to appreciate duckweed as far more than a simple floating aquatic plant. Its rapid growth, high protein-producing capacity, and ability to accumulate starch under specific stress conditions reveal remarkable biological versatility and significant potential for applications in food, feed, and biomass production. These characteristics inspired me to explore how nutrient regulation influences duckweed growth, composition, and nutritional value.

My research focuses on two closely interconnected objectives: identifying duckweed germplasm with high protein content and developing economically viable, reproducible cultivation systems. Following the screening of germplasm for protein accumulation, I investigate the effects of different culture-medium formulations on duckweed growth and protein production. These formulations include self-designed compound-fertilizer media and media prepared from agriculture-grade straight fertilizers. A primary objective is to identify compound-fertilizer formulations that provide an optimal balance among nutrient availability, cultivation performance, cost, and operational stability, while evaluating single-nutrient fertilizer strategies for maximizing protein productivity. In parallel, I manipulate nitrogen-to-phosphorus ratios in the culture medium to examine their effects on nutrient uptake, utilization, and protein biosynthesis. Through this approach, I aim to improve our understanding of the physiological and biochemical mechanisms underlying nutrient-regulated protein accumulation in duckweed.

My research experience has also strengthened my understanding of experimental reproducibility and the challenges involved in translating laboratory findings into practical cultivation systems. During the development of a compound-fertilizer medium, I encountered a substantial decline in duckweed growth following the introduction of a new fertilizer batch from the manufacturer. Because the same cultivation system had previously produced consistent results, I systematically reassessed the culture conditions and fertilizer composition. Comparative analysis indicated that the increased proportion of urea in the new fertilizer batch could have contributed to the observed growth inhibition. I subsequently replaced the fertilizer with a formulation containing a different nitrogen source and conducted repeated experiments, which restored the expected growth performance.

This experience highlighted an important distinction between demonstrating biological efficacy under controlled experimental conditions and developing a robust cultivation strategy suitable for practical application. Agriculture-grade fertilizers may exhibit variation in chemical composition among production batches, and such variability can influence cultivation outcomes. Consequently, the development of scalable duckweed production systems requires consideration of not only growth and protein productivity but also input cost, material consistency, availability, and experimental reproducibility. These considerations have broadened my research perspective from

identifying conditions that maximize protein accumulation to developing cultivation strategies that are robust enough for application beyond controlled laboratory environments.

More broadly, my research is motivated by the growing need for sustainable and resource-efficient protein production systems. Population growth, resource constraints, and increasing environmental pressures are intensifying the demand for alternative protein resources. At the same time, reliance on imported soybean-based protein resources presents challenges for long-term resource security and sustainable agricultural development in China. Duckweed possesses several characteristics that make it a promising candidate for further investigation, including rapid biomass production, relatively high protein content, and efficient nutrient utilization. However, realizing this potential requires systematic evaluation of germplasm diversity, optimization of cultivation conditions, and mechanistic understanding of nutrient regulation. My research therefore seeks to contribute to the scientific foundation required for the development of duckweed as a potential alternative protein resource.

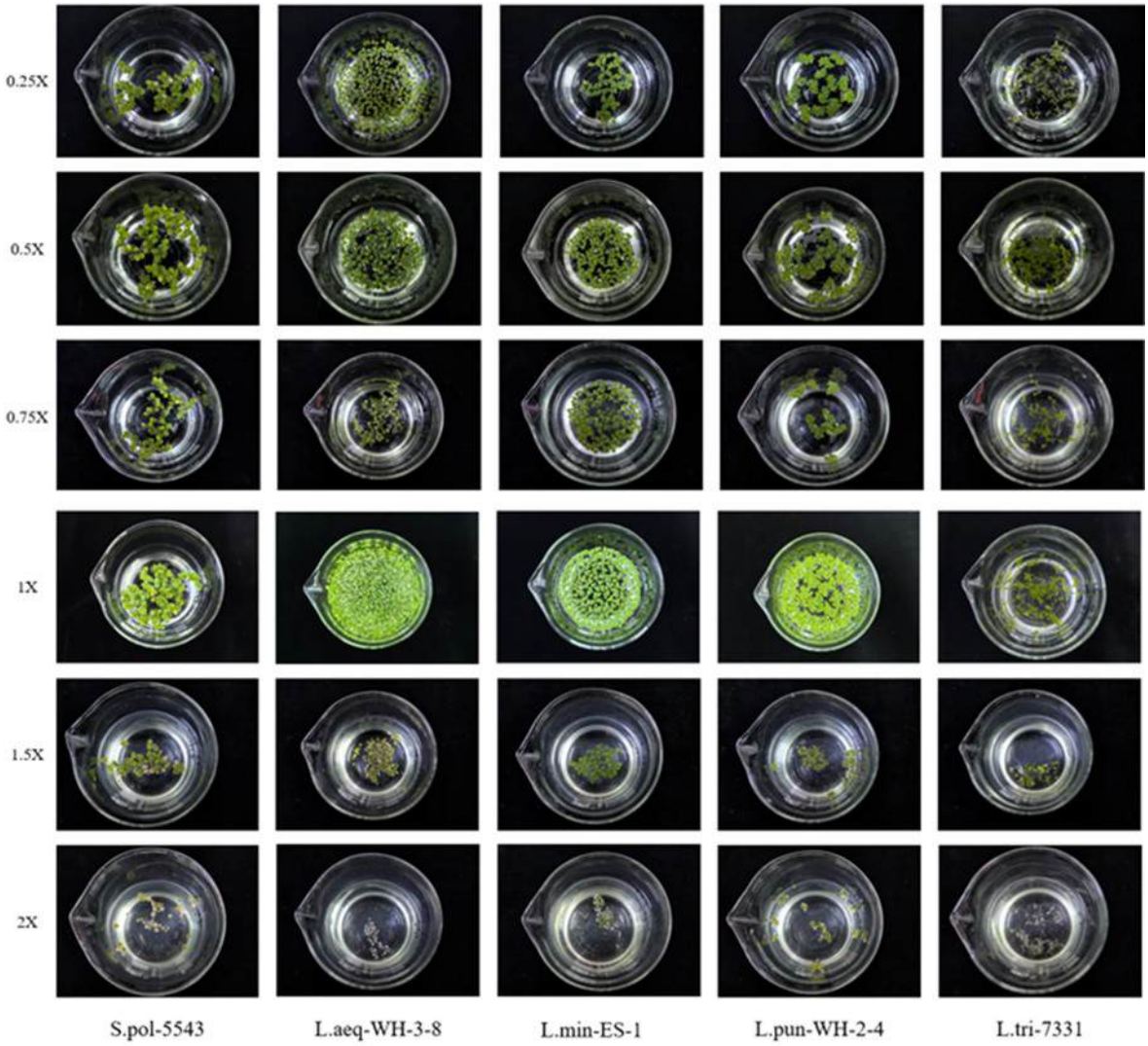
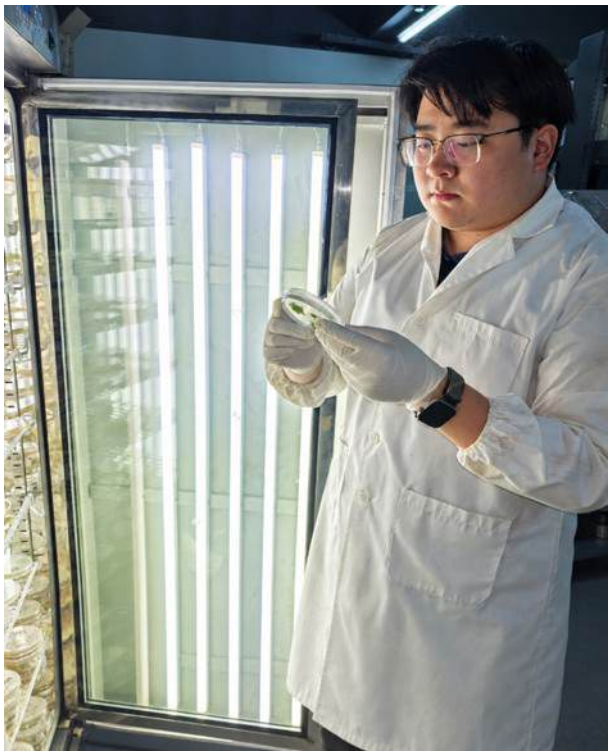


Figure 1. Growth responses of five duckweed strains to different strengths of a low-cost fertilizer-based culture medium

To move from germplasm screening towards a practical cultivation system, I compared the responses of five duckweed strains to a low-cost fertilizer-based medium at six concentrations (Figure 1). The 1× formulation gave the most consistent growth and was therefore selected as the starting point for further optimization.

Beyond my laboratory research, I value opportunities to engage with different cultures and academic environments. Traveling, visiting museums, caring for plants, and interacting with people from diverse backgrounds have broadened my perspectives and strengthened my appreciation for interdisciplinary and cross-cultural learning. My interest in plants also extends beyond my research activities, as observing their responses to environmental variation continues to reinforce my curiosity about plant adaptation and physiological regulation.



In the next stage of my research, I intend to investigate the effects of different chemical forms and concentrations of nitrogen and phosphorus on protein accumulation in duckweed. Particular attention will be given to the relationships among nutrient sensing, nutrient uptake, assimilation, and protein biosynthesis. By integrating physiological and biochemical measurements with molecular approaches, I hope to identify key regulatory processes associated with nutrient-induced changes in protein accumulation. Such knowledge could provide a mechanistic basis for optimizing nutrient management and developing more targeted cultivation strategies. I also plan to establish a systematic evaluation framework that integrates biomass productivity, protein yield, nutrient-use efficiency, and cultivation cost. This framework would enable a more comprehensive assessment of cultivation strategies by distinguishing treatments that increase protein concentration from those that improve overall protein productivity and economic feasibility. In the longer term, I hope that this research will contribute to the development of scientifically validated cultivation systems for the production of duckweed biomass for applications in animal feed and alternative-protein production.

Looking ahead, I aspire to pursue a PhD and further investigate the physiological, biochemical, and molecular mechanisms underlying nutrient regulation and protein accumulation in duckweed. My research experience has taught me that scientific progress is not always linear and that unexpected results and experimental setbacks can provide valuable opportunities for deeper investigation. Each challenge has strengthened my ability to critically evaluate evidence, refine experimental approaches, and persist in pursuing meaningful scientific questions. Beginning with a small aquatic plant, I hope to expand my research toward broader questions of sustainable biomass and protein production, ultimately contributing to scientifically sound and environmentally responsible solutions.

From the Database

We checked in almost all publications whether the species identity of the investigated duckweeds/ water lentils was given following sufficient reliable investigation or whether relevant references were cited. We gave it at the end of the abstracts as "DF comment".

Highlights

Light-triggered ROS signaling regulates abaxial cuticle permeability in developing duckweed fronds

Kim, K; Kang, J; Jeon, W-T; Lee, J-M; Woo, J; Je, S; Yamaoka, Y; Lee, Y. (2026) Journal of Experimental Botany DOI10.1093/jxb/erag360.

The cuticle is an ancient innovation that enabled plants to colonize land by providing a protective yet selectively permeable barrier. Although its composition is well characterized, the developmental and environmental regulation of cuticle permeability remains unclear. Here, we used duckweed, a tiny aquatic plant that absorbs nutrients through a permeable abaxial frond surface, as a model to investigate the formation of water-permeable cuticles. During frond development, the abaxial surface gradually acquires permeability upon the emergence of the maternal pouch. Structural and biochemical analyses showed that this permeable cuticle is thinner, less compact, and depleted of very-long-chain alkanes compared to the adaxial surface. Transcriptomic profiling revealed the distal enrichment of light-responsive and cuticle-modifying genes, suggesting light-regulated cuticle remodeling. Consistent with this, light, rather than direct water contact, served as the key external cue; exposure of daughter fronds to unfiltered light without overlying tissue enhanced permeability, whereas a reduced red-to-far-red ratio attenuated it. This transition required the accumulation of reactive oxygen species in the abaxial epidermis, as inhibition of reactive oxygen species production suppressed permeable cuticle formation. Together, these findings show that duckweeds co-opt a light-quality sensing mechanism, reminiscent of terrestrial shade-avoidance signaling, to regulate reactive oxygen species-mediated cuticle permeability, revealing how developmental programs and environmental cues integrate to dynamically remodel cuticle properties in aquatic plants.

DF comment: Putative *Lemna minor* plants were collected from a park in South Korea and typed using plastidic barcodes. Unfortunately, as have been demonstrated by in-depth studies over the past years, the method used is insufficient to distinguish between *L. minor* and its hybrids, such as the nothospecies *Lemna x japonica*. This ambiguity can be resolved using TBP barcoding approach.

Examining the potential of aquatic plant duckweed biomass as a sustainable feedstock for bioplastics: Effect of duckweed species and loading content on properties of PHBV/PBAT/plasticized duckweed biomass blends

Dang, KM; Yoksan, R; Matsumoto, K; Morikawa, M. (2026) Food and Bioproducts Processing 159: 287-299.

Duckweed, a fast-growing aquatic plant rich in carbohydrates and proteins, is a promising renewable resource for sustainable materials. This study examined the potential of duckweed biomass as a raw material for making sheets based on plasticized duckweed biomass and biodegradable polyesters, specifically poly(3hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(butylene adipate-co-terephthalate) (PBAT). Plasticized duckweed materials were prepared from two species, *Lemna minor* (PLE) and *Wolffia globosa* (PWO), chosen for their different carbohydrate-to-protein ratios. Ternary PHBV/PBAT/plasticized duckweed biomass blends were compounded using a twin-screw extruder and subsequently converted into sheets by cast sheet extrusion. The PHBV:PBAT ratio was 50:50, while PLE or PWO contents varied at 8, 16, 24, and 32 wt%. The effects of the duckweed species and loading level on processability and properties of the blends were systematically studied. Incorporation of plasticized duckweed biomass facilitated sheet extrusion by decreasing surface stickiness and accelerating melt solidification. Among the formulations investigated, the PHBV/PBAT blend containing 8 wt% PLE exhibited the highest tensile strength of 21 MPa and Young's modulus of 1404 MPa, together with lower water uptake, glycerol migration, and degradation of PHBV and PBAT during processing and hydrolytic exposure than the corresponding blends containing PWO or higher duckweed contents. In contrast, the blend containing 16 wt% PLE exhibited the greatest elongation at break of

122% and impact strength of 4.1 J/mm. These findings demonstrate the potential of duckweed biomass as a low-cost renewable feedstock for the development of bioplastic materials.

Aquaculture/ Agriculture

Role of cultivated plants in bio-regenerative life support systems

Tripathi, A; Pandey, P; Burduhos, P. (2026) Extreme Life, Biospeology & Astrobiology 18: 24-24.

Bioregenerative life support systems (BLSS) represent a critical technological pathway toward sustaining long-duration human space exploration by enabling the closure of material loops for air, water, food, and waste. This mini-review synthesizes current knowledge on the role of cultivated plants within BLSS architectures, emphasizing their multifunctional contribution as primary producers of edible biomass, oxygen generators, and agents of water purification. Key candidate species—including staple crops such as wheat, rice, potato, and soybean, as well as fast-growing plants like *Wolffia globosa* and microalgae—are evaluated in terms of productivity, nutritional value, and adaptability to space conditions. The review further examines nutrient recycling strategies, particularly nitrogen and phosphorus recovery from waste streams, and highlights the operational principles of major BLSS programs such as NASA's CELSS and ESA's MELiSSA. Special attention is given to the challenges of species selection under extraterrestrial constraints, including microgravity, radiation, and limited resources, as well as to emerging solutions involving extremotolerant microorganisms and in situ resource utilization (ISRU). Despite significant advances, current BLSS designs remain limited by incomplete nutrient closure and insufficient dietary completeness. Future progress will depend on the integration of biological and physicochemical subsystems, the optimization of crop systems for both yield and nutrition, and the validation of fully closed-loop systems at operational scale.

Species-specific growth responses and biomass accumulation in native Korean duckweeds under diverse cultivation conditions

Kang, J; Kim, B; Lee, Y. (2026) Journal of Plant Biology DOI10.1007/s12374-026-09530-x.

Duckweeds are small, fast-growing aquatic monocots with increasing potential as sustainable bioresources. However, their effective use requires species-level characterization to guide rational species selection and cultivation optimization. In Korea, native duckweed species remain poorly characterized, and comparative analyses among species are limited. Here, we compared four representative native Korean duckweeds-*Spirodela polyrhiza*, *Landoltia punctata*, *Lemna minor*, and *Wolffia globosa* - under different photoperiod, temperature, and light-source conditions. The four species exhibited distinct growth strategies, reflected in differences in biomass accumulation, frond expansion, clonal propagation, and stress-related traits. *L. minor* maintained relatively stable growth across diverse conditions, including fluctuating low-temperature regimes, suggesting broad environmental tolerance. By contrast, *L. punctata* showed pronounced condition-dependent responses, particularly under continuous illumination and LED-based light conditions, indicating sensitivity to light-associated stress. Low-temperature treatments further revealed species-specific differences in turion formation, highlighting distinct strategies for balancing active growth with stress survival under unfavorable conditions. Together, these findings establish a species-level framework for matching native Korean duckweed species to cultivation environments and production goals, supporting the strategic use of local duckweed resources for scalable and sustainable biomass production.

DF comment: These authors used plastidic DNA barcodes for species identification of their collected duckweed strains. While this method is sufficient for *Spirodela* and *Landoltia* species, they have been demonstrated to be insufficient for *Lemna minor* (Michael et al. Comm. Biol., 2026), and likely also problematic for *Wolffia globosa* (Borisjuk et al. *Plants*, 2015). An alternative barcode strategy such as TBP would be required.

Decoding *Wolffia*: A predictive model for duckweed growth and nutrient uptake for resource recovery from wastewater in tropical countries

Pasos-Panqueva, J; Yulistyorini, A; Baker, A; Camargo-Valero, MA. (2026) ACS ES & T Engineering 6: 2402-2415.

Duckweed-based wastewater treatment systems have attracted increasing attention for sustainable water pollution control, yet predictive tools to optimize their operation under real-world conditions remain limited. Here, we develop and validate a temperature- and nutrient-dependent kinetic model for the duckweed species

Wolffia angusta, integrating nutrient limitation (Monod model), temperature dependence (Arrhenius model) and internal nutrient reserve dynamics. The model was parametrized and validated through laboratory-scale batch experiments (20-30°C), in small cylindrical plastic vessels, under varying phosphorus and nitrogen supplies, including nutrient-limiting scenarios. Temperature predominantly drives biomass production, with optimal growth observed at 25-30°C (maximum relative growth rate of 0.427 d^{-1}), while nutrient availability more strongly governs biological nutrient uptake and removal efficiency. Maximum nutrient removal rates reached 6.85 mg N L^{-1} d^{-1} and 1.85 mg P L^{-1} d^{-1} under nutrient-replete conditions. Biomass accumulation increases at the higher end of optimum temperature values, but specific nitrogen uptake rates decrease, indicating metabolic constraints. Model calibration markedly improved predictive accuracy (R^2 > 0.9; RPD > 2), even under nutrient-limited conditions. The model was applied to simulate a duckweed-based pond systems to polish the effluent from a hybrid anaerobic baffled reactor (HyABR) processing domestic wastewater under tropical conditions (Indonesia). Beyond predicting effluent nutrient concentrations, the model was used as a process design tool to optimize system configuration to meet local effluent discharge consents. Simulations showed that increasing the number of duckweed ponds in series reduces total hydraulic retention time and total land area while lowering per-pond productivity, with an optimal configuration of two to three ponds balancing treatment performance and resource recovery. This work advances the mechanistic understanding of *Wolffia*'s growth and nutrient uptake, and delivers a practical, transferable modeling framework for design and optimization of duckweed-based nutrient control units for decentralised wastewater treatment, particularly in tropical regions. The approach represents a significant step toward integrating nature-based systems into circular, sustainable water management strategies.

Duckweed as a multifunctional industrial biomass: Advances in cultivation, processing, valorization, and circular bioeconomy potential

Song, YJ; Liu, SZ; Yang, T; He, RM; Yang, XY; Li, S; Luo, SS; Hu, ZL; Lu, YL (2026) Industrial Crops and Products 251: 124246.

Duckweed (Lemnaceae) is increasingly recognized as a multifunctional industrial biomass that can link wastewater-based nutrient recovery with feed production and downstream valorization. Recent studies have moved beyond basic biomass cultivation to evaluate duckweed in resource-recovery systems, where nitrogen and phosphorus-rich effluents are converted into protein-rich biomass while reducing wastewater pollutant loads. The harvested biomass has potential applications in animal feed, bio-based products, nutrient recycling, and integrated biorefinery schemes, thereby supporting circular bioeconomy models that connect waste treatment, biomass generation, and product development. However, industrial deployment remains constrained by variability in biomass composition, contaminant accumulation during wastewater cultivation, inefficient harvesting and drying technologies, uncertain cost performance, and incomplete regulatory frameworks for feed grade use. This review synthesizes recent advances in duckweed cultivation, biomass processing, nutritional and functional attributes, wastewater-based production, environmental performance, and techno-economic feasibility. Unlike previous reviews focused mainly on controlled cultivation, food-oriented applications, bioactive nutrient extraction, or general biorefinery potential, this review provides a feed-oriented circular biomass framework that integrates wastewater-to-feed resource recovery, biomass safety, animal-feed deployment, processing requirements, life-cycle implications, and techno-economic feasibility.

Biotechnology

Co-digestion as a strategy to optimize anaerobic digestion without pretreatment: Implications for Methane yield and process stability

Akan, AP; Dalkilic, K; Ugurlu, A (2026) Fermentation 12: 389.

Rapid population growth, urbanization, and industrialization are continuously increasing global energy demand while intensifying climate change associated with fossil fuel consumption. In this context, renewable energy production from organic waste has gained increasing attention as a sustainable and environmentally friendly strategy. Anaerobic digestion (AD) offers significant potential for simultaneous waste stabilization and biomethane generation. However, many previous studies investigating lignocellulosic or nutrient-rich substrates have relied on physical, chemical, or thermal pretreatment methods to enhance biodegradability, despite their additional operational costs, energy consumption, and environmental impacts. Therefore, developing low-cost and pretreatment-free co-digestion strategies remains an important research need. This study investigated the biomethane production potentials of untreated chicken manure (CM) and duckweed

(*Lemna minor*-LM) collected from the final sedimentation tanks of wastewater treatment plants under mono-digestion and co-digestion conditions. The study hypothesized that rapidly growing and widely available LM biomass could enhance methane production without requiring pretreatment. Among all reactors, CM0.75 (75% of the total TS derived from CM and 25% from LM and inoculum) achieved the highest performance with a cumulative biogas production of 5350 mL (74.2% of CH_4) and a methane yield of 327 mL CH_4/g VS, while mono-digestion of CM resulted in the lowest methane yield of 104 mL CH_4/g VS. The results demonstrated that LM biomass naturally proliferating in wastewater treatment plants can be directly utilized as an effective co-substrate to improve biomethane production from poultry wastes. The proposed approach provides a cost-efficient, eco-friendly, and circular-economy-oriented alternative by eliminating the need for pretreatment while simultaneously valorizing problematic biomass generated in wastewater treatment facilities.

DF comment: The duckweed "particles" used in this work was obtained from waste water plant. While it is claimed to be *Lemna minor*, no justification for this species designation was provided aside from some visual characteristics.

Enzyme-minimized bioethanol production from wild-harvested *Lemna minor* using dilute sulfuric acid pretreatment with reduced reagent demand

Hsieh, PC; Wu, CL; Kung, Y. (2026) Sustainable Energy & Fuels DOI10.1039/d6se00880a

This study evaluated an enzyme-minimized pathway for converting wild-harvested *Lemna minor* into ethanol using dilute sulfuric acid pretreatment followed by direct yeast fermentation. Pretreatment with 2% (w/v) H_2SO_4 at 90°C for 10 h reduced the detectable starch content by more than 99% within 4 h and produced a maximum reducing-sugar concentration equivalent to 4.22 mg glucose per mL after 10 h. Fermentation of the acid hydrolysate by *Saccharomyces cerevisiae* produced an apparent ethanol yield of 0.133 g g^{-1} dry biomass, corresponding to 74% of the theoretical maximum. Incorporating a cellulase-polishing step (0.495 U g^{-1} dry biomass, 50°C, 72 h) increased the apparent ethanol yield only slightly to 0.137 g g^{-1} (+3% relative), while extending the hydrolysis time by 72 h and adding enzyme input. More than 95% of the final apparent ethanol yield was achieved within the first 12 h of fermentation, indicating rapid sugar utilization. Compared with selected enzyme-intensive or steam-based duckweed conversion workflows, the acid-only route reduced supplemental cellulase use and avoided high-pressure pretreatment equipment under the tested bench-scale conditions. These findings support process simplification and enzyme minimization, but they do not by themselves establish economic feasibility or environmental superiority.

DF comment: The "wild" duckweed used in this work was harvested from a channel near an experimental farm. Its identification as the species *Lemna minor* was not supported by any reported evidence with currently accepted methods and is thus questionable and by extension, how these results can be broadly related to other known studies is uncertain.

Sustainable epoxy coatings with *Lemna minor* extract-loaded diatomaceous earth for enhanced corrosion protection

Hasanzadeh, A; Udoh, I; Dam-Johansen, K. (2026) Materials Today Sustainability 36: 101450.

Marine corrosion of mild steel (MS) demands protection that is both durable and sustainable. We prepared an epoxy (EP) coating that carries a plant-derived inhibitor from *Lemna minor* (LM) by loading the extract into diatomaceous earth (DE) to form a DE@LM reservoir. FTIR, LC-MS, XPS, DLS, and BET characterizations verified polyphenolic/N-donor functionality in LM and its entrapment within the mesoporous DE. TGA quantified the LM loading at 13.1 wt%. pH-dependent release measurements further confirmed controlled inhibitor delivery from DE@LM. In 3.5 wt% NaCl, DE@LM outperformed LM alone: weight-loss tests gave inhibition efficiencies up to 84.8% at 48 h, and EIS showed a steady rise in total resistance (RT) from 1.90 to 8.18 k Omega cm^2 at 72 h, consistent with sustained release. Potentiodynamic polarization indicated mixed-type inhibition for DE@LM with the lowest corrosion current (1.82 times; 10^{-6} A cm^{-2}), whereas LM shifted Ecorr positively and acted mainly anodically. DE@LM also increased surface hydrophobicity (water contact angle, 103.5 degrees). When incorporated in EP, EP/ DE@LM exhibited the highest impedance, improved long-term intact-coating stability, and self-healing at scratches (Rct42 -> 157 k Omega cm^2), reaching a self-healing efficiency of 73.5% at 24 h. Salt-spray exposure and SEM confirmed a dense, adherent layer that suppressed under-film corrosion. Together, enhanced barrier properties and controlled inhibitor release convert an

otherwise problematic biomass into an environmentally friendly anticorrosive coating for steel in saline environments.

DF comment: The duckweed used in this work was isolated from a freshwater lake and then cultivated. While it is claimed to be *Lemna minor*, no justification for this species designation was provided.

New sight on co-pyrolysis of phytoremediation plant and duckweed: The interaction mechanisms between phosphorus and multi-metals

Li, G; Cheng, W; Zhang, SH. (2026) Bioassay & Bioenergy 217C: 110045.

Phytoremediation offers an eco-friendly and cost-effective approach to soil remediation but may cause secondary pollution due to heavy metal accumulation in plants. In this study, the co-pyrolysis of poplar (PL) and duckweed (DW) was investigated to elucidate the transformation and stabilization mechanisms between Zn, Cd, Pb, and P. The results showed that DW addition enriched oxygen-, nitrogen-, and phosphorus-containing functional groups and promoted the formation of C-O-P structures and crystalline phases including $Zn_2P_2O_7$, $Zn_3(PO_4)_2H_2O$, and $K_2PbP_2O_7$. In raw PL, 76.16% of Zn and nearly 80% of Cd occurred in the exchangeable fraction (F1), whereas F1 of Zn was nearly eliminated after co-pyrolysis. The Fe-Mn oxide-bound fraction of Cd exceeded 80% at 500°C, and its residual fraction reached 76.78% at 700°C. Meanwhile, the organic matter-bound fraction of Pb increased to 78.88% at 700°C, indicating an overall transformation of heavy metals toward less labile forms. Increasing temperature also promoted the redistribution of phosphorus from relatively labile fractions to HCl-extractable and residual fractions. SEM-EDX analysis of representative regions yielded characteristic P/Zn and K/Zn slopes of 1.76 and 2.11, respectively, with R^2 values above 0.98, indicating a close association among Zn, P, and K on the biochar surface. TCLP and DTPA tests further showed that the environmental behavior of heavy metals was strongly temperature-dependent. The DTPA extractable fraction of Zn decreased to its minimum at 600 degrees C and then increased slightly at 700 degrees C, whereas Cd and Pb exhibited their lowest extractable fractions at approximately 400°C.

DF comment: The duckweed (DW) used in this work was reported as supplied by the Chengdu Institute of Biology, China. However, no description of the species for the duckweed biomass used was provided or known.

The trade-off between growth and starch deposition of duckweed as a food crop: a review

Sun, ZL; Li, FJ; Chen, Y. (2026) Future Foods 14: 101169.

Starch is a major carbohydrate widely used in food and industrial applications, but its production remains largely dependent on terrestrial food crops, raising concerns over competition between food security and industrial utilization. Duckweed is a fast-growing aquatic plant with remarkable starch-accumulating ability and is increasingly recognized as a promising non-grain feedstock. However, the practical value of duckweed starch cannot be evaluated only by starch content or biomass productivity. For food-oriented utilization, key quality attributes such as amylose/amylopectin ratio, granule morphology, gelatinization and pasting behavior, extraction purity, sensory constraints, and safety must also be considered. In this review, we summarize the current understanding of starch accumulation in duckweed under diverse environmental and physiological conditions, including light regime, trophic mode, CO_2 enrichment, nutrient limitation, temperature, cation treatment, exogenous hormones, and pH variation. We further integrate food science parameters, extraction and purification requirements, formulation performance, consumer acceptance, regulatory safety, and commercialization barriers. By connecting physiological regulation with processing functionality and industrial feasibility, this review positions duckweed starch as a complementary aquatic starch platform rather than a direct replacement for commodity starches such as maize, potato, cassava, wheat, and rice.

The role of phenolic profile of salt-stressed duckweed (*Lemna minor*) in synthesis and biological activity of green ZnO nanoparticles

Stamenkovic, N; Nikolic, F; Matic, A; Reljin, DA; Milovancevic, M; Paunovic, D; Radulovic, O. (2026) Molecules 31: 2326.

This study investigated whether salinity during cultivation of the aquatic plant *Lemna minor* (duckweed) influences the phytochemical composition of plant extracts and the properties of green-synthesized zinc oxide nanoparticles (ZnO NPs). Duckweed was cultivated under 0, 10, and 100 mM NaCl, followed by Orbitrap metabolomic profiling, nanoparticle synthesis, physicochemical characterization, and evaluation of antioxidant

and antimicrobial activities. Orbitrap analysis revealed pronounced salinity-dependent changes in extract composition, including increased abundance of several flavonoids, glycosylated flavones, and hydroxycinnamic acid derivatives in the order 0 < 10 < 100 mM. ZnO nanoparticle formation was supported by UV-Vis spectroscopy, which showed characteristic absorption features around 360 nm, and by powder X-ray diffraction (PXRD), which indicated the predominance of the hexagonal wurtzite ZnO phase in all samples. SEM-EDS analysis revealed Zn- and O-rich materials consisting of micron-scale aggregates and finer submicron structures. Raman spectra were dominated by fluorescence, which increased with salinity treatment and may reflect differences in surface-associated phytochemicals rather than substantial changes in the ZnO crystal structure. Nanoparticles synthesized using extracts from salt-stressed duckweed exhibited higher total phenolic content (up to 66.79 ± 0.15 μM GAE g^{-1}), antioxidant activity (up to 55.01 ± 0.21%), and antimicrobial activity against *Staphylococcus haemolyticus* D4-2-100/1 (inhibition zone up to 1.55 ± 0.05 cm). Although the mechanisms underlying these differences remain to be fully elucidated, the results suggest that salinity-induced changes in duckweed metabolism may influence the biological properties of the resulting nanomaterials. Overall, this study highlights the potential of manipulating cultivation conditions to modulate plant extract composition and, consequently, influence the characteristics and functionality of green-synthesized ZnO nanoparticles.

DF comment: the species designation of the duckweed used in this work is unclear. Tracing the citations related to this material's origin, it is likely that it originated from the the nursery garden of the Institute for Biological Research "Siniša Stanković", University of Belgrade. But its origin and methods of species determination is unclear.

Ecology

Effect of ultraviolet radiation (type B) and titanium dioxide nanoparticles on *Microcystis aeruginosa* and *Lemna minor* growth rate and potential allelopathic interactions

Samuel, SA; Dauda, S; Otogo, RA; Lorenzi, AS; Ibrahim, ZA; Abdulmalik, E; Cordeiro-Araujo, MK; Chia, MA. (2026) Aquatic Ecology 60: 105.

Ultraviolet-B (UV-B) radiation, intensified by ozone depletion, is a major stressor for aquatic primary producers. Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in industrial and consumer products, leading to their increasing discharge into aquatic systems. These particles interact with cyanobacteria (e.g., *Microcystis aeruginosa*) and macrophytes (e.g. *Lemna minor*). This study examined the interactive effects of UV-B radiation (0.8 W/m^2 and 2 W/m^2) and TiO_2 NPs (48 mg/L) on the physiological responses and allelopathic interactions between *M. aeruginosa* and *L. minor*. Our findings showed that low UV-B exposure significantly reduced chlorophyll a and total protein levels in *M. aeruginosa*, accompanied by increased microcystin, hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) levels under UV-B exposure. High UV-B particularly upregulated peroxidase (POD) activity in *M. aeruginosa*. In *L. minor*, low UV-B enhanced chlorophyll a, whereas high and low UV-B and TiO_2 exposure suppressed pigment and growth. Co-culturing *M. aeruginosa* with *L. minor* under low UV-B and TiO_2 exposure further suppressed algal growth and reduced protein content. UV-B and TiO_2 increased oxidative damage, reduced algal biomass and toxin production, and potentially disrupted allelopathic interactions. Exposure of *L. minor* to *M. aeruginosa* and high UV-B increased H_2O_2 levels and POD activity. Our results show that allelopathic interactions between *L. minor* and *M. aeruginosa* are highly sensitive to UV-B radiation and are further increased when TiO_2 nanoparticles are present. Additionally, UV-B radiation and TiO_2 nanoparticles exert synergistic physiological stress on *M. aeruginosa* and *L. minor*, potentially changing species interactions and reshaping community structure in freshwater ecosystems.

DF comment: The duckweed used were collected in a pond at the university where the work is conducted, and then identified as *L. minor* at the Herbarium of its Botany department. However, no description of the basis for its identification was reported. This would be very important to include in future studies by these researchers.

Design of carbon capture system utilizing *Wolffia globosa* and CaO derived from waste seashells with continuous real-time IoT-based monitoring

Nukunudompanich, M; Suriyachai, N; Leeabai, N; Kunbuala, N; Pontong, S; Anuwongpinit, T. (2026) Engineering Reports 8: e71044.

This study presents the design and implementation of a prototype-scale hybrid CO_2 capture system that combines *Wolffia globosa* cultivation, mineral carbonation with waste seashell-derived CaO, and real-time IoT-based monitoring. Waste seashells were calcined at 900°C to produce CaO, which was characterized by XRD and morphological FESEM and EDS analysis and was selected for mineral carbonation. The IoT system monitored changes in CO_2 concentration above the *W. globosa* tank, which were influenced by photosynthesis and respiration cycles. It also tracked CO_2 concentration after the mineral bed, where a consistent decrease of approximately 300 ppm relative to the inlet was observed under the tested conditions, with outlet readings approaching the lower bound of the sensor's specified accuracy (± [50 ppm + 5% of reading]). This decrease was attributed to CaO hydration and subsequent carbonation. Continuous measurements of CO_2, pH, and temperature provided useful diagnostic information for interpreting system behavior. The results demonstrate the feasibility of integrating locally available waste-derived CaO with biological cultivation and IoT-based monitoring in a small-scale laboratory prototype. However, a full net carbon balance, including calcination emissions and operational energy demand, was not included in this study. Therefore, the observed CO_2 decrease should be interpreted as local CO_2 uptake during the mineral carbonation step rather than confirmed net carbon removal.

Feed & Food

Duckweed (*Wolffia globosa*, *Wolffia arrhiza*, and *Lemna minor*) as novel therapeutic food for acute malnutrition and famine alleviation in low- and middle-income countries and humanitarian crisis settings

Hernani-Rodrigo, AE; Waters, SF; Figueroa, LSV; Msemakweli, JG; LaRuta-Rosas, N; Buhezo-Chamon, M; Gilman, RH (2026) Frontiers in Nutrition 13: 1835959.

Acute malnutrition remains a leading cause of under-five mortality in low- and middle-income countries (LMICs), particularly in humanitarian crisis settings where conventional therapeutic food supply chains are frequently disrupted. There is an urgent need for locally cultivable, affordable, and nutritionally adequate alternatives to imported ready-to-use therapeutic foods (RUTFs). In this review, we examine the nutritional composition, food safety, and production scalability of three duckweed species, namely: *Wolffia globosa, Wolffia arrhiza*, and *Lemna minor*, as candidate therapeutic foods in famine-affected regions. Compositional analyses indicated that these species consist of a high protein content (up to 48.2, 50.9, and 39.1% dry weight for *W. globosa, W. arrhiza*, and *L. minor*, respectively), a comprehensive essential amino acid profile, and an array of micronutrients and immunomodulatory compounds. As indicated by a Protein Digestibility-Corrected Amino Acid Score (PDCAAS) of 89% for *W. globosa* and the Digestible Indispensable Amino Acid Score (DIAAS) of 0.75 for *W. arrhiza*, the protein quality of duckweed is considered high. Furthermore, as one of the fastest-growing plants on earth, duckweed can double their biomass in only 4-5 days, reach harvest size within 2-5 days, and produce up to similar to 177 g of biomass per square meter per day, with water requirements up to 90% lower per kilogram of protein produced as compared to conventional crops. Although antinutritional factor levels are comparable to common vegetables and pathogen risks are manageable via solar drying and boiling, two primary limitations restrict their immediate application. First, a low-fat content (0.5%-9.6% DW) that confines energy density relative to standard RUTFs and, second, a susceptibility to accumulating heavy metals from growth medium. Additionally, the current evidence base may be constrained by absence of data regarding cultivation failures or adverse implementation outcomes. Consequently, rigorous, context-specific risk-benefic assessments are required. Controlled cultivation trials in LMICs, integrated with randomized controlled trials in malnourished cohorts, are necessary to objectively determine the feasibility of these species for acute malnutrition management.

Effect of phosphorus-containing additive and natural biomass on co-pyrolysis characteristics and heavy metal migration of phytoremediation plant: A comparative study

Li, G; He, YY; Cheng, W; Jiang, H; Yang, HP; Chen, HP; Zhang, SH (2026) Journal of Analytical and Applied Pyrolysis 200: 108103.

The disposal of biomass waste after soil remediation still faces severe challenges. In this study, poplar wood (PW) harvested from contaminated soil was co-pyrolyzed with duckweed (DW). The influences of DW and three phosphorus-based additives on product distribution, biochar properties, heavy metal migration behavior, and ecological risk were systematically compared. The results showed that the solid and gas yields rose with

the increased DW blending ratio, whereas the liquid yield declined. The product regulation performance of PW blended with 30% and 50% DW was comparable to that with the addition of phosphoric acid (PA) and calcium dihydrogen phosphate (CDP), respectively. Both DW and phosphorus-based additives could enrich the surface functional groups of biochar, enhance its inorganic crystal structure, improve porosity, and strengthen the immobilization capacity of heavy metals during co-pyrolysis with DW. Among them, DW exhibited the best performance in enhancing stability and the toxicity characteristics according with the TCLP leaching procedure, due to the participation of endogenous phosphorus in mineral phase reconstruction. Overall, co-pyrolysis with DW provided an effective strategy for the clean utilization of phytoremediation plant.

DF comment: Duckweed used in this study was collected from a contaminated site in Hunan province, China. No further biological information in terms of its population uniformity or species identification are provided in this publication. This omission makes it difficult to related to future work on this topic.

Wolffia globosa-fortified hydrogels for extrusion-based 3D food printing: Effects of particle microstructure and process parameters on dimensional fidelity

Baothong, T; Sanklong, N; Kaewsuwan, D; Pakawanit, P; Pitayachaval, P. (2026) Applied Sciences 16: 8867.

This study investigated and optimized the operational process parameters of an extrusion-based 3D food printing system to maximize the dimensional fidelity of newly developed *Wolffia globosa* (duckweed) starch hydrogel constructs relative to a nominal target specification of 30 times; 30 times; 30 mm. Prior to parameter optimization, synchrotron X-ray tomographic microscopy (SR-XTM) was used to characterize *W. globosa* particle size and dispersion within the starch matrix, showing that grinding eliminated large particle agglomerates (up to approximately 150 μm) and was necessary for smooth, continuous extrusion; the ground formulation was accordingly selected for all printing trials. A full factorial experimental configuration was executed to examine the synchronized effects of three core process parameters: print-head traverse speed (5-15 mm/s), extrusion speed (5-15 steps/mm), and layer height (1.9-3.7 mm). Experimental responses were evaluated via Three-Way Analysis of Variance (ANOVA) and Response Surface Methodology (RSM) using triplicate measurements (n = 3) at each of the 27 tested parameter combinations. Residual diagnostics indicated an approximately normal distribution for the height model (Shapiro-Wilk p = 0.716), whereas the width and length models showed some departure from normality ($p < 0.01$), consistent with the significant lack-of-fit detected for these two responses. Three-way ANOVA confirmed that print-head (nozzle) speed was the dominant factor governing the in-plane dimensions (width and length; partial eta(2) approximate to 0.98), while height was jointly governed by all three factors, with layer height and print-head speed contributing the largest effects. With the statistical power afforded by replicate measurements, all two-and three-way interactions among the three factors were also statistically significant for width and length ($p < 0.001$), refining the single-replicate interaction pattern reported previously. Empirical second-order polynomial equations explained a substantial share of the variance in each dimension (R^2 = 0.70-0.85), although formal lack-of-fit testing indicated that higher-order interactions not captured by the quadratic terms remained statistically significant, and the equations should therefore be interpreted as descriptive rather than as precise predictive tools. Based on the triplicate means, a print-head speed of 5 mm/s, extrusion speed of 15 steps/mm, and a layer height of 1.9 mm minimized the mean cumulative absolute error to 4.01 mm, yielding a mean dimensional profile of 28.63 ± 0.78 mm width, 27.76 ± 0.96 mm length, and 30.41 ± 0.70 mm height (mean ± SD, n = 3).

DF comment: No description of the origin of the *Wolffia globosa* used in this work was provided.

Wolffia globosa as a multifunctional sustainable bioresource: Applications in aquaculture nutrition, functional foods, and circular bioeconomy

Rajesh, SD; Yadav, NK; Angom, J; Kumari, N; Campal, EI; Kalaiselvan, P; Kumar, P; Kakakhel, MA; Patel, AB. (2026) Aquaculture Journal 6: 38.

The rapid expansion of global aquaculture and increasing demand for sustainable protein sources have intensified the search for alternatives to conventional fish meal. *Wolffia globosa*, the smallest and fastest-growing flowering plant, has emerged as a promising yet underutilized aquatic resource with significant potential in aquaculture nutrition and sustainable food systems. Despite growing interest in aquatic plant-based proteins, comprehensive evaluation of *W. globosa* across nutrition, cultivation, and application systems

remains limited. This review synthesizes current knowledge on its biology, nutritional composition, functional properties, and application potential in aquaculture and circular bioeconomy systems. *W. globosa* exhibits rapid vegetative propagation, high biomass productivity, and minimal resource requirements, enabling efficient cultivation in nutrient-rich environments. It contains high-quality protein (20-48% dry weight), essential amino acids, polyunsaturated fatty acids, dietary fiber, vitamins, minerals, and diverse bioactive compounds with antioxidant and immunomodulatory properties. Feeding studies demonstrate its potential to enhance growth performance, feed efficiency, and physiological health in aquatic species. Additionally, its capacity to absorb nitrogen and phosphorus supports nutrient recycling and water quality improvement in integrated aquaculture systems. However, variability in nutrient composition, limited large-scale cultivation data, insufficient long-term feeding trials, and uncertain economic feasibility at industrial scale remain key challenges for its commercial adoption. Overall, *W. globosa* represents a promising but still developing biomass resource with strong potential to reduce dependence on fish meal while supporting environmentally efficient aquaculture and future food systems. Future research should focus on optimizing cultivation practices, developing efficient processing technologies, and validating long-term feeding performance to facilitate its large-scale adoption.

Duckweed as a novel functional food-implications for gut microbiota modulation

Bandyopadhyay, A; Vashisht, I; Pal, T. (2026) Comprehensive Physiology 16: e70245.

The gastrointestinal microbiota has a pivotal role in human health, orchestrating metabolic homeostasis, immunological activity, and nutrient bioavailability. Dietary modulation of gut microbiota is the simplest and most effective approach to preventing and managing metabolic and inflammatory disorders. Duckweed, particularly *Wolffia* species, has recently emerged as a sustainable, nutrient-dense functional food with potential microbiome-mediated benefits. Beyond its high-quality protein content, duckweed provides fermentable fibers, polyphenols, minerals, and micronutrients including vitamin B12-that potentially influence gut microbial composition and metabolic activity. Evidence from compositional analyses, in vitro human colonic fermentation models, animal studies, and controlled dietary interventions suggests that duckweed-derived substrates support short-chain fatty acid production, generate bioactive phenolic metabolites, and interact with probiotic taxa, positioning duckweed as a prebiotic-like matrix with synbiotic potential. Clinical studies within whole-diet frameworks, such as green-Mediterranean dietary patterns incorporating *Wolffia globosa*, report favorable metabolic outcomes alongside microbiome-associated signals. This review synthesizes mechanistic, preclinical, and clinical evidence to reframe duckweed not just as another alternative protein, but as a microbiota-accessible, synbiotic-ready food matrix, while identifying the critical evidence gaps that currently limit clinical translation and regulatory constraints.

Untargeted metabolomics reveals functional bioactive metabolite networks in green rice milk yogurt enriched with *Wolffia globosa*

Kiatprasert, P; Punyauppa-Path, S; Taesuk, N; Maneerat, S; Kanchanarach, W; Najomtien, P; Phupaboon, S. (2026) Life 16: 1385.

This study aimed to illustrate the effects of plant-based yogurts made from bio-fermentative green rice milk yogurt (GRMY), enhanced with *Wolffia globosa* powder (WGP) and infused with Thai green tea. Different formulations (F1 to F4) were analyzed to assess the impact of varying weight ratios of WGP, ranging from 0% to 15%. The goal was to improve nutritional values and bioactive components. The F4 formulation of plant-based yogurt demonstrated enhanced nutritional values, including higher levels of dietary fiber, starch, protein, and fat contents, which were 42.45, 26.55, 22.48, and 2.29% DW, respectively. It was increased by bioactive compounds such as total phenolic content (52.99 mg GAE/g), total flavonoid content (60.45 mg QUE/g), and beta-carotene (1.81 mg/g). Additionally, the F4 formulation exhibited the highest antioxidants in terms of DPPH activity (5.48 mg TROE/g), ABTS activity (424.15 mg TROE/g), and FRAP reducing capacity (106.35 mg TROE/g) and antidiabetic activity of alpha-glucosidase inhibition in IC_{50} at 1.41 mg/mL. The yogurts were evaluated through metabolomic network analysis as a complex bioactive food system, wherein fermentation-derived metabolites synergistically interact with tea polyphenols, amino acids, phytosterols, and carotenoids. The prevailing metabolic profiles indicate an increase in antioxidant capacity, a reduction in inflammatory burden, enhanced glucose metabolism, alterations in gut microbiota, cardiovascular protection, and neuroprotective potential. These findings suggest that the product represents a promising plant-based functional food, offering numerous health benefits. This assertion is supported by a diverse metabolite network identified through UPLC-MS/MS metabolomic research.

Integrated characterization of *Wolffia globosa* as a potential food ingredient: Nutritional and physicochemical properties, selected elements, and preliminary in vitro responses

Tongyai, A; Pisprasert, V; Tudpor, K; Toontom, N. (2026) Foods 15: 2919.

Wolffia globosa (water meal) is a potential plant-based food ingredient. This study characterized its nutritional and physicochemical properties, elemental levels, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical-scavenging activity, IL-6 production in H_2O_2-stimulated RAW264.7 macrophages, and preliminary cell-viability responses. On a dry-weight basis, the tested *W. globosa* sample contained 25.83% protein, 4.56% fat, 16.58% ash, 8.80% crude fiber, and 44.22% carbohydrate. Arsenic and zinc were detected below their limits of quantification, whereas cadmium, copper, and lead were below their method detection limits. All analytical reporting limits were below the corresponding EFSA specification values. The extract had TPC and TFC values of 0.85 ± 0.03 mg GAE/g sample DW and 0.52 ± 0.02 mg QE/g sample DW, respectively, and showed weak DPPH radical-scavenging activity (IC_{50} = 7.58 ± 0.32 mg DW-equivalent/mL). At 0.1 mg DW-equivalent/mL, the H_2O_2-stimulated extract-treated group showed a lower IL-6 concentration than the H_2O_2-stimulated extract-free model control. Weak reductions in cancer-cell viability occurred only at high concentrations (IC_{50} values of approximately 38-42 mg DW-equivalent/mL). The biological findings remain preliminary. Overall, the tested sample showed promising nutritional and physicochemical characteristics, low selected-elements levels, and preliminary in vitro responses.

Evaluation of the potential of watermeal (*Wolffia arrhiza* L.) as a plant-based protein boost for rice seasoning powder

Saeiam, K; Hirunyophat, P; Poolcharoensil, N. (2026) African Journal of Food, Agriculture, Nutrition and Development 26: 29944 – 29963.

Watermeal (*Wolffia arrhiza* L.), known as the smallest aquatic plant in the world, has recently gained attention as a promising plant-based protein source for its high protein content, essential amino acids, minerals, vitamins, and antioxidants. Its rich nutrient profile highlights its potential for use in health-oriented food innovations. This study aimed to investigate the feasibility of incorporating dried watermeal (WM) as a partto enhanced in rice seasoning powder (furikake) to enhance its nutritional value, particularly its protein content. The rice seasoning powders were prepared following a modified standard recipe, which involved heating the seasoning mixture at 90°C, drying at 60°C, cooling, and subsequently blending with either dried seaweed or dried WM before packaging. Five formulations were developed by substituting seaweed with WM at levels of 0%, 3%, 6%, 9%, and 12%, and their physicochemical properties, antioxidant activity, microbial quality, and sensory acceptance were evaluated. The results demonstrated that increasing WM substitution significantly elevated protein, fiber, and mineral contents compared to the control (0% WM). All formulations achieved food safety standards and indicated suitability for extended storage. However, higher WM levels led to notable changes in appearance, aroma, taste, and texture. Among the tested formulations, WM-3 (3% WM) achieved the most desirable balance between nutritional enhancement and consumer acceptability across all sensory attributes. Antioxidant activity was reduced in WM-substituted samples, likely due to thermal and processing effects that influenced the stability of bioactive compounds. Overall, the findings confirm that WM can serve as a functional ingredient that effectively enriches the nutritional profile of furikake without compromising food safety or overall consumer acceptance when used at low substitution levels. The incorporation of WM presents an opportunity to develop plant-based, nutrient-dense seasoning products that align with current market trends toward sustainable and health-oriented diets.

DF comment: The water meal used in this work was purchased from a shop in Kalasin province in Thailand. No additional information is provided to validate its identification as *W. arrhiza*, which is not a native plant of Thailand. Similar to early work describing *W. arrhiza* from Thailand, it may be likely that this is *Wolffia globosa* instead.

Effects of dietary supplementation with *Wolffia globosa* and *Limosilactobacillus reuteri* KUB-AC5 on health parameters and gut microbiota composition in dogs

Areerat, S; Kovitvadhi, A; Jenjirawatn, K; Khamma, S; Chapanon, P; Chundang, P; Praditrungwatana, N; Pliantiangtam, N; Patumcharoenpol, P; Akrimajirachoote, N; Nakphaichit, M; Charoensiddhi, S; Lertwanakarn, T; Palaseweenun, P. (2026) Biology 15: 1067.

The aquatic plant *Wolffia globosa* and the probiotic *Limosilactobacillus reuteri* KUB-AC5 have been proposed as candidate synbiotic components targeting gut microbiota modulation. In this study, we investigated the effects of *W. globosa* supplementation, alone or in combination with *L. reuteri* KUB-AC5, on the gut microbiota and health status in healthy adult dogs. For a 28-day feeding trial, 24 healthy dogs were randomly assigned to three dietary treatment groups: control, prebiotic (PRE; 3.5% *W. globosa* powder), and synbiotic (SYN, 3.5% *W. globosa* powder combined with *L. reuteri* KUB-AC5 at 1 times; 10^8 CFU/day). No significant differences were observed in body weight, body condition score, fecal consistency, feed intake, or blood parameters among groups ($p > 0.05$). Although overall gut microbial diversity remained unchanged, linear discriminant analysis effect size analysis revealed the selective enrichment of specific bacterial taxa. The PRE group exhibited higher relative abundances of *Parabacteroides merdae*, *Romboutsia lituseburensis*, *Subdoligranulum variabile*, and *Phocaeicola sartorii*, whereas the SYN group showed increased levels of *Escherichia coli* and *Terrisporobacter glycolicus* and reduced levels of *Anaerobiospirillum succiniciproducens* and *Campylobacter upsaliensis* ($p < 0.05$). In conclusion, *W. globosa*, alone or combined with *L. reuteri* KUB-AC5, was safely used to modulate gut bacterial composition as a potential functional ingredient.

Effects of duckweed and pigments on the chemical composition, physicochemical properties, and sensory attributes of plant-based patties

Weini, W; Radzi, NRSM; Mokhtar, WMFW; Manab, A; Andriani, RD; Huda, N; Ismail, I (2026) Malaysian Applied Biology 55: 64-71.

The present study evaluated the influence of duckweed incorporation (0%, 1%, and 2%) and two pigment types (beet and soy leghaemoglobin, 2%) on the chemical composition, physicochemical properties, visible appearance, and sensory attributes of plant-based patties (PBPs). Increasing duckweed levels improved protein content and moisture retention while reducing cooking loss. Soy leghaemoglobin enhanced redness (a*), whereas beet pigment intensified yellowness (b*). This was also evident in the visible appearance, with leghaemoglobin formulations appearing noticeably redder. Higher duckweed concentrations decreased hardness but lowered sensory acceptance, whereas 1% duckweed, particularly with beet pigment, improved flavour, colour, and overall liking. These results indicate that strategic duckweed inclusion combined with natural pigments can enhance the nutritional value and physicochemical properties of PBPs, although excessive duckweed may compromise sensory quality. Thus, this work offers a new approach to developing PBPs by replacing common ingredients and pigments, supporting advances in future food product development.

DF comment: Duckweed used in this work was purchased from a hypermarket (RT-Mart) in Hefei, China. No description of its composition, origin, or species identification is provided.

General Topics and Conferences

Editorial: Harnessing the potential of duckweed: biological insights and ecological applications

Xu, ZH; Zhao, H; Bai, SN; Lam, E; Yang, L (2026) Frontiers in Plant Science 17: 1887238.

https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2026.1887238/full

Dual-purpose biological systems: Enhancing wastewater treatment and biogas generation with duckweed and microorganisms-a systematic review

Grzegorzek, M; Jurga, A; Rodziewicz, T; Zimoch, I; Kalka, J; Lobos-Moysa, E; Kazmierczak, B (2026) Sustainability 18: 6372.

At present, treated wastewater may still contain residual nutrients and micropollutants, including heavy metals, pharmaceuticals, and dyes, which can negatively affect receiving water bodies. Increasingly stringent environmental regulations, including Directive (EU) 2024/3019, require both enhanced removal of these contaminants and greater integration of renewable energy sources in wastewater treatment plants. This paper presents a review of biomass-based wastewater polishing technologies employing biological agents such as microalgae, fungi, bacteria, co-cultures and duckweed for the removal of residual contaminants from treated

effluents. The compiled data indicate that while optimal conditions can drive pollutant removal efficiencies beyond 90%, system performance varies widely depending on species selection, wastewater characteristics, and operational conditions (e.g., pH, temperature, salinity, nutrient availability, and light intensity). In addition to effluent polishing, the produced biomass can be valorized for bioenergy generation, contributing to renewable energy production and supporting circular economy principles in wastewater treatment plants. Despite these benefits, biomass harvesting remains a major technical and economic bottleneck, often representing a significant share of operational costs and limiting large-scale implementation. Overall, biomass-based treatment technologies are a promising approach for improving effluent quality and supporting renewable energy objectives; however, further advances in biomass recovery are required for broader application.

Duckweed: Research Meets Applications—2nd Edition

Oláh V, Appenroth KJ, Sree KS. Eds. (2026) Special Issue of the journal Plants. doi.org/10.3390/books978-3-7258-8956-3.

https://www.mdpi.com/journal/plants/special issues/YM4X6P629M

Interaction with other organisms

Indole-3-acetic acid production by *Klebsiella variicola* from watermeal and its role in enhancing watermeal growth

Ladthaisong, T; Gateta, T; Seemakram, W; Kokaew, U; Jangpromma, N; Boonlue, S. (2026) Plants 15: 2642.

Plant growth-promoting bacteria (PGPB) enhance plant development through mechanisms such as the production of indole-3-acetic acid (IAA). However, endophytic bacteria associated with aquatic plants, particularly watermeal (*Wolffia* spp.), remain poorly explored. This study investigated the potential of bacterially derived crude IAA extract for enhancing watermeal growth and accumulation of secondary metabolites in watermeal. This study isolated and screened endophytic bacteria from watermeal for IAA production, identifying *Klebsiella variicola* W1/6 as the most efficient producer. Optimization using response surface methodology (RSM) revealed optimal conditions at pH 5.84, 5 days of incubation, and 10.63% (v/v) inoculum size. Crude IAA extract significantly enhanced watermeal growth under both laboratory and greenhouse conditions. Notably, the combination of 0.5 ppm crude IAA extract with 50% chemical fertilizer produced growth comparable to that of the full fertilizer treatment, indicating reduced fertilizer requirement. These findings highlight the potential of bacterially derived IAA from *K. variicola* W1/6 as a microbial biostimulant for sustainable aquatic plant cultivation.

DF comment: This work utilized mainly the Salkowski assay for IAA determination. While this method can readily detect indole-containing compounds, it is also confounded by its tactionon of many others that do not exhibit auxin-like behaviors. Thus, the use of this assay as a proxy for IAA should be used with caution.

Host-associated taxonomic and functional diversity of plant growth-promoting bacteria in three duckweed species: *Spirodela polyrhiza*, *Lemna minor*, and *Wolffia globosa*

Ruenpham, K; Mori, K; Tanaka, Y; Thamchaipenet, A; Morikawa, M; Toyama, T (2026) Bioresource Technology Reports 35: 102989.

Duckweed is an aquatic plant that simultaneously enables biomass production and wastewater treatment. Associated bacteria substantially influence duckweed growth, highlighting the importance of understanding host-associated bacterial interactions for optimizing duckweed-based systems. However, how bacterial taxa and plant growth-promoting (PGP) traits vary among duckweed species remains insufficiently characterized, and culture-based evidence of host-associated taxonomic and functional differentiation among duckweed-associated bacteria remains limited. This study investigated the taxonomic and functional diversity of bacteria associated with three phylogenetically and morphologically distinct duckweeds - *Spirodela polyrhiza*, *Lemna minor*, and *Wolffia globosa*. A total of 54 strains were isolated from axenic duckweeds following controlled co-cultivation with environmental water samples containing indigenous bacterial communities; of these, 47 strains were taxonomically identified, spanning 26 genera across four phyla. Genus-level bacterial community composition exhibited host-associated patterns, whereas phylum-level patterns were similar among duckweed

species. These 54 isolates were assessed for PGP traits; specifically, 74.1%, 51.9%, and 61.1% of isolates produced indole-3-acetic acid (IAA) and siderophores, and exhibited potential nitrogen-fixing activity, respectively. Furthermore, 72.2% of isolates promoted duckweed growth, whereas 14.8% inhibited it. Plant growth-promoting bacteria were prevalent among isolates from *S. polyrhiza* and *L. minor*, while isolates from *W. globosa* included a relatively high proportion of plant growth-inhibiting bacteria. Notably, IAA production was the only trait significantly associated with growth promotion. These findings provide culture-based evidence of host-associated taxonomic and functional differentiation among duckweed-associated bacteria. The results advance our understanding of duckweed-microbe interactions and may contribute to the future development of microbial inoculants for biomass production and phytoremediation.

DF comment: This work utilized mainly the Salkowski assay for IAA determination. While this method can readily detect indole-containing compounds, it is also confounded by its tactionon of many others that do not exhibit auxin-like behaviors. Thus, the use of this assay as a proxy for IAA should be used with caution.

Isolate-specific modulation of growth, carbon allocation, and transcriptomic responses in *Spirodela polyrhiza* by duckweed-associated bacteria

Ruenpham, K; Mori, K; Tanaka, Y; Thamchaipenet, A; Morikawa, M; Toyama, T. (2016) Microorganisms 14: 1850.

Duckweed-associated plant growth-promoting bacteria have attracted attention for their potential to enhance duckweed biomass production. However, the mechanisms underlying isolate-specific growth promotion are unclear. This study compared the effects of two duckweed-associated bacterial isolates, *Terrimicrobium* sp. PS02 and *Aeromicrobium* sp. PS05, on the growth, biomass composition, colonization behavior, and transcriptomic responses of *Spirodela polyrhiza*. Biomass composition and transcriptome analyses were performed to characterize the host responses. Both isolates enhanced duckweed biomass; PS02 increased it 1.2-fold, whereas PS05 induced a significant 1.4-fold increase compared to the uninoculated control. PS05 established bacterial populations approximately one order of magnitude higher than those of PS02 and formed dense extracellular polymeric substance-mediated microcolonies on the surface. Transcriptome analysis revealed that PS05 induced 1108 differentially expressed genes, compared with 454 in PS02, indicating greater host transcriptomic reprogramming. Functional enrichment of transcriptome responses showed that PS05 preferentially regulates carbohydrate biosynthesis, central carbon metabolism, and starch biosynthesis, significantly enhancing starch accumulation and turion formation without reducing protein or photosynthetic pigment content. This study provides evidence linking bacterial colonization, host transcriptomic regulation, carbon allocation, and biomass production in duckweed, offering a basis for engineering high-performance duckweed-microbiome systems for sustainable biomass production.

The metabolic and anatomical complexity of root microhabitats modulate their interaction with the microbiota

Frene, JP; Custódio, V; Rouco, H; Martinez-Jarquin, S; Gao, YQ; di Bari, V; Bakhoum, N; Northen, TR; Bowen, BP; Louie, KB (2026) Nature Commnication 17: 9738.

Plant roots constantly communicate with their microbiota, adapting their anatomy to facilitate microbial colonisation under abiotic stresses. Microbes, in turn, can reshape root anatomy once they establish. However, the mechanisms that coordinate this interplay remain largely unknown. Working with the aquatic plant family Lemnaceae, we reveal that the inherent complexity of root anatomy determines root plasticity in response to microbial colonisation. This microbiota-driven anatomical plasticity enhances plant survival in nutrient-competitive environments. By combining synthetic root models with real roots, we also find that anatomical plasticity is associated with metabolic reprogramming during microbial establishment. Moreover, we identify a plant metabolite, N6,N6,N6-Trimethyl-L-lysine, that regulates anatomical plasticity in response to microbial colonisation. Our work generalizes the importance of microhabitat complexity for microbiome recruitment under challenging environmental conditions.

Composite symbiotic bacteria enhance wastewater purification and feed value of *Spirodela*

Li, GX; Liu, XZ; Wu, SH; Lv, DW (2026) Sustainability 18: 6495.

The present study aims to address critical research gaps in duckweed-microbe symbiotic systems specifically applied to high-load livestock and poultry breeding wastewater. These gaps include the insufficient development of well-characterized, multi-functional, complex microbial consortia adapted to complex livestock wastewater matrices, and the technical challenge of achieving simultaneous efficient wastewater purification and duckweed feed quality enhancement. This study is motivated by the pressing issue of agricultural non-point source pollution, which is caused by large-scale livestock and poultry breeding wastewater discharge, and the high external dependence of the feed industry on protein raw materials. The present study utilised *Spirodela* as the fundamental material, and a functionally complementary complex symbiotic bacterial consortium consisting of *Bacillus subtilis*, *Bacillus tequilensis* and *Pseudomonas fluorescens* was screened and constructed. An experiment was conducted over a 14-day period in which a range of inoculation ratios were systematically explored. The aim of this experiment was to ascertain the purification efficiency of the duckweed-bacteria symbiotic system on high-load livestock and poultry breeding wastewater. Furthermore, the experiment sought to determine the effect of this purification process on the feed value of duckweed. The results demonstrated that complex bacterial inoculation significantly enhanced wastewater purification efficiency. The final removal rate of ammonia nitrogen in all treatment groups exceeded 90% after 14 days, and the maximum removal rates of total nitrogen and total phosphorus reached 67.0% and 58.9%, respectively, thereby demonstrating superior purification performance in comparison to the control group. The inoculation ratio of 10:1 was identified as the optimal parameter for wastewater purification, while the 5:1 ratio was found to be the maximum for crude protein accumulation in duckweed. The maximum dry-based crude protein content recorded was 38.9% on day 14, representing an increase of 26.3% in comparison with the control group. The established duckweed-bacteria symbiotic system has the capacity to simultaneously achieve the efficient purification of livestock and poultry breeding wastewater and the high-value utilisation of duckweed. The optimal process parameters for a range of application scenarios have been determined. This study contributes to the theoretical framework of aquatic plant-microbe symbiotic remediation and provides technical support for the recycling of wastewater resources and the sustainable development of the livestock and poultry breeding industry.

For colonization success, should hosts and microbes travel alone, together, or swap partners along the way?

Usui, T; Yu, JC; Frederickson, ME (2026) New Phytologist DOI10.1111/nph.71372

Microbiomes that enhance the performance of host plants are likely to be co-introduced with their host during colonization because of their intimate association. Yet, it is unclear how co-introduced microbes will impact host colonization, as both the microbiome and its effects could vary upon introduction into a new habitat. Using the duckweed *Lemna japonica* - a cosmopolitan, freshwater angiosperm - and its microbiome, we tracked the colonization of both plants and microbes during an experimental co-introduction in the wild. We tested how plant performance varied during colonization when plants were co-introduced with microbes from their home habitat or with microbes local to the introduced habitat. We found that plant performance was substantially reduced when plants were co-introduced with microbes from their home habitat (i.e. with microbes that are non-local to the introduced habitat), relative to hosts with a local microbiome. Moreover, negative impacts from the initial, non-local microbiome persisted for multiple host generations despite a rapid turnover in microbiome composition. Our results suggest that the initial microbiome plants are co-introduced and can leave lasting impacts on plant performance during colonization. Considering the identity of the co-introduced microbiome will therefore be critical to predicting plant colonization dynamics in an era of global change.

Morphology

Mechanism of duckweed morphological plasticity under gradient phosphate concentrations

Li, ZF (2026) Theoretical and Natural Science 187: 185-191.

Phosphorus is a key limiting nutrient triggering aquatic eutrophication, and duckweed with simple structure and sensitive phenotypic changes is an ideal model to explore plant phosphorus adaptive responses. This paper focuses on the morphological plasticity of duckweed exposed to low, moderate and high phosphate gradients and constructs a multi-index evaluation system for its growth status. Low phosphate restrains duckweed cell division and chlorophyll synthesis, leading to shrunken pale fronds and elongated roots for

nutrient capture. Moderate phosphate supports balanced frond proliferation, stable root development and continuous biomass accumulation to form optimal growth state. High phosphate brings short-term rapid propagation but induces intraspecific competition and long-term aquatic ecological risks. Single frond counting generates biased analysis, while joint assessment of frond area, leaf color, root length and dry weight can accurately judge phosphorus stress. This study provides theoretical support for applying duckweed as bio-indicator and ecological remediation material in phosphorus-polluted water bodies.

Physiology & Stress

Tolerance for heat stress in *Lemna minor* reflects both local adaptation and acclimation over time

Blanco-Sánchez, M; Sultan, SE; Verhoeven, KJF. (2026) Environmental and Experimental Botany 250: 106459.

Assessing intraspecific variation in thermal stress tolerance is key to predicting plant responses and long-term persistence under climate change, yet its underlying sources and temporal dynamics remain poorly understood. Using a common garden experiment with four ecologically-relevant temperatures (18, 23, 28, and 33°C), we evaluated the sources and temporal dynamics of variation in temperature stress tolerance of 18 *Lemna minor* clonal lines from contrasting climates. Our results showed that past adaptation, physiological acclimation, and within-line variation jointly contributed to variation in performance. The study provides the first evidence of adaptive genetic differentiation in heat stress tolerance in this ecologically widespread freshwater species, with lines from warmer regions showing higher growth under heat stress. However, these differences were transient and diminished under prolonged exposure. Specifically, adaptive differentiation in heat stress tolerance (as indicated by the correlation between performance and temperature of origin) was detected at 33°C only during the first week of exposure ($R = 0.74$; $P < 0.001$). Experimental lines also showed acclimation over time, but these responses were strongly temperature-dependent and occurred only under sub-optimal conditions. Finally, replicates within some lines exhibited divergent performance trajectories under sustained heat stress, suggesting the emergence of novel phenotypic variation, potentially mediated by epigenetic mechanisms. These results show that heat stress tolerance in *L. minor* arises from multiple interacting sources and is dynamically shaped by both selective history and immediate exposure time, suggesting a more nuanced, multi-layer understanding of variation in heat stress tolerance.

Duckweeds under salt stress: a meta-analysis of growth inhibition in relation to morphological and physiological traits and a systematic review of molecular responses

Bueno, SB; Pita-Barbosa, A; Borella Jr. (2026) New Phytologist DOI10.1111/nph.71591.

Salinity is one of the main environmental factors limiting the performance of duckweeds and despite growing ecological and biotechnological interest in these macrophytes, there is a lack of quantitative analyses of their responses to sodium chloride. To address this gap, we conducted a meta-analysis and a systematic review of 38 articles to quantify the effects of NaCl on growth, photosynthetic pigments, carbohydrate metabolism, osmotic adjustment, ionic homeostasis, oxidative stress, antioxidant defense and molecular responses in duckweeds. NaCl consistently reduced growth-related traits and photosynthetic pigments while increasing proline, oxidative stress, Na^+ accumulation, K^+ depletion and altered starch metabolism. Antioxidant responses varied among enzymes, with SOD, APX, GPX and GR generally showing positive responses under saline conditions. Species-specific analyses identified *Spirodela polyrhiza* and *Lemna minor* as having greater bioenergetic potential, whereas *S. polyrhiza* and *Landoltia punctata* showed lower ionic disturbances and stronger osmoprotective responses. Available molecular studies indicate the involvement of ABA-, ROS- and Ca^{2+}-mediated signaling pathways, and individual studies highlight Ca^{2+} and K^+ supplementation as promising strategies to enhance salt tolerance. Overall, duckweed salinity tolerance emerges from the coordinated interaction of ionic, osmotic and redox regulation, providing a mechanistic framework for future applications in saline environments and biomass-based technologies.

Differential physiological and antioxidant responses in roots and fronds of *Spirodela polyrhiza* (L.) Schleiden under pulp and paper mill effluent stress

Sharma, A; Malaviya, P (2026) International Journal of Phytoremediation DOI10.1080/15226514.2026.2720091.

Spirodela polyrhiza, a free-floating aquatic macrophyte, is widely used in toxicity and effluent studies because of its rapid growth and tolerance to adverse conditions. This study compared the responses of roots and fronds of *S. polyrhiza* exposed to 50% and 100% pulp and paper mill effluent over five days. Effluent exposure caused concentration-dependent reductions in growth and photosynthetic pigments, accompanied by increased oxidative stress and membrane injury. Roots exhibited greater electrolyte leakage and lipid peroxidation, indicating higher physiological stress due to direct effluent contact, whereas fronds showed pronounced pigment reductions while retaining comparatively higher protein and soluble sugar contents. Increased activities of catalase, ascorbate peroxidase, and guaiacol peroxidase, together with elevated proline, hydrogen peroxide, and malondialdehyde, indicated activation of antioxidant and stress-response mechanisms. However, these responses were insufficient to prevent severe membrane destabilization and metabolic disturbance at higher effluent concentrations. Principal component analysis distinguished growth-related variables from stress-associated parameters, with root observations showing a stronger association with oxidative injury. The root-frond comparison revealed greater oxidative and membrane injury in roots and stronger impairment of photosynthetic and metabolic functioning in fronds, providing a clearer basis for interpreting plant stress and selecting indicators for future duckweed-based wastewater treatment studies.

Effect of increasing temperatures on duckweed growth, photosynthetic performance and frond ontogenesis

Irfan, M; Chaudhry, MA; Bog, M; Szabó, S; Mészáros, I; Jansen, MAK; Oláh, V (2026) Plant Cell Reports 45: 249.

Duckweeds offer a suitable platform to study the effects of elevated temperatures on aquatic plants, and chlorophyll fluorescence imaging can reveal frond-level distribution of temperature stress. Temperature rise associated with climate change poses a major challenge for aquatic plants. It has a strong influence on their physiological performance and, consequently, their competitive ability and fitness. Yet comparative studies of impacts on different species remain scarce. This study investigated the effects of four different temperature regimes between 20 and 35°C on five duckweed species (*Spirodela polyrhiza*, *Landoltia punctata*, *Lemna gibba*, *Lemna minor* and *Lemna minuta*) by integrating growth- and photosynthesis-based parameters. We also recorded spatial patterns of photosystem II efficiency within fronds using chlorophyll a fluorescence imaging to understand how temperature stress is distributed throughout the frond development. The results highlighted substantial inter-specific variation in thermal responses and underscore the importance of integrating physiological and growth-based indicators to predict aquatic plants' responses to climatic warming. Through simultaneous measurement of growth performance, photosynthetic efficiency and within-frond heterogeneity, this study provides mechanistic insights into how rising temperatures may influence duckweeds. Such knowledge is essential for predicting future shifts in freshwater primary productivity, species dominance, biological invasions and ecosystem stability under the current global warming scenario.

Sucrose as a stress protectant: A novel application of *Lemna minor* L. under fluoride contamination conditions

Kaminski, J; Piotrowicz-cieslak, AI. (2026) Fluoride 59: e474.

Fluorine (F) is an element whose environmental levels have increased due to human activities, and it has been shown to exert negative effects on plants. Plants were cultivated on Murashige and Skoog (MS) medium supplemented with sucrose at 3, 12, 21, and 30 g/L and exposed to NaF concentrations ranging from 120 to 200 mg/L. We analyzed cell viability and metabolic activity (WST-1 Assay), sucrose synthase (SUS) and acid (A-Inv). While sucrose alone did not adversely affect plant growth, the optimal biomass accumulation under non-stress conditions occurred at 12 g/L, with higher concentrations reducing frond size, likely due to osmotic effects. Under fluoride stress, higher sucrose concentrations (21-30 g/L) significantly enhanced plant survival, reduced chlorosis, and maintained frond area, indicating a protective effect. Metabolic analysis revealed that sucrose synthase (SUS) activity increased in a dose-dependent manner and was further enhanced under fluoride stress, directing sucrose-derived carbon toward anabolic pathways. Acid invertase (A-Inv) activity rose under low sucrose supply during stress, promoting immediate energy availability, whereas neutral invertase (N-

Inv) exhibited moderate modulation. These results demonstrate that exogenous sucrose not only serves as a metabolic substrate but also acts as a regulatory molecule, reprogramming carbon metabolism to sustain growth and viability under fluoride toxicity. The study highlights the concentration-dependent dual role of sucrose in *Lemna minor*: optimal growth under normal conditions and stress mitigation under fluoride exposure.

DF comment: The *L. minor* culture used in this work was from a University in Olsztyn, Poland. However, it is unclear what is its origin, how it was typed, and whether it has been confirmed with the latest method using TBP barcoding to confirm its species identity.

Phytomedicine

Optimization and metabolomic profiling of *Wolffia globosa* extract reveal multitarget neuroprotective activity against Alzheimer's disease with in vitro, in silico, and in vivo validation

Temviriyanukul, P; Inthachat, W; Laowanitwattana, T; Buacheen, P; Suttisansanee, U; Pitchakarn, P. (2026) International Journal of Molecular Sciences 27: 8266.

Wolffia globosa, the world's smallest flowering plant, is a sustainable protein- and phytochemical-rich food source with emerging neuroprotective potential. This study presents the first comprehensive characterization that integrates extraction optimization, multitarget bioactivity profiling, drug synergy assessment, high-resolution metabolomics, molecular docking, and in vivo validation in *Drosophila melanogaster*. Ethanol-based extraction was optimized using a Box-Behnken design and response surface methodology (RSM), yielding optimal conditions of 32% ethanol, 1:40 (g/mL) solid-to-solvent ratio, 60°C, and 28 min. The optimized extract exhibited a total phenolic content (TPC) of 15.17 ± 0.27 mg GAE/g DW, a total flavonoid content (TFC) of 22.34 ± 1.71 mg QE/g DW, and an exceptional ORAC antioxidant activity of 4374.33 ± 395.65 µmol TE/g DW. Potent and selective BACE-1 inhibition was observed (IC_{50}: 1.59 ± 0.11 mg/mL), alongside moderate AChE (IC_{50}: 7.42 mg/mL) and BChE (IC_{50}: 6.55 mg/mL) inhibition. Synergistic interactions with donepezil were confirmed by the Chou-Talalay combination index method, with combined AChE, BChE, and BACE-1 inhibitions reaching 60.3%, 77.0%, and 82.2%, respectively-substantially exceeding theoretical additive values. Multi-platform metabolite profiling by HPLC-QTOF-MS/MS and LC-ESI-MS/MS identified luteolin, apigenin, caffeic acid, isovitexin, schaftoside, and pinolenic acid as the principal bioactives. Molecular docking predicted favorable binding of luteolin (delta-G: -10.24 kcal/mol, AChE) and naringenin (delta-G: -8.76 kcal/mol, BACE-1) against crystal structures of human AChE (PDB: 7E3H), BChE (PDB: 4TPK), and BACE-1 (PDB: 6EQM). In vivo, the *W. globosa* extract significantly improved locomotor performance and suppressed brain BACE-1 activity in a Drosophila AD model over 28 days. These findings establish *W. globosa* as a multifunctional nutraceutical candidate for neurodegenerative disease prevention.

DF comment: The smallest Lemnaceae species is *Wolffia angusta*

Phytoremediation

From invasive aquatic biomass to high-efficiency adsorbent: valorization of duckweed into KOH-activated biochar for rapid dye remediation

Letchiminan, S; Rajoo, KS; Hii, TT; Seng, KWK; Chung, ELT; Andou, Y; Zheng, ALT. (2026) Journal of Iranian Chemical Society 23: 349.

Dye-contaminated wastewater from textile and related industries poses persistent risks to aquatic ecosystems and water quality. This study evaluated KOH-activated duckweed-derived biochar (BC) prepared at 500°C and 700°C, denoted as 500CDW and 700CDW, for the adsorptive removal of methylene blue (MB) from aqueous solution. The adsorbents were prepared by carbonization followed by potassium hydroxide (KOH) activation and characterized using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and point of zero charge (pHpzc) analysis. Batch adsorption experiments examined the effects of initial MB concentration, contact time, solution pH, adsorbent dosage, and temperature. Among the tested materials, 500CDW showed faster MB uptake than 700CDW and was selected for subsequent adsorption studies. The pHpzc of 500CDW was 6.79, indicating that electrostatic attraction

contributed to the enhanced uptake of cationic MB under alkaline conditions. Nonlinear kinetic analysis showed that the pseudo-first-order (PFO) model provided the best overall fit across the investigated MB concentrations, although fitting error increased at 20 mg/L, indicating more complex adsorption behaviour at higher dye loading. The nonlinear equilibrium data were best described by the Temkin model, followed closely by the Langmuir model. Thermodynamic analysis suggested spontaneous and endothermic adsorption within the studied temperature range. A preliminary laboratory-scale cost analysis estimated the production cost of 500CDW at RM 3.13 g^{-1}. Overall, 500CDW showed promise as a low-cost duckweed-derived adsorbent for MB removal, although further textural characterization, regeneration testing, and real-water validation are needed to confirm its practical applicability.

DF comment: The duckweed, assumed to be *Lemna minor*, was apparently collected from a pond in Bintulu, Malaysia. The method for species determination should be reported since there are multiple *Lemna* spp. that can look very similar and easy to be confused.

Agro-industrial wastewater treatment and biomass production using macrophyte *Lemna minor* in sequencing batch reactor experiments

Xenaki, A; Gatidou, G; Stasinakis, AS. (2026) International Journal of Phytoemediation DOI10.1080/15226514.2026.2736171.

Conventional methods often fail to treat high-load agro-industrial wastewater, whereas plant-based systems offer superior pollutants removal and high-value biomass. This study investigated the performance of *Lemna minor* in sequencing batch reactors for treating dairy, citrus juice and chili sauce wastewater. It also evaluated the effect of NH_4-N addition and wastewater dilution with a chemical medium or tap water on pollutants removal and biomass characteristics. According to the results, COD removal ranged between 59 ± 12% and 91 ± 6% while nitrogen supply did not significantly affect the plant's ability to remove organic load. NH_4-N removal exceeded 60% in all bioreactors. Citrus juice and chili sauce were the most suitable types of wastewater for biomass production while nitrogen addition enhanced the biosynthesis of proteins, lipids and lutein. The use of a chemical medium to dilute the agro-industrial wastewater in the bioreactors at a ratio of 1:3 (25% wastewater and 75% medium) enhanced macrophyte growth, resulting in higher growth rates. The biochemical composition of the biomass varied depending on the wastewater types used with total protein and lipid content ranged up to 36% and 54%, respectively. The lutein concentrations ranged between 2.52 and 9.61 mg g^{-1}. *L. minor* showed high assimilation efficiency in treating nutrient-rich agro-industrial wastewater, simultaneously generating biomass with significant potential for commercial valorization.

A comparative study of response surface methodology (RSM) and artificial neural networks (ANN) based optimization and modeling of paper mill effluent treatment using *Lemna minor*

Goala, M; Kumar, V; Bachheti, A. (2026) Journal of Applied and Natural Science 18: 1275-1286.

Industrial effluents are a major source of water pollution, often leading to ecological imbalance and human health risks. Among these, paper mill effluent is particularly challenging to manage due to its high pollution load. This study aimed to evaluate the phytoremediation potential of *Lemna minor* for paper mill effluent and optimize operational conditions using Response Surface Methodology (RSM) and Artificial Neural Networks (ANN). For this, effluent samples were collected from an industry located in Saharanpur, India, and exposed to *L. minor* at varying biomass densities (10, 20, 30 g) in 20 L containers under controlled greenhouse conditions for 16 days. Three wastewater concentrations (0%, 50%, 100%) were tested. Both RSM and ANN models were developed to predict BOD and COD removal and biomass production, and statistical validation was performed using selected metrics. Results indicated severe pollution in the effluent, with maximum pollutant removal and biomass production occurring at moderate wastewater concentration (50–70%) and higher plant density (20–30 g). Specifically, optimized conditions of 69.03% wastewater and 29.89 g plant density yielded a model-predicted near-complete BOD removal (~100%; the polynomial model extrapolated to 100.81% and is therefore reported as ~100%), COD removal of 88.23%, and biomass of 102.95 g (predicted), closely relating to experimental values (BOD 96.48%, COD 85.21%, biomass 97.42 g). In this, ANN consistently outperformed RSM in predictive accuracy (R2 > 0.999, lower ME, and lower MSE and RMSE), effectively capturing nonlinear interactions among variables. The study showed that *L. minor* is an effective and sustainable candidate for industrial effluent treatment, contributing significantly to pollutant removal and biomass yield.

Phytoremediation of Cadmium, Nickel and Lead from wastewater by *Lemna gibba* using response surface methodology: Optimization study

Mohammed, AJ. (2026) Natural Resources for Human Health 6: 1433-1444.

Water pollution caused by toxic heavy metals remains a critical global issue, driving the search for sustainable and low-cost treatment technologies. This study explores an approach for removing cadmium (Cd), nickel (Ni), and lead (Pb) from industrial wastewater using phytoremediation with aquatic plants. Aquatic species *Lemna gibba* (*Lemna* G.) was selected for its availability and potential as green remediation agent. In the phytoremediation experiments, synthetic wastewater solutions containing Cd, Ni, and Pb were exposed to the aquatic plant. Metals concentrations were monitored daily, while the percentage removal, was calculated to assess uptake efficiency. Optimization using response surface methodology (RSM) and central composite design (CCD) identified conditions that achieved high removal efficiencies across biomass load, exposure time, and initial concentrations. The plant, *Lemna* G. exhibited the highest and fastest removal rates, particularly for Pb and Cd, classifying it as a hyperaccumulator. Overall, results demonstrate that the combined use of *Lemna* G. for phytoremediation provides an effective, low-cost, and environmentally sustainable strategy for removing heavy metals from industrial wastewater. The dual use of biomass not only maximizes remediation efficiency but also reduces plant waste, offering a circular and eco-friendly treatment solution. The data was analyzed using the response surface methodology (RSM) in order to support activities that optimize the phytoremediation process. Three variables were included in a central composite design (CCD), which included 20 trials with 6 axial points and 6 repetitions at the center point. The most significant component in each experimental design response was determined by the analysis of variance (ANOVA). The prediction error between the experimental and predicted results for the removal of Cd, Ni, and Pb was 2.4, 1.8 and 0.3, respectively. This study recommended to use *Lemna gibba* for the removal of Cd, Ni, and Pb from wastewater.

Comparative phytoremediation efficiency of *Lemna minor* and *Hydrilla verticillata* for removing linear alkylbenzene sulphonate (LAS)

Wiratno, EN; Buwono, NR; Lintang, A. (2026) Bioremediation Journal DOI 10.1080/10889868.2026.2730316.

Linear alkylbenzene sulfonate (LAS), a widely used anionic surfactant, can adversely affect aquatic systems. This study compared the LAS-removal performance of the floating macrophyte *Lemna minor* and the submerged macrophyte *Hydrilla verticillata* using a 3 & times; 3 factorial design (0, 20, and 40 ppm LAS; 0, 5, and 10 days; n = 3). After 10 days, both plant-associated systems removed more than 80% of LAS. *L. minor* achieved the highest observed removal (90.12% at 40 ppm), compared with 81.90% for *H. verticillata*, but this performance came at the cost of greater biomass loss. Biomass declined by approximately 28% in *L. minor* and 25% in *H. verticillata* at 40 ppm, and biomass was negatively associated with removal efficiency (Spearman rho = -0.82 and -0.79, respectively; $p < 0.05$). Future work must incorporate abiotic/plant-free controls, increased replication, complete LAS mass balances, and analyses of microbial communities, biofilms, and transformation products, alongside mixed-species or staged systems. Validation using real detergent wastewater under flow-through or pilot-scale conditions is essential to assess treatment robustness, harvesting needs, and practical operational constraints.

DF comment: The authors described their plants material as "*L. minor* and *H. verticillata* were obtained from healthy laboratory cultures." Without identification of the plant species, the experimental results are not useful.

Boron attenuation using magnetically recoverable carboxymethyl cellulose-carbon nanotube beads and *Lemma gibba*: Adsorption mechanisms and toxicity buffering

Yakar, A; Baran, NY; Türker, OC. (2026) Journal of Environmental Chemical Engineering 14: 124845.

Boron contamination in aqueous environments presents a persistent remediation challenge, primarily because boron predominantly occurs as uncharged boric acid under near-neutral pH, limiting the efficiency of conventional treatment approaches. To address this limitation, a hybrid adsorption-phytoremediation system was developed by integrating magnetic carboxymethyl cellulose-carbon nanotube (CMC-CNT-Mag) nanocomposite beads with *Lemna gibba*, enabling coupled physicochemical and biological boron attenuation. To the best of our knowledge, this is among the first to integrate a magnetically recoverable, CMC-based nanocomposite for boron removal with the toxicity-buffering capacity of a living macrophyte. Among the synthesized composites, CMC-CNT-Mag exhibited the highest adsorption performance, with maximum theoretical capacity of 31.18 mg/ g. Kinetic and isotherm analyses were consistent with a chemisorption-dominated uptake involving heterogeneous, multilayer interactions. Regeneration experiments showed that the

removal efficiency declined from 33.24% in the first cycle to 10.00% by the fifth, indicating limited reusability while suggesting persistent boron retention within the sorbent. The magnetic recoverability of CMC-CNT-Mag also enabled straightforward separation from the aqueous phase. This configuration, the adsorptive phase, acted as a primary buffering component, reducing acute boron stress on *L. gibba* and supporting plant viability. The biological component accumulated boron up to 3340.33 mg/kg, with a maximum apparent bioconcentration factor of 49.58 at 8 mg/L, while the system achieved a maximum removal efficiency of 72.38% at 8 mg/L. These findings demonstrate that the CMC-CNT-Mag-*L. gibba* hybrid system combines nanocomposite adsorption, plant-mediated accumulation, and magnetic recovery for boron-rich industrial and mine-influenced waters.

A duckweed-red light system for phosphate removal and recovery from low phosphate polluted water coupled with nutrient-rich biomass production

Wei, CC; Hu, ZB; Liu, YQ; Zhao, LY; Xiao, JL; Gao, Y; He, KZ; Jin, YL; Yi, ZL; Fang, Y; Xiao, WJ; Jian, XB; Zhao, YG; Zhao, H. (2026) Journal of Environmental and Chemical Engineering 14: 124741.

Eutrophication remains a major global challenge, particularly because algal blooms can occur at very low inorganic phosphate (Pi) concentrations. However, efficient removal and recovery of low-concentration Pi remain technically challenging. To identify suitable plant material for low-Pi water treatment, we first screened five duckweed strains with different endogenous Pi-P statuses. Among them, *Lemna minor* 0258 (Lm0258) with endogenous Pi-P ≤ 0.2 mg g^{-1} fresh weight showed the highest Pi-P removal and was selected for subsequent experiments. Based on this selected material, we developed a Duckweed-Red Light (DRL) system using red-light supplementation at an average photosynthetic photon flux density of approximately 110 μmol m^{-2} s^{-1}. Under laboratory conditions, the DRL system reduced Pi-P from similar to 0.4 to similar to 0.01 mg L^{-1}, achieving 97.65% removal, with 98.14% of the removed P recovered in harvested duckweed biomass, and a treatment capacity of 333.12 L m^{-2} d^{-1}. Under field conditions, Pi-P decreased from similar to 0.35 to similar to 0.08 mg L^{-1}, with 76.65% removal, 56.23% recovery of removed P, and a treatment capacity of 178.71 L m^{-2} d^{-1}. Laboratory and field biomass productivities reached 8.49 and 7.19 g m^{-2} d^{-1}, respectively. Red-light treatment was associated with higher expression of Pi-starvation-response and starch-biosynthesis genes and increased related enzyme activities. The DRL system enables low Pi-P water purification while supporting P recovery and production of starch-and protein-rich duckweed biomass. Further evaluation under seasonal and water-quality variability is needed to define its long-term stability and field applicability.

Evaluation of environmental parameters on remediation efficiency: Mathematical modeling for coliform removal

León-Suárez, R. (2026) Enfoque Ute 17: 1-12.

Phytoremediation using aquatic macrophytes is a sustainable strategy for the removal of coliforms and *Escherichia coli* in wastewater, although its efficiency is subject to variations in environmental parameters such as pH, temperature, and total dissolved solids (TDS). This study developed a first-order kinetic mathematical model, incorporating an environmental penalty mechanism based on a Gaussian function to dynamically adjust the removal coefficient (k) according to physicochemical conditions. Six species of macrophytes (*Azolla, Salvinia, Pistia, Ceratopteris, Spirodela*, and *Eichhornia*) were experimentally evaluated alongside a positive control (*Lemna*) and a negative control (no plant), with daily measurements of bacterial concentrations and environmental parameters over seven days. The results showed that the model adequately described removal trends in several treatments, especially for *E. coli*. The environmental penalty allowed pH, temperature, and TDS effects on k to be evaluated, producing more conservative predictions. *Ceratopteris* and *Azolla* showed the best fits for coliforms (R-2 > 0.99), while *Spirodela, Salvinia*, and *Eichhornia* exhibited low performance. It is concluded that the model is a useful tool for predicting removal efficiency under variable environmental conditions, though further research into species-specific thresholds and microbial variables is required to optimize its applicability in real-world scenarios.

Phytotoxicity

Temperature-dependent toxicity of insecticide chlorantraniliprole (Altacor®) in the aquatic primary producers *Raphidocelis subcapitata* and *Lemna minor*

Marinho, AF; Antunes, SC; Rodrigues, S. (2026) Aquatic Botany 207: 104040.

Anthropogenic activities and climate change are intensifying multiple environmental stressors that may interact and threaten aquatic ecosystems. Among these, pesticides represent a major concern due to their frequent detection in surface waters. This study evaluated the combined effects of the insecticide chlorantraniliprole (CAP; the active ingredient of Altacor (R)), and elevated temperature on two standard aquatic primary producers: the microalgae *Raphidocelis subcapitata* and the macrophyte *Lemna minor*. Organisms were exposed to a range of CAP concentrations (0.007-10.5 mg L^{-1}) at 24 ± 1°C (standard condition) and 28 ± 1°C (projected climate change scenario). Relative growth rates, photosynthetic pigments (chlorophylls and carotenoid contents), and oxidative stress biomarkers (catalase activity and lipid peroxidation) were measured. Temperature significantly modulated CAP toxicity, with interactions between the two factors for most endpoints evaluated. *R. subcapitata* showed minimal growth inhibition but significant alterations in pigment content, whereas *L. minor* exhibited moderate to high physiological and biochemical effects, particularly enhanced catalase activity and lipid peroxidation at 28°C. An increase in temperature generally intensified oxidative stress and pigment imbalances. Overall, these findings highlight significant temperature-dependent interactions between pesticide exposure and warming, posing risks to primary producers and, consequently, aquatic food webs. The results reinforce the need to integrate climate-related variables into environmental risk assessments and to promote more sustainable pesticide management under global warming scenarios.

Assessing the ecological toxicity of institutional greywater to *Lemna minor*

Sackey, LNA; Aidoo, IA; Adomako, B; Jaiteh, F; Josephine, G; Adjei, PA. (2026) Next Sustainability 8: 100528.

Greywater, generated from domestic activities such as bathing, laundry, and dishwashing, represents a significant portion of wastewater on university campuses. If discharged untreated, it poses ecological risks to aquatic ecosystems through contaminants, including nutrients, surfactants, and heavy metals. The greywater generated on the main campus of Kwame Nkrumah University of Science and Technology (KNUST) is not routed to a specific location before being released into the environment. This study, therefore, assessed the ecological toxicity of the greywater from residential halls at KNUST using *Lemna minor* (duckweed) as a bioindicator. This study provides novel insight into the ecological toxicity of untreated campus-derived greywater in Kwame Nkrumah University of Science and Technology by employing *Lemna minor* as a bioindicator to evaluate its potential environmental risks. Greywater samples were analyzed for physicochemical parameters and heavy metal concentrations, followed by bioassays to evaluate duckweed growth, chlorophyll content, and frond area across varying concentrations. Physicochemical results revealed that pH ranged from 6.3 to 7.2, while electrical conductivity and total dissolved solids showed wide-ranges. Heavy metals such as chromium (0.12 mg/L), lead (0.09 mg/L), and nickel (0.05 mg/L) were detected but within Ghana EPA limits. Duckweed growth showed concentration-dependent inhibition, with higher greywater concentrations (50% and 100%) suppressing increase frond number, chlorophyll content, and frond area. The median inhibitory concentration (IC_{50}) for chlorophyll inhibition was determined at 95.5 mg/L, indicating potential toxicity. The findings showed that untreated greywater can impair primary producers like duckweed, with potential cascading effects on aquatic ecosystems. There should be institutional greywater management policies, treatment interventions, and further research into long-term ecological impacts.

DF comment: The source of the duckweed was not identified and the method for its species identification is lacking in this report.

Europium toxicity in *Lemna minor* cannot be explained by phosphorus deficiency alone

Maturana, SA; Macfie, SM. (2026) Aquatic Toxicology 301: 108021.

The rapid expansion of mining for rare earth elements, including europium (Eu), has raised concerns about potential contamination of aquatic ecosystems. The aquatic macrophyte *Lemna minor* was used to test the hypothesis that Eu toxicity is caused by phosphorus deficiency. Chemical equilibrium modeling predicted one-to-one binding of Eu and phosphate in the growth medium, suggesting no soluble Eu ions and a 20% reduction in phosphate ions. The concentration of Eu used was not environmentally relevant; it was chosen for its potential to bind enough phosphate to elicit a deficiency response in the plants. Despite the predicted lack of soluble Eu, plants grown for 15 days in media with 1 mM Eu and 4 or 5 mM total phosphate accumulated 19.3 ± 3.2 or 27.5 ± 8.0 µg/g Eu, respectively. Symptoms of Eu toxicity included 34% fewer fronds, 45% smaller frond area and 35-55% reduced biomass relative to control. In contrast, plants given 80% phosphate to mimic

Eu-phosphate complexation were only 16-32% smaller than control plants. The activity of acid phosphatase in the growth medium increased by 44% relative to control for plants treated with Eu or 80% phosphorus, whereas activity was equal to control for plants treated with Eu and 120% phosphorus. This enzyme may have liberated both Eu and phosphate from the complex, which would explain the ability of Eu-treated plants to accumulate as much phosphorus as control plants. In conclusion, the impact of Eu toxicity on *L. minor* was greater than could be explained by Eu-induced phosphorus deficiency.

DF comment: The *L. minor* clone #490 used in this study was sourced from the Canadian Phycological Culture Center (CPCC). This clone was also propagated with the ID of 8434 via Anne Stomp. However, using TBP barcoding and whole genome sequencing, this clone was recently found to be a diploid hybrid of *Lemna japonica* (Michael et al. Nature Communication 2026). It may be of interest to confirm the *L. x japonica* identity of this clone in the CPCC collection by using TBP.

Ecological toxicity of illegal mining on selected rivers at Obuasi in Ghana using *Daphnia magna* and *Lemna minor*

Sackey, LNA; Quansah, YM; Aidoo, I; Mensah, EF. (2026) Environmental Toxicology 41: 1069-1085.

Illegal mining activities pose a growing threat to aquatic ecosystems through the release of toxic substances which affect the aquatic organisms within the ecosystem. This has become a growing concern because of the rise of illegal mining in certain parts of Ghana. Therefore, this research seeks to assess the effect of illegal mining on selected rivers at Obuasi in the Ashanti Region using *Daphnia magna* and *Lemna minor*. Obuasi was selected as the study area because it is an illegal mining hotspot. Three rivers were randomly selected from Obuasi; Kwabrafoso river (KB), Pompom river (PO), and Diewuo river (DE) to assess their level of toxicity to aquatic organisms. The heavy metals were analyzed using the Agilent 4210 Microwave Plasma Atomic Emission Spectrometer (MP-AES) and the physicochemical properties by using the OHAUS Starter 3100m. Duckweed (*L. minor*) and Daphnia (*D. magna*) were used as the test organisms following OECD 211 and OECD 202 protocols respectively. The pH of all river samples was alkaline (7.10-7.49) and within the World Health Organization (WHO) permissible limit. All river samples exceeded the WHO standard for Electrical conductivity and Turbidity. Kwabrafoso showed the highest concentrations of Zn (0.46mg/L), Pb (0.18mg/L), and As (0.62mg/L). Bioaccumulation showed Zn and As as the most bioavailable metals to *Daphnia*. IC_{10} and EC_{50} were measured in the toxicity bioassays for duckweed and *Daphnia*, respectively, with a toxicity gradient of PO < KB < DE ranging from (4.90-11.48mg/L) for IC_{10} and DE < PO < KB ranging from (15.15-31.9mg/L) for EC_{50}. All the river samples showed confirmed toxicity, indicating the impact of illegal mining on aquatic ecosystems. This highlights the need for monitoring and strong regulations to protect aquatic ecosystems.

DF comment: The duckweed used in this work was collected in the wild from a stagnant pond near a botanical garden of a university in Ghana, Africa. No effort to validate its species designation as *L. minor* and no effort to derive a clonal population from the collected duckweed was reported.

Unraveling the responses of methylparaben on bioconcentration, gas exchange regulation, photosystem II dynamics and scavenging activity in *Lemna trisulca*

Elbasan, F; Yildiztugay, A; Tarhan, I; Ozfidan-Konakci, C; Yildiztugay, E. (2026) Environmental Research 309: 125774.

Methylparaben (MP) is a pseudo-persistent emerging contaminant ubiquitously detected in aquatic environments, where its endocrine-disrupting activity threatens both ecosystem function and human health, driving interest in sustainable phytoremediation approaches using submerged macrophytes such as *Lemna trisulca*. This study presents a comprehensive assessment of physiological, biochemical, and remediation responses of *L. trisulca* exposed to MP at Control, 10, 50, 100, 250, 500, and 1000 mg L^{-1} for 14 days. Tissue MP accumulation, quantified by HPLC, showed a dose-dependent increase from 6.98 to 496.92 mg kg^{-1} fresh weight, while the bioconcentration factor decreased from 9.97 to 0.58 and removal efficiency declined from 93.6% to 14.9%, indicating efficient paraben removal at concentrations up to 50 mg L^{-1}, where high bioconcentration coincided with preserved physiological integrity. Relative growth rate remained unchanged at low doses but markedly decreased at 500 and 1000 mg L^{-1}, where photosynthesis was severely impaired and PSII reaction-centre inactivation was evident. Oxidative stress, reflected by H_2O_2 and lipid peroxidation markers, emerged progressively above 100 mg L^{-1}, defining a clear stress threshold. Antioxidant enzyme responses were dose-dependent and stressor-specific: catalase and peroxidase provided substrate-independent H_2O_2 detoxification across all doses, whereas GPX and GST activities, although elevated, were rendered functionally inefficient by progressive glutathione depletion at higher concentrations. At low MP doses, autonomous

ascorbate recycling within the ascorbate-glutathione cycle compensated for glutathione arm collapse and sustained cellular protection, whereas above 100 mg L^{-1} this compensatory capacity was lost, resulting in uncontrolled oxidative damage. These findings establish *L. trisulca* as a promising nature-based solution for paraben-contaminated waters and demonstrate that the functional integrity of the ascorbate-recycling arm of the ascorbate-glutathione cycle, sustained by adequate substrate availability, is a key biochemical determinant of phytoremediation performance under organic contaminant stress.

Differential sensitivity of *Lemna minor* and *Moina macrocopa* to polyethylene terephthalate (PET) microplastic fibers: hazard characterization and scenario-based risk assessment

Jayasinghe, VS; Samarakoon, T; Hemachandra, C. (2026) Aquatic Toxicology 301: 108023.

Polyethylene terephthalate (PET) microfibers present a common contaminant in freshwater environments, yet their toxicological sensitivity across distinct trophic levels remains poorly studied. This study assessed the comparative toxicity of PET microplastic fibers on the duckweed *Lemna minor* and the cladoceran *Moina macrocopa*. Healthy *L. minor* fronds were exposed to several PET fiber concentrations for seven days, and the frond number, frond area, root length, and chlorophyll a and b contents were determined. For *M. macrocopa*, an acute immobilization test was performed for 48 h, and the survival, growth, and heartbeat of the neonates were assessed. A chronic reproduction test was also conducted for 7 days using several sublethal PET fiber concentrations. Frond area was significantly reduced in *L. minor* at PET fiber concentrations ≥ 50 mg/L ($p < 0.05$). Average frond number was significantly reduced at ≥ 150 mg/L, while chlorophyll a and b contents were significantly reduced at 200 mg/L ($p < 0.05$), while root length was unaffected. Acute exposure of *M. macrocopa* significantly reduced survival, growth, and heartbeat at ≥ 5, >= 10, and ≥ 100 mg/L, respectively ($p < 0.05$). Chronic exposure significantly reduced total reproduction at ≥ 5 mg/L ($p < 0.05$). A dose-dependent effect was observed on other reproduction-related endpoints, although none were statistically significant. These findings demonstrate differential sensitivity of freshwater primary producers and consumers to PET microplastic fiber exposure, with reproductive effects in *M. macrocopa* occurring at lower concentrations than most growth- and physiological responses in *L. minor*. An initial scenario-based exploratory ecological risk assessment indicated negligible-to-low current ecological risk under surface water concentrations, though long-term microfiber accumulation and chemical co-exposure pose ongoing ecological concerns.

DF comment: The duckweed used in this work originated from a pond of a home garden in Sri Lanka. No effort to validate its species designation as *L. minor* and no effort to derive a clonal population from the collected duckweed was reported.

Comparison of herbicide responses between root re-growth in *Lemna aequinoctialis* and root development during turion germination in *Spirodela polyrhiza*

Lee, H; Han, T; Park, J (2026) Toxics 14: 791.

Root length is increasingly used as a rapid endpoint in duckweed ecotoxicology, yet biologically distinct assays are often treated as equivalent. Two 72 h root-length tests are now in use: root re-growth after excision in mature *Lemna aequinoctialis* fronds, and root development during germination of rootless *Spirodela polyrhiza* turions exposed directly to the test solution. Both begin from zero initial root length, but for different biological reasons. We asked whether they produce comparable herbicide responses. Thirteen herbicides were drawn from a screening campaign in which both assays were run under matched exposure conditions. Nine compounds returned quantified EC_{50} estimates (with the *L. aequinoctialis* coefficient of variation below 40%) and formed the primary comparison. EC_{50} ratios (*S. polyrhiza*/*L. aequinoctialis*) ranged from 0.75 to 430 (median 2.76). Five compounds agreed within about three-fold, whereas four compounds spanning several target-site classes diverged by 74- to 430-fold, and potency rankings were not significantly correlated (Spearman rho = 0.43, p = 0.24). For the common reference toxicant, the two assays differed by only about two-fold, and in the opposite direction; comparable reference-toxicant performance therefore did not predict comparable herbicide responses. The two assays should not be treated as interchangeable root-length tests. However, they differ not only in the biological origin of the measured root but also in species and developmental starting condition, and the present design cannot separate these factors; the observed differences therefore demonstrate assay-level non-equivalence without identifying its biological cause.

Bioaccumulation and physiological responses of common duckweed (*Lemna minor* L.) treated with legacy and emerging per-and polyfluoroalkyl substances (PFAS) under acute and chronic exposure conditions

Groffen, T; Drenckhan, W; Prinsen, E; Jansen, MAK (2026) Aquatic Toxicology 301: 108002.

Per- and polyfluoroalkyl substances (PFAS) are persistent and bioaccumulative contaminants of emerging concern in aquatic ecosystems, yet their uptake dynamics and physiological effects on macrophytes remain poorly understood. This study investigated PFAS bioaccumulation and physiological responses of *Lemna minor* exposed to seven PFAS compounds under both acute and chronic conditions. Longer exposure durations resulted in detectable accumulation of a greater number of PFAS, particularly at lower exposure concentrations. Concentration-dependent accumulation was observed, although the direction and magnitude of these relationships varied among PFAS types. PFBS showed the highest accumulation, with concentrations up to 8736 ± 5715 ng/g dw detected after 10 days of exposure to 100,000 ng/L. When expressed as bioconcentration factors (BCFs), an inverse relationship with exposure concentration indicated higher uptake efficiency at environmentally relevant concentrations. The highest BCF (6076 ± 898 L/g) was observed for PFBS after 20 days of exposure to 1 ng/L. Differences in accumulation and BCF values were attributed to functional group and chain length, which affect solubility and hydrophobicity. Despite measurable bioaccumulation, physiological responses were generally minor, as neither growth nor photosynthetic efficiency (Fv/Fm and Y(II)) showed consistent impairment. Growth stimulation was observed in a limited number of treatments, suggesting potential hormetic effects. Overall, these findings highlight that both exposure duration and concentration range should be considered when assessing PFAS bioaccumulation in macrophytes, and that integrating time-dependent accumulation into risk assessments is essential for accurately evaluating PFAS behaviour and ecological risks.

Integrated ecotoxicological assessment of metal nitrates in *Lemna minor* based on growth inhibition, biochemical responses, and species sensitivity comparison

Iachimov-Datcu, AD; Istrate, DM; Tobosaru, LM; Doczi, GG; Bulancea, CC; Roman, DL; Agachi, BV; Vulpe, CB. (2026) Toxics 14: 742.

Heavy metals in water are a global concern due to their persistence, toxicity, and bioaccumulation potential, highlighting the need to assess their effects on aquatic organisms. The ecotoxicity of eight potentially toxic metal nitrates (Cd (0.002 mg/L), Co (0.02 mg/L), Cr (0.02 mg/L), Cu (0.001 mg/L), Mn (0.04 mg/L), Ni (0.005 mg/L), Pb (0.002 mg/L), Zn (0.1 mg/L) up to 1000 mg/L for all metals) was assessed on the aquatic macrophyte *Lemna minor*, in a multilevel study. Growth responses, measured as frond number and average specific growth rate, were used to generate dose-response curves, but also to calculate EC_{50} (Median Effective Concentration) and ErC_{50} (50% Effect Concentration based on growth rate) values. Based on EC_{50}, toxicity followed the order Cd > Co > Ni > Zn > Cu > Cr > Pb > Mn, while ErC_{50}-based ranking was Cd > Ni > Co > Zn > Cu > Cr > Pb > Mn. Metals were classified as highly toxic (Cd, Ni; Co based on EC_{50}), moderately toxic (Co based on ErC_{50}, Zn based on EC_{50}), slightly toxic (Cu, Cr; Zn based on ErC_{50}, Pb based on EC_{50}), or practically non-toxic (Pb based on ErC_{50}, Mn). Biochemical responses regarding photosynthetic pigments, primary metabolites, and oxidative-stress-related enzymes were investigated, revealing physiological effects for most metals. Species sensitivity distribution-based comparison indicated that *L. minor* sensitivity varies across metals, ranging from typical to relatively low within the broader species sensitivity distribution. Although the toxicity of potentially toxic metals to *L. minor* has been extensively investigated, data on ErC_{50} values for several of the metals investigated in the present study remain limited, highlighting the need for further ecotoxicological research.

DF comment: The duckweed used in this study was reported as to have been obtained from a stock culture but no further information was given. This makes future comparison with other studies rather difficult. As such, it is unclear how the species identification was performed.

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.

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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).
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- 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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Please let us know via email to the Chair of ISCDRA, Dr. K. Sowjanya Sree: kssree9@bhu.ac.in