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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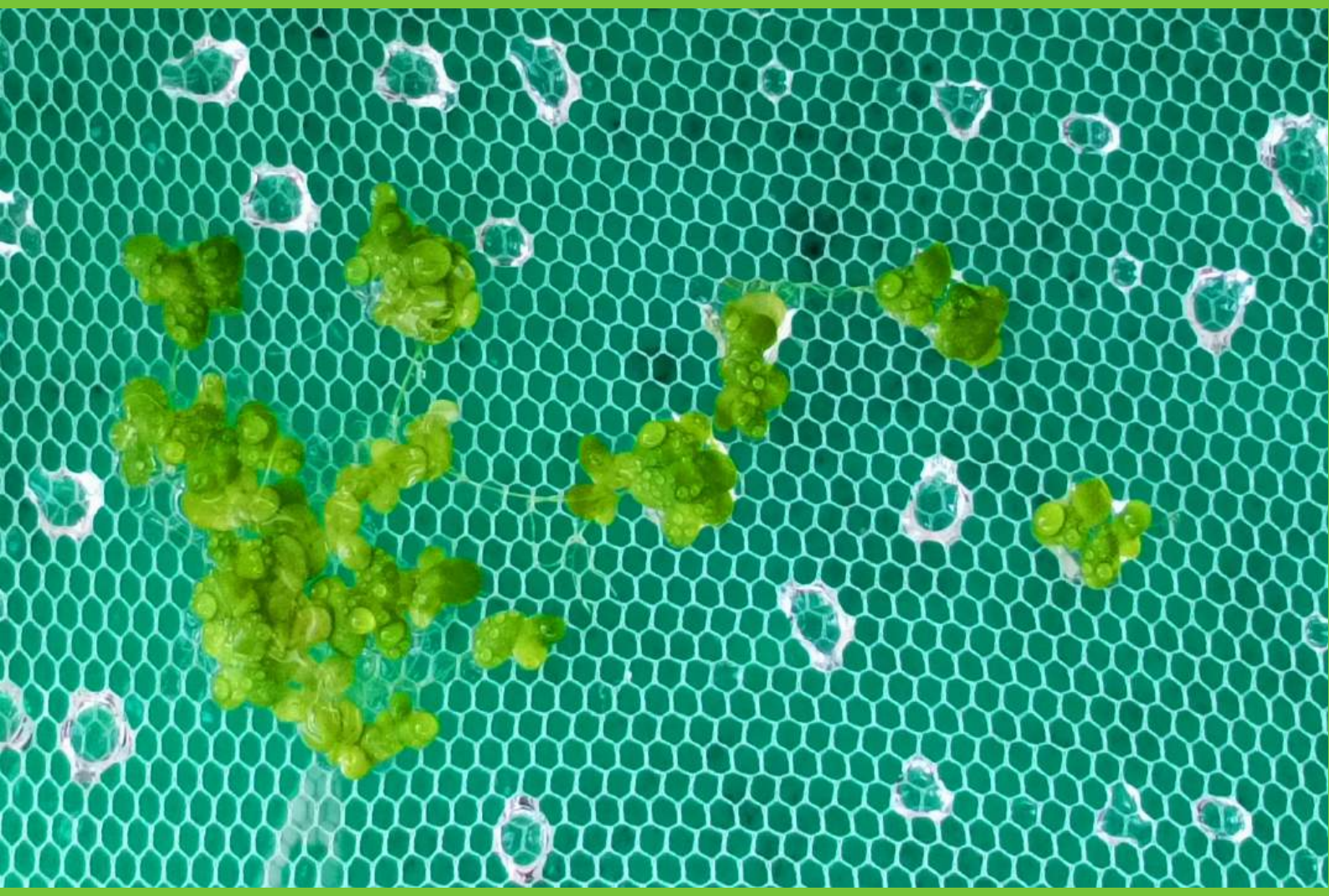

Lemna minor grown under fog-o-ponic conditions

Cover page

Lemna minor grown under fog-o-ponic conditions: *Lemna minor* "Blarney" grown on a layer of fabric under fog-o-ponic conditions (Photo courtesy: Prof. Marcel Jansen, University College Cork, Ireland)

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

Letter from the Editor:

July 30th, 2026

Dear Duckweed Community,

Greetings to all. The eagerly awaited time to prepare for the get-together at the Duckweed meeting in Italy is nearing. The organizers are keenly decking up the stage for all of us to have a successful meeting and a comfortable stay in Italy. The detailed conference announcement in this issue provides further information about the registration, accommodation and travel to the venue. It is suggested to go through this announcement and plan accordingly.

In this issue, two interesting commentaries are presented, from the authors of the articles selected as highlights in the previous DF issue. One of them highlights a new technique for cultivation of duckweeds, fog-o-ponics, which can become a part of applications like phytoremediation. The authors also discuss the road ahead dealing with several issues like minimizing contamination of the duckweed biomass, finally leading towards an automated fog-o-ponics system. The other commentary highlights the progress made in the field of transformation of duckweeds. The authors established a transformation platform using a specific clone of *Spirodela polyrhiza* and demonstrated its utility for genetic modifications via different techniques. This issue also offers an interesting experiment by students from University of Lethbridge, Canada which started with an accidental rip-off of the parafilm used for securing the duckweed cultures. Several of our laboratories use the micropore tape but this experimental data is a proof that we are on the right track. And, Students from the Weizmann Institute of Science highlight their research on using duckweed for molecular farming in their student spotlight in this issue.

Currently, we have a discussion forum open about the conditions required for optimal growth of the duckweed species, *Lemna trisulca*. Those who have experience growing this species are welcome to share it. All the pooled knowledge will be published in the next DF issue. Very soon, also a special issue on Duckweeds will be open for article submissions in the journal *Plants*. Contributions are welcome from several different fields of duckweed research and applications. The very useful Database section of the DF provides us with the updates in the field over the last three months.

The election of the members to the 7th International Steering Committee on Duckweed Research and Applications (ISCDRA) is now open until September 15, 2026. We encourage all of you to actively participate in the voting process in order to have a robust steering committee. The election procedure and the link to the e-ballot are detailed in this issue. Further, We are glad to have received four applications, from China, Belgium, Australia and Vietnam, who will be contesting for hosting the next ICDRA-2028. This voting for the venue will take place offline during the conference in Italy.

Looking forward to our Duckweed meeting,

Sincerely,
K. Sowjanya Sree
Chair, ISCDRA

Commentary: Fog-o-ponic cultivation of duckweed (*Lemna minor* L.): an innovative technique for phytoremediation

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An ecological prelude

"Lemnaceae are a family of free-floating freshwater plants". This sentence is commonly found in the introductory section of research papers but is actually not quite correct. Firstly, not all duckweed species float, for example *Lemna trisulca* is a duckweed species with a more submerged lifestyle. Secondly, in some habitats duckweed species can take on a partially terrestrial lifestyle, certainly not involving "free-floating". The inspiration for the work by Ivan Loaiza Alamo and Marcel Jansen (Loaiza and Jansen, 2026) goes back to observations of duckweed displaying such a semi-terrestrial lifestyle. Figure 1 shows *Lemna minor* as part of a vegetation comprised of mosses and liverworts, photographed in the south of Ireland. This observation of a semi-terrestrial lifestyle was not new as Prof Elias

Figure 1. *Lemna minor* growing under semi-terrestrial conditions in Tramore, Co. Waterford, Ireland. Red arrows point at clusters of *Lemna minor*.

Landolt already described duckweed growing on wet rocks of the Andean Yungas in Bolivia in 1998 (Landolt, 1998). The plants noted by Landolt grew at 2350 m a.s.l. in a cloud forest, on rough granitic rocks covered by a thin film of water and further kept wet by a spray of mist droplets from a waterfall. Initially, the duckweed species was referred to as *Lemna yungensis*, but later molecular studies identified the species as *Lemna valdiviana* (Bog *et al.,* 2020). It is clear, that *Lemna minor* in these habitats is not a "free-floating" aquatic plant, rather, the plant thrives thanks to a consistently wet substrate that supplies the plants with both water and nutrients. Undoubtedly, the relatively high humidity above wet substrate will further help to prevent plant desiccation. Previously, the role of humidity in preventing duckweed desiccation was explored as part of our studies on bird-mediated duckweed dispersal. These studies yielded quantitative data on the association between humidity (and temperature) and duckweed survival (Coughlan *et al.,* 2015). It was found that at a relative humidity (RH) of 38%, *Lemna minuta* taken out of the water column dies in just 2 hours. In contrast, at an RH of 85% *Lemna minuta* can be out of the medium for more than 8 hours without apparent negative impacts on survival (Coughlan *et al.,* 2018).

These varied observations on the role of humidity in keeping duckweed plants alive triggered the speculation that it should be possible to cultivate duckweed in a fine mist of droplets. Indeed, a well-developed, commercially relevant, horticultural technique is to grow plants in a mist of nutrient-containing droplets (Suganob *et al.,* 2024). Typically, only roots of horticultural crops are exposed to mist droplets, and cultivation takes place in the absence of soil. To test the hypothesis that duckweed can similarly be cultivated using mist, we borrowed a domestic humidifier and involved some enthusiastic graduate students. They showed that various duckweed species can be grown in a mist of half-strength Hutner's medium, although growth rates were poor.

Perhaps research would never have progressed beyond this point if it wasn't for the development of stacked duckweed cultivation systems. These systems were described in publications by Petersen *et al.* (2022) and Coughlan *et al.* (2023) and enabled cultivation of multiple layers of duckweed per area of floor space. While this development was critically important from the perspective of upscaling indoor duckweed production, the stacked cultivation systems had to be heavily engineered to hold the weight of trays with water on which duckweed floats. At this stage, the idea that duckweeds can instead be cultured on thin, light-weight, layers of mesh or fabric while being exposed to a continuous stream of nutrient-laden mist or fog became very attractive.

The foggy journey

Developing a duckweed fog-o-ponic system involved a variety of considerations such as identifying the most suitable type of fabric, light intensity, humidity, and fog-nutrient concentration. It was hypothesized that waterproof (hydrophobic) fabrics might be particularly suitable as they repel mist and stay moist longer than more absorbent fabrics. As a result of its water repellence, a thin layer of fluid is retained on top of the fabric, providing a permanently wet surface not unlike the natural soil surface in figure 1. This wet surface was hypothesized to facilitate duckweed survival and especially duckweed growth. To test this hypothesis, a simple fog experiment was set up, testing growth of *Lemna minor* on different hydrophobic and non-hydrophobic fabrics (Figure 2). In these pioneering experiments, fabrics were positioned over otherwise empty Magenta boxes, and *Lemna minor* colonies were placed on top of the fabrics. The Magenta boxes were placed within a larger container connected to a mister filled with half-strength Hutner's medium. A Valoya LED-unit (AP67 R-series, Valoya, Finland) was positioned above the system to generate Photosynthetically Active Radiation (PAR). It was quickly realised that the light intensity in the system was very low as the fog blocked light, with a decrease in light intensity of some 10-fold

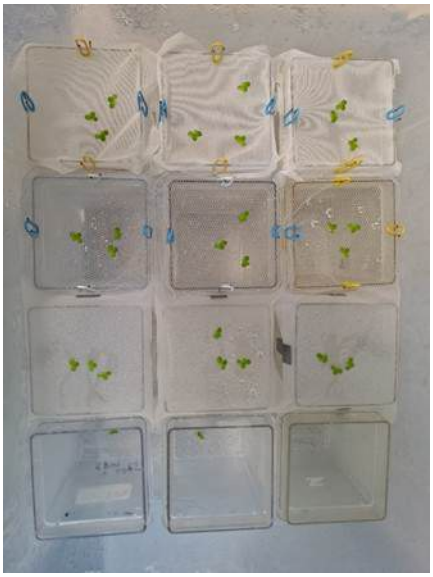

Figure 2. A simple set-up to test suitability of specific fabrics for fog-o-ponic cultivation of *Lemna minor*.

(from 50 µmol m^{-2} s^{-1} PAR to 4 µmol m^{-2} s^{-1} PAR). This was considered a real problem for fog-o-ponic cultivation of duckweed, especially as a multi-layer system was considered the final goal of this research project. In a multilayered system, both fog droplets and consecutive layers of fabric will block light. To alleviate the problem, it was decided to utilize fabrics that were transparent for light, albeit still hydrophobic. Separately, the light intensity was increased about ten times to provide enough PAR to facilitate growth.

A pertinent question was whether to run the mister continuously, or whether generation of fog can be done intermittently. In this respect we recognised two distinct considerations; does the RH remain high enough to avoid plant desiccation and is the supply of mist droplets sufficient to meet plant nutrient requirements. To explore this question, the mister was run continuously, on/off for 30 min : 30 min, or on/off for 15 min : 45 min. It was found that in all three operating modes the humidity levels remained high enough (> 95%) to avoid desiccation and no negative effects on *Lemna minor* survival were observed, not even when the mister was on a cycle of 15 min on / 45 min off. However, under the latter conditions plants did not grow well, suggesting that nutrient supply was sub-optimal. Attempts to increase the fog nutrient concentration were not successful, possibly as a result of osmotic stress, and therefore it was decided to cultivate plant under a continuous stream of mist droplets. Conversely, in situations where rapid growth is not required, i.e. retention of stock cultures, intermittent supply of fog combined with a low light intensity may be appropriate.

The overall aim of the work was to develop a high capacity, compact, stacked system that avoids the heavy engineering associated with stacked systems using trays with liquid. Following optimization of operational parameters such as the choice of fabric, misting regime, nutrient supply and light intensity, we decided to design a ten-layer stacked fog-o-ponic system. At this stage, additional design questions came up such as, should the fog inlet be at the top, the middle of the bottom of the system? And is the distribution of nutrients and light equal across culture layers? Nutrient concentrations across the system were compared by capturing fog droplets using petri dishes located throughout the stacked fog-o-ponic system. Overall, nitrogen and phosphorus concentrations were similar throughout the system, although light intensities decreased markedly towards the lower cultivation layers. Through trial-and-error we obtained an optimized multi-layer stacked fog-o-ponic system (Figure 3) that facilitated good growth of *L. minor* as detailed in "Loaiza, I. and Jansen, M.A.K., 2026. Fog-o-ponic cultivation of duckweed (*Lemna minor* L.): an innovative technique for phytoremediation. *International Journal of Phytoremediation*, pp.1-11".

Figure 3. A multi-layer stacked fog-o-ponics system for cultivation of *Lemna minor*. Left panel; a single layer of fabric, measuring 58 by 39 cm, covered by *Lemna minor* plants. Right panel; two containers that each contain some 10 layers of fabric for growing *Lemna minor*. Containers are closed at the top with a colour-less film to maintain humidity. A small drain is positioned in the bottom of each container.

What next; a fog-o-ponic system for the future

The current fog-o-ponic system has been used to produce duckweed biomass using 7, 14 or 21 cultivation cycles (Loaiza and Jansen, 2026). Yields of the top layers are comparable to those

achieved on liquid cultures; however productivity was low at the lowest layer of the stacked system, and this is most likely due to low PAR intensities. Thus, further engineering is required to improve the homogeneity of light supply, possibly involving the use of fibre optics fed in from the sites of the container. Use of light fibres has the added advantage of avoiding the exposure of light sources to fog. Furthermore, better control of mist droplet size, and especially creation of micro-droplets (1-10 µm), may improve homogeneity of nutrient supply (as used in horticultural applications), and allow for an intermittent fog exposure regime, in turn improving light penetration.

A second point for action concerns adaptation of the system for axenic operation. The current fog-o-ponic system does easily get contaminated with algal and microbial growth. Microbial contamination will make biomass unsuitable for human consumption, but also result in competition for nutrients, while algae can block light, particularly where fabric gets covered. There are two possible routes to address contamination issues; construction of closed fog-o-ponic systems less prone to microbial exposure, or operation of a standard fog-o-ponic system in a controlled clean room with rigorous air filtration, UV light, and sterilization. The current experiments were performed in an industrial hall with ample contaminants present. Ideally, future versions of the system have an autocleaning routine, facilitating periodical disinfection.

Perhaps the largest challenge for fog-o-ponic cultivation concerns harvesting of duckweed biomass, which should be automated for operation on an industrial scale. One possible design is a semi-automated process, whereby the fabrics turn over a roller to let the duckweed drop over the edge for harvesting at the bottom of the system.

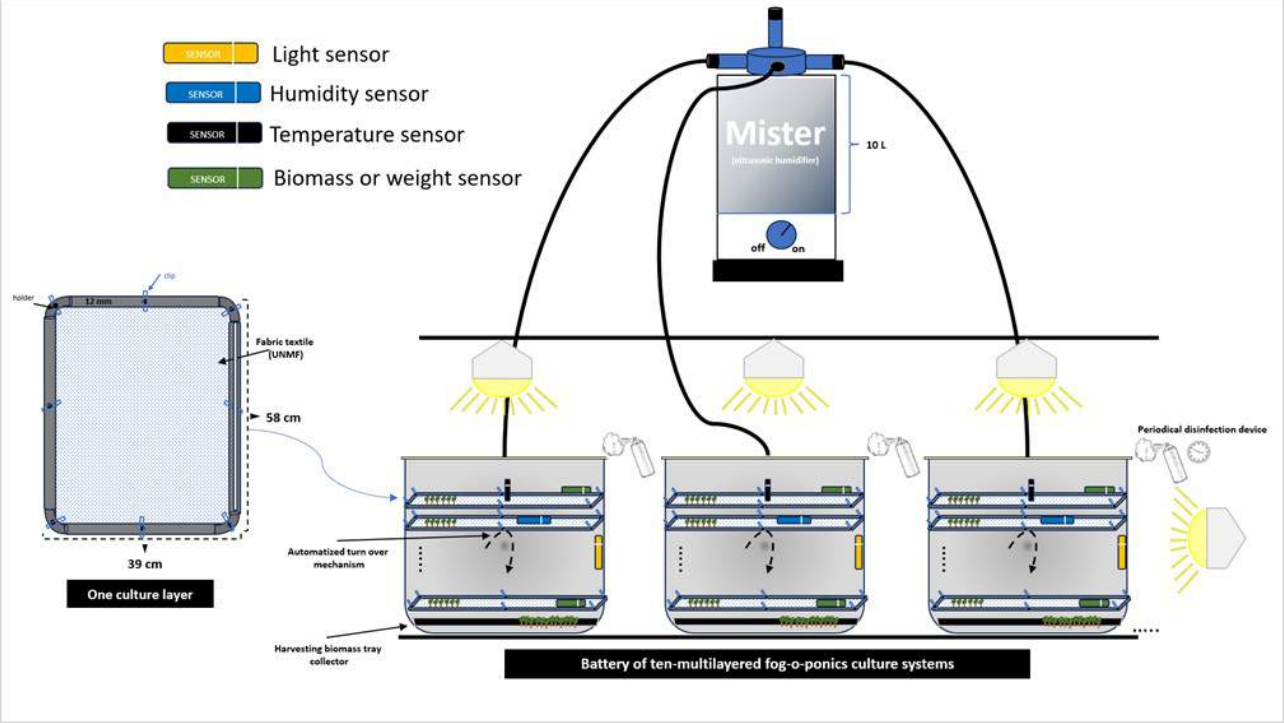

Figure 4. Schematic presentation of a future fog-o-ponic, multi-layered, system.

Finally, modern and up-to-date engineering will need to integrate sensors monitoring and controlling key operational parameters, e.g. light, humidity and nutrients. Ideally, sensors will also monitor biomass accumulation, i.e. weight or surface area, and instigate a harvesting protocol when a threshold biomass has been reached. The ultimate aim is to develop autonomous multi-layered, compact fog-o-ponic systems (Figure 4) for use on cruise liners, submarines, isolated research centres (e.g. Arctic or Antarctic) and other isolated places where these systems may supply clean protein-rich vegetables for human consumption.

In conclusion

Fog-o-ponics has been shown to be a realistic, relatively easy, cultivation method for *Lemna minor* and similar Lemnaceae species. This new method adds to the portfolio of existing cultivation methods and is particularly suitable for upscaling under indoor conditions where space requirements necessitate a multi-layered system.

Acknowledgements

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ICDRA-8: Announcement

Università degli Studi di Napoli Federico II

CNR IBBA Istituto di Biologia e Biotecnologia Agraria

8th International Conference on Duckweed Research and Applications

Model plant and novel crop, the thousand faces of duckweed

ICDRA 2026 | Portici (Naples), Italy | 28 September – 2 October 2026

Co-organized by: Department of Agricultural Sciences – University of Naples "Federico II" · Institute of Agricultural Biology and Biotechnology – CNR

Reminder: Registration & Abstract Submission

The deadline for **registration and abstract submission is 27 July 2026** — please check the conference website for any extension. Confirmation of oral and poster presentations will follow by **31 August 2026**. Registration must be completed *and paid* before an abstract can be evaluated.

Category	Registration fee
Students	400 €
Academics	500 €
Privates / Companies	700 €

Included in the fee: all lunches and coffee breaks · apericena · pizza dinner · conference dinner · guided tour of the Royal Palace of Portici, the conference venue itself, kindly offered by MUSA – Museum of Agricultural Sciences, which also hosts several conference spaces · guided visit to the Herculaneum excavations (included for students; € 30 supplement for other participants).

Registration: indico.unina.it/e/8thICDRA · **Abstract submission:** indico.unina.it/event/117/abstracts/

A contribution from FAO

We are pleased to announce that **Dr Jogeir Toppe**, Fishery Officer and aquaculture feed expert at the Food and Agriculture Organization of the United Nations (FAO, Rome), has accepted our invitation and will take part in the conference. His participation brings the perspective of an international organisation to the discussion of duckweed as an alternative protein source for feed and food systems, and we look forward to the exchange it will open with the duckweed research community.

Travelling to the venue

Piazza Garibaldi is the main hub of the city — whether you arrive by air or by rail, your journey to Portici begins there.

- **By air.** Flights land at Naples Capodichino Airport (NAP). Take the **Alibus** shuttle directly to Napoli Piazza Garibaldi.
- **By rail.** High-speed trains from Rome, Milan and other Italian cities (Frecciarossa, Italo) terminate at Napoli Centrale / Piazza Garibaldi.
- **From Piazza Garibaldi to the venue.** Take the **Trenitalia metropolitan line towards Salerno** and alight at **Portici-Ercolano**. Trains run roughly every half hour; the journey takes about 20 minutes, followed by a 10-minute walk. A single ticket costs approximately € 1.80–2.20 (station machines, Trenitalia app, or trenitalia.com). Daily commuters may find a weekly Unico Campania / TIC pass more economical.
- **By car.** A3 Napoli–Salerno motorway, Portici exit.

Please avoid the Circumvesuviana line. Although it also serves Portici and Ercolano, we strongly advise against it: ageing rolling stock, frequent delays and recurring comfort and safety issues make it unsuitable, particularly for travellers with luggage. **Use Trenitalia instead.**

Accommodation

Participants are asked to arrange their own accommodation. A curated list of options within approximately thirty minutes of the venue, grouped by zone and with direct booking links, is available at www.8thicdra.info/accommodation-options.

- **Zone 1 — Portici:** 5–15 minutes on foot from the venue; the closest option.
- **Zone 2 — Ercolano / Miglio d'Oro:** 15–25 minutes on foot along Via Roma.
- **Zone 3 — Naples city centre (Piazza Garibaldi):** approximately 30 minutes in total — a 20-minute train ride, followed by a 10-minute walk from Portici-Ercolano station. Please note that the last train from Portici departs at 22:42.

Early booking is strongly recommended.

Saturday excursion — Royal Palace of Caserta, 3 October 2026

A full-day excursion to the Royal Palace of Caserta, a UNESCO World Heritage site, is planned for **Saturday 3 October 2026**, including a guided tour of the palace and its gardens, and lunch. **The excursion is subject to a separate fee.** Registration and further details will be published on the conference website in the coming days. This excursion is distinct from the tour of the Royal Palace of Portici, which is included in the registration fee.

The Royal Palace of Caserta was commissioned in 1752 by Charles of Bourbon and designed by Luigi Vanvitelli, one of the greatest Italian architects of the eighteenth century, conceived to rival Versailles and Madrid. The palace has five floors and 1,200 rooms, including the Court Chapel, the Palatine Library and a theatre modelled after the Teatro San Carlo in Naples. Its gardens are among the last of the great European landscape compositions: a chain of fountains and basins extends for more than three kilometres from the palace to a waterfall in the woodland. The complex was inscribed on the UNESCO World Heritage List in 1997, together with the Vanvitelli Aqueduct and the silk-manufacturing complex of San Leucio.

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On behalf of the Organising Committee, 8th ICDRA

DUCKWEED FORUM
ISCDRA International Steering Committee on Duckweed Research and Applications

Let ‘er rip: Accidental rips in sealing film inspire a lab exercise investigating the role of gas exchange in duckweed population growth

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Introduction

Duckweeds are a valuable study system in a wide variety of fields (Lam, 2015; Acosta et al., 2021), including: biochemistry (Klein et al. 2025); genetics, genomics, metabolomics, and microbiomics (Wang et al., 2014; Van Hoeck et al., 2015; Acosta et al. 2020); development (Hillman, 1976; Cox et al., 2022); ecology and evolution (Laird and Barks, 2018); ecotoxicology (Wang, 1990; Ziegler et al. 2019); bioremediation (Ziegler et al., 2016; Ekperusi et al. 2020); and human food, animal feed, and biofuel production (Bhanthumnavin and McGarry, 1971; Cheng and Stomp, 2009; Cui and Cheng 2014). Due to their small stature, ease of cultivation, and rapid growth, they are also well suited for exercises in the secondary school and undergraduate laboratory (e.g., Robinson, 1988; Appenroth, 2015a, b, 2016a, b; Science and Plants for Schools, 2026). One of us (VCT), in her capacity as a Graduate Teaching Assistant in our department’s Research Internship Concentration (RIC), has used the duckweed *Lemna minor* L. to demonstrate principles of experimental design and execution for undergraduate students. In one such demonstration, students serendipitously noticed that populations of *L. minor* appeared to grow much more rapidly when the paraffin-based sealing film (hereafter ‘Parafilm’) used to seal their growth vessels was accidentally overstretched and ripped, compared to when it remained intact. The students hypothesized that this was due to poorer gas

exchange in vessels with intact Parafilm; in particular, they focused on the potential role of CO_2 depletion and the subsequent inhibition of photosynthesis and growth (Mueller et al. 1977; Andersen et al. 1985; Mohedano et al. 2016).
We followed up the students' 'accidental experiment' with a true randomized experiment in which we compared *L. minor* populations grown in vessels that were sealed in a manner that either inhibited or freely allowed gas exchange. Our aim for this study was to provide a low-cost, accessible model for secondary and undergraduate instructional labs, demonstrating how a fortuitous observation can drive formal inquiry into fundamental physiological processes.

Methods

We selected 40 *L. minor* plants from a stock culture (Canadian Phycological Culture Centre strain CPCC 492; originally collected from Elk Lake, BC, Canada, 48° 31'30" N, 123° 23'18" W; GenBank accession numbers for DNA barcoding primers *atpF-atpH* and *psbK-psbI* are MG000373 and MG000446, respectively). We placed these plants individually into petri dishes (nominal diameter × height: 60 mm × 15 mm; 25384-090, VWR, Radnor, PA, USA) containing 10 mL of quarter-strength (0.8 g L^{-1}) Schenk and Hildebrandt liquid growth medium (cat. no. S6765, Sigma-Aldrich, St. Louis, MO, USA). We replaced the growth medium in the dishes weekly. We placed dishes across two wire trays in a randomized manner. To grow the plants, we placed them on a growth shelf, with plants located 23.5 cm under a light fixture (cat. no. FLT46, Agrobrite Hydrofarm, Petaluma, CA, USA) with six 122-cm grow bulbs (T5, 54 W, 6400 K). We utilized a 15:9 (light:dark) photoperiod, and measured the photosynthetically active radiation (PAR) at 156.2 µmol m^{-2} s^{-1} using a Hobo Micro Station data logger and PAR sensor (Hoskin Scientific, Edmonton, AB, Canada). The mean temperature of liquid in the petri dishes was 26.9 °C. We randomized tray position daily.
We performed a pre-experiment procedure to ensure genealogical homogeneity among our experimental subjects, with the aim of mitigating any effects of parent or recent ancestor age at the time of offspring detachment (Barks and Laird, 2015). To this end, we marked the initial 40 'progenitor' plants with a dot of dilute India ink, and observed their lineages daily over multiple clonal generations, to obtain descendant 'focal' plants with which to found our populations. We selected the first offspring produced by each progenitor plant as it detached from its parent plant. We marked that offspring, then discarded its parent (the progenitor). We repeated this retain-discard process four additional times on each subsequent offspring plant to obtain a focal plant that was known to be a first-born plant, as well as the descendant of ancestors that were also first-born plants, back through three clonal generations.
On the day the focal plant detached from its parent, we placed it into a clear glass jar (S-21739, ULine, Pleasant Prairie, WI, USA) that contained 200 mL of the same growth medium previously described. Half of the jars (n = 20) were randomly assigned to be sealed with Micropore medical tape (cat. no. 1530-3, 3M, St. Paul, MN, USA), that allowed the free diffusion of gases through it, but not liquids or contaminating microorganisms (hereafter 'Micropore treatment'). The other half (n = 20) were sealed with a layer of Parafilm M paraffin-based sealing film (cat. no. HS234526B, Heathrow Scientific, Vernon Hills, IL, USA), which allowed much less diffusion of gases through it (hereafter 'Parafilm treatment'). The Micropore tape was more opaque than the Parafilm, so we placed a layer of Micropore tape on top of the Parafilm layer, such that both treatments had similar shading of the plants in the jars. The mean PAR in the Micropore treatment (mean ± SEM = 94.1 ± 5.0 µmol m^{-2} s^{-1}) was not significantly different from that in the Parafilm treatment (96.9 ± 2.1 µmol m^{-2} s^{-1} ; Wilcoxon rank-sum test, W = 11, p = 0.84, n = 5 per treatment). We marked the level of the surface of the medium on each jar, to observe any evaporation that occurred. Then we placed each jar into a randomized position on the same growth shelf on which the petri dishes were placed. Shelf air temperature was measured daily, with a mean value of 24.1 °C throughout the experiment. The mean growth medium temperature in the Micropore treatment (28.8 ± 1.0 °C) was not significantly different from that in the Parafilm treatment (28.4 ± 1.3 °C; Wilcoxon rank-sum test, W = 7.5, p = 1.0, n = 4 per treatment).
After founding, we allowed the populations to grow for 21 days before harvesting them. At this time, we marked the surface level of the medium, and measured the distance between the initial and final

marks. We collected each population into a pre-weighed aluminum weigh boat, and dried it in an oven at 60 °C until a constant weight was achieved. We then weighed the populations to obtain their dry mass.

Our initial sample size was reduced due to a deviation from the experimental protocol that caused three jars to be harvested late, and thus have an increased growth time; these populations were excluded. In addition, one population did not have an initial mark to measure the surface level of medium against, and so does not have a measurement of change in surface level; and one population was lost during the drying phase, and thus does not have a mass measurement. The final sample size was $n = 18$ for each treatment, for both measurements.

Data and R code (version 4.4.0; R Core Team, 2024) are archived at the Federated Research Data Repository (Chmilar et al. 2026).

Results and discussion

Populations in the Micropore treatment were significantly larger after three weeks' growth than populations in the Parafilm treatment (Fig. 1a, b; Wilcoxon rank sum test, $W = 324$, $p = 3.22 \times 10^{-7}$, $n = 18$ per treatment). Additionally, populations in the Micropore treatment showed significantly greater decreases in growth medium depth compared to populations in the Parafilm treatment (Fig 1c; Wilcoxon rank sum test, $W = 324$, $p = 2.15 \times 10^{-7}$, $n = 18$ per treatment).

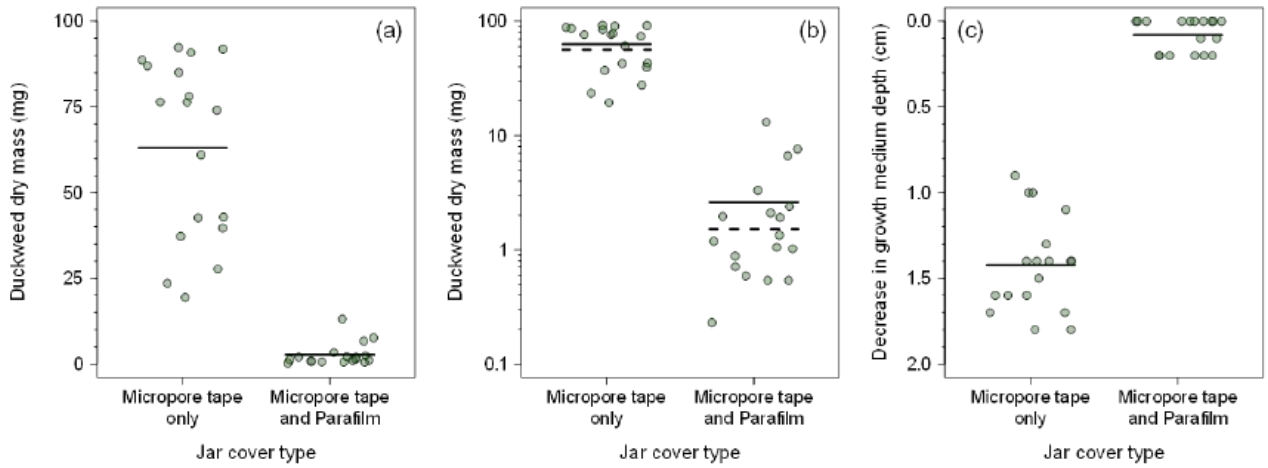

Fig. 1. (a, b) Duckweed dry mass in mg versus jar cover type, and (c) decrease in growth medium depth in cm versus jar cover type. (a) and (b) show the same data on an arithmetic vertical axis in (a) and on a logarithmic vertical axis in (b) – the latter to facilitate visualization of variation in dry mass in the 'Micropore tape and Parafilm' jar cover type. Note the reversed vertical axis direction in (c). Solid horizontal line segments are arithmetic means. Dashed horizontal line segments are geometric means (in (b) only, befitting the logarithmic axis).

Our results are consistent with the RIC students' hypothesis that the facilitation of gas exchange – in particular, the replenishment of access to CO_2 – explains why their original *L. minor* populations grown in vessels with ripped Parafilm grew at a faster rate than those grown in vessels with intact Parafilm. Could there be alternative explanations? Certainly! For example, could the greater evaporation in the Micropore treatment have led to more nutrient-concentrated growth medium, and therefore faster population growth? This seems unlikely given that quarter-strength Schenk and Hildebrandt growth medium has high enough N and P concentrations such that small increases probably would not affect population growth rates appreciably (Pasos-Panqueva et al. 2024). Nevertheless, this alternative explanation could be tested by crossing the current pair of jar cover-type treatments with another pair of treatments for which jar volume was topped-up daily with plain water, thereby preventing the artificial concentration of nutrients caused by evaporation. In following these types of 'future directions' – among others such as the roles of higher humidity and ethylene

accumulation in better-sealed vessels (Ku et al., 1970; Färber et al., 1986) – lab studies like this one could help engage students with the finer points of experimental design and the iterative nature of science.

This study highlights a successful transition from anecdotal observation to empirical data, a critical step in the authentic scientific process that is often missing from 'cookbook' style instructional labs (Brownell et al. 2012). More broadly, we wholeheartedly endorse and recommend the use of *L. minor* and other duckweeds in secondary and undergraduate research labs and demonstrations.

Acknowledgements

We thank the University of Lethbridge Research Internship Concentration students (Winter 2025 semester) for their curiosity. This experiment was funded by a Natural Sciences and Engineering Research Council (Canada) Discovery Grant to RAL (grant no. RGPIN-2021-02967).

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Discussion Forum: How to cultivate *Lemna trisulca* L.?

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The species *Lemna trisulca* (Figure 1) prefers cooler regions but is distributed over a huge area from North America, Europe, Africa, Northern and Western Asia, Southern and Eastern Asia, to Australia (Landolt, 1986). It is submerged in water, I saw it in many countries, e.g. in Germany, Netherlands, Poland, Ireland and Albania. It survives cold winters just under a layer of ice without being trapped within the ice layer. Thus, this species is by far not seldom. Moreover, it belongs to the few species of Lemnaceae that can be recognised easily even directly in nature. Nevertheless, it is the only species, which I could not induce to propagate quickly. In most cases growth is stunted although the fronds remain alive. As a consequence, I could not sterilize this species because it did not regenerate after treatment with bleach.

Figure 1: *Lemna trisulca* L.

I did not investigate the influence of external factors systematically. However, over the time, I tested several conditions: temperature of 17 °C instead of 25 °C, light intensity of 30 instead of 100 µmol m^{-2} s^{-1}, different nutrient media like N-medium, according to Steinberg, Murashige-Skoog, Schenk-Hildebrand, and Hoagland (cf. Appenroth, 2015). Moreover, I diluted the N medium (0.5 or 0.1-fold) and even diluted specific components, especially the nitrate and phosphate concentrations – all without success.

As far as I am aware, the relative growth rates were not really measured. Landolt (1957) mentioned that growth rate is low and that organic supplements are required for mixotrophic growth.

I am therefore addressing the duckweed community, asking whether someone has positive experience in cultivating *L. trisulca*. This would be interesting for many duckweed enthusiasts and researchers.

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Commentary: Unlocking the Giant Duckweed: Establishing a New Model for Plant Biology

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Duckweeds possess many of the characteristics that define successful model plants: rapid clonal propagation, simple morphology, and short generation time. As monocots, they are also evolutionarily close to major crops such as wheat, maize, and rice, making them attractive systems for both fundamental and applied research. Despite these advantages, duckweeds have never achieved the widespread adoption of established model plants. A major obstacle has been the lack of a robust and reproducible genetic toolkit. Although genetic transformation and genome editing have been reported for several duckweed species (Chhabra et al., 2011; Vunsh et al., 2014; Yang et al., 2018; Liu et al., 2019), many protocols remain slow, genotype-dependent, or difficult to transfer across laboratories. Consequently, duckweeds have remained promising but underutilized experimental systems (Tek and Villot, 2026).

Among duckweeds, the giant duckweed (*Spirodela polyrhiza*) stands out as an especially attractive model species. It combines exceptionally fast growth with a compact diploid genome —the smallest and simplest among duckweeds (Wang et al., 2014)— a comparatively large frond surface, and an exceptionally low spontaneous mutation rate (Xu et al., 2019). In addition, *S. polyrhiza* possesses a naturally reduced RNA-directed gene-silencing machinery (Dombey et al., 2025), which may contribute to more stable and long-lasting transgene expression. Together, these characteristics make *S. polyrhiza* a particularly promising experimental platform—a "yeast-like" model system for plant biology.

Despite its promise, *S. polyrhiza* lacked one key feature shared by successful model systems: a reliable and reproducible genetic toolkit. Our study, published in *New Phytologist* (Barragán-Borrero et al., 2026), addresses this long-standing bottleneck by establishing a robust, reproducible, and scalable platform for genetic transformation and genome editing in *S. polyrhiza* (Figure 1). The method was developed through a collaborative effort involving three independent laboratories, with Arturo Mari-Ordonez's laboratory leading its development and optimization. Together, we identified a transformable genotype, SP162, that supports *Agrobacterium*-mediated transformation of calli and subsequent regeneration of whole plants. Importantly, calli can be maintained in culture for at least one year, increasing the scalability and throughput of the transformation platform. Using this platform, we demonstrated transient and stable transgene expression, CRISPR-Cas9-mediated gene knockouts, and RNA interference-mediated gene knockdowns, thereby establishing a versatile

genetic toolkit for duckweed research. In addition, transient transgene expression proved to be remarkably long-lasting, likely reflecting the reduced RNA-mediated gene-silencing machinery of *S. polyrhiza* (Dombey et al., 2025). This characteristic may make the species particularly attractive not only for basic research but also for biotechnological applications requiring sustained transgene expression.

The impact of the developed genetic toolkit extends well beyond molecular biology. These tools enable researchers to investigate the genetic basis of species interactions, community dynamics, and adaptation; unravel the regulation of clonal growth, dormancy, and flowering; and engineer duckweeds as sustainable production platforms for proteins, specialized metabolites, and other high-value products. By enabling precise genetic manipulation, they allow hypotheses to be tested directly rather than inferred from comparative or correlative studies, bringing duckweeds much closer to the experimental versatility of established model systems.

To help the research community adopt this platform, we made SP162 publicly available, having deposited the strain in collections and laboratories in Germany, Italy, Austria, USA, and China. In addition, we released a near-chromosome level genome assembly of SP162 together with an open-access web server for genome exploration, gene discovery, and CRISPR guide design (https://agxu.uni-mainz.de/SP162/). We are also making an expanding collection of transgenic *S. polyrhiza* lines publicly available (see https://plant-evolutionary-ecology.uni-mainz.de/#duckweed-engineering for a list of available lines). Researchers interested in additional transgenic lines or collaborative development of new genetic resources are encouraged to contact us. Ultimately, our goal is not only to expand the genetic toolkits, but also to foster an open and collaborative community that accelerates the use of duckweeds as model systems for plant biology and biotechnology.

Although our transformation platform represents an important step forward, significant challenges remain before duckweeds can fully realize their potential as plant model systems. Most notably, transformation still depends on callus induction, selection, and plant regeneration, a process that takes several months and can lead to somaclonal variation, epigenetic reprogramming, and regeneration-associated growth defects (Krishna et al., 2016; Larkin PJ & Scowcroft WR,1981; Miguel & Marum, 2011). Developing faster transformation methods and, ultimately, regeneration-independent transformation strategies should therefore be a major priority for the field, whether through improvements to tissue-culture protocols or entirely new approaches. A second challenge is the limited number of transformable genotypes. Additional transformable genotypes representing diverse genetic backgrounds will broaden the applicability of duckweeds for both research and biotechnology. Finally, regenerated plants frequently exhibit reduced growth and smaller frond size compared with their wild-type counterparts. Understanding and overcoming these transformation-associated effects will be essential, both to improve experimental reproducibility and to realize the full biotechnological potential of duckweeds.

Beyond improving transformation efficiency, the next challenge is to expand the duckweed biotechnology toolbox. One important goal is to achieve transgene-free edited plants. Current CRISPR-based approaches typically integrate the editing machinery into the nuclear genome. In sexually reproducing plants, these transgenes can be removed through crossing, leaving only the desired edit (He and Zhao, 2019). In *S. polyrhiza*, however, this strategy is largely precluded by the species' predominantly clonal reproduction and infrequent flowering (Khurana and Maheshwari, 1986). Developing efficient transient editing systems or gaining better control over sexual reproduction would therefore represent a major advance for both basic research and biotechnology.

Another important frontier is plastid engineering. Compared with nuclear transformation, plastid engineering enables precise DNA integration through homologous recombination, exceptionally high levels of recombinant protein accumulation, and natural transgene containment through the maternal inheritance of plastids (Bock, 2015). Establishing plastid transformation in duckweeds would therefore greatly expand their potential as platforms for both basic research and molecular farming.

For decades, duckweeds have offered enormous potential as experimental systems, yet they have lacked the genetic toolkit needed for widespread adoption (Tek and Villot, 2026). Our work represents an important step towards closing this gap by providing a standardized transformation

platform, demonstrating modern genome-editing approaches, and establishing open genetic and genomic resources for the community. Equally important, the study illustrates how collaborative efforts across multiple laboratories and open communications in the duckweed community can overcome long-standing technical barriers and establish shared resources that benefit an entire research field. We hope these advances will encourage researchers to use duckweeds as model systems, while inspiring the development of new transformation and genome-editing approaches. By continuing to develop these resources together as a community, we believe duckweeds can become a true powerhouse of modern plant biology and biotechnology.

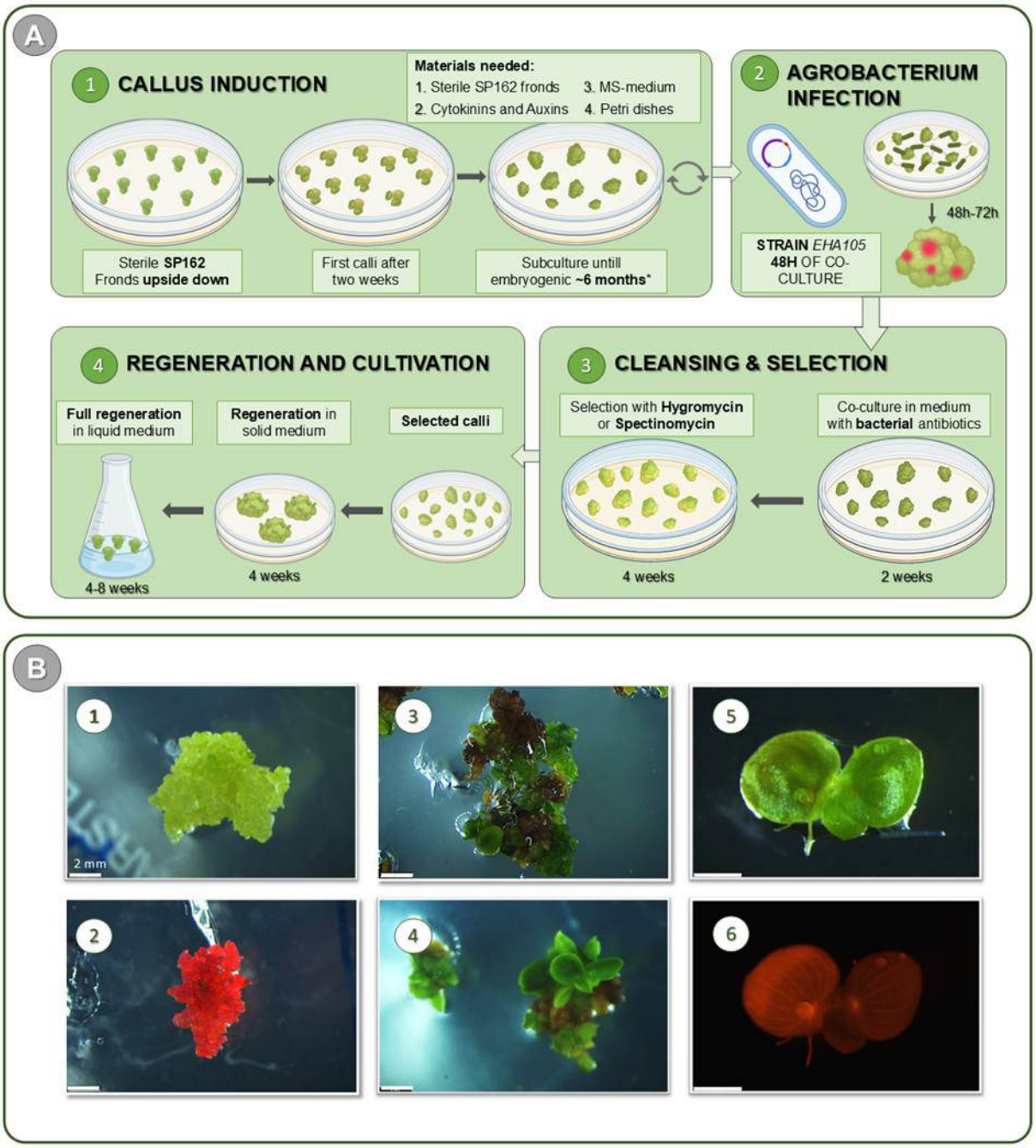

Figure 1. Standardized genetic transformation and regeneration for *Spirodela polyrhiza* SP162.

(A) Simplified schematic of the *Agrobacterium*-mediated transformation protocol optimized for *S. polyrhiza*. Sterile fronds of the SP162 genotype are placed abaxial side (upside down) on induction medium to produce embryogenic callus (1). Once obtained, this embryogenic callus culture can be subcultured and propagated for years, providing a continuous, long-term source of transformable tissue that bypasses the initial induction period. Following callus preparation, the protocol

proceeds through *Agrobacterium* infection and co-cultivation (2), tissue cleansing and antibiotic selection (3), and subsequent regeneration of transgenic tissue into whole fronds (4). Scale bar = 2 mm.

(B) Key experimental stages of *S. polyrhiza* SP162 transformation and marker expression: (1) Wild-type (WT) SP162 embryogenic callus; (2) Transgenic SP162 callus stably overexpressing the DsRed fluorescent reporter; (3, 4) Transformed calli during active organogenesis and frond regeneration; (5) Fully regenerated transgenic DsRed plant under standard white (common) light; (6) The same regenerated plant under an RFP (Cy3) filter.

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Special Issue Information

Dear Colleagues,

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

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 a 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 are now goinng to launch a third volume of the Special Issue "Duckweed: Research meets Applications III", along the lines of the 8th International Conference on Duckweed Research and Applications which will be held in Portici (Naples), Italy from 28 of September to 2nd of 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 also encourage reports on large-scale cultivation and applications as well.

Guest Editors

Dr. Viktor Oláh,
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Election of the 7th ISCDRA: Now open

Election of members to the 7th International Steering Committee on Duckweed Research and Applications is now open until 15 September, 2026.

Link to the e-ballot: https://forms.gle/JUS5GdPdszTUwi8S7

Eligibility for voting: You must belong to at least one of the categories listed below to be eligible to Vote.

• Attended any of the past two ICDRA meetings.

• Will attend ICDRA 2026 in Italy.

• A principal investigator who is working with duckweed in his/her laboratory.

• A researcher/postdoc/student who is working with duckweed in their research (i.e. not just reading about it, but actually doing a project).

• An entrepreneur who is working to commercialize a duckweed-based technology and/or product.

• A research scientist who is working on a commercial venture that aims to develop a duckweed-based technology and/or product.

• A venture capital principle who has invested significantly into duckweed-based technology and/or Product.

• A worker in a commercial venture involved with a duckweed-based technology and/or product.

• An administrator in a University or Funding Agency who has sufficient interest in the duckweed community to provide funding for one or more duckweed-based activity/project/venture.

List of candidates for the election to 7th ISCDRA is the following:

Prof. Asaph Aharoni; Weizmann Institute of Science, Rehovot, Israel
https://www.weizmann.ac.il/plants/aharoni/home; https://www.linkedin.com/in/asaph-aharoni-664b3a14/

PD Dr. Klaus-Jürgen Appenroth; Friedrich Schiller University Jena, Jena, Germany
https://www.bio.uni-jena.de/9812/mitarbeiter
https://www.researchgate.net/profile/Klaus-J-Appenroth

Prof. Hongwei Hou, iEAT, Shenzhen University of Science and Technology (SUAT), Shenzhen, China
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Prof. Robert Laird, University of Lethbridge, Lethbridge, Canada
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Dr. Ingrid M. van der Meer, Wageningen University & Research, Wageningen, The Netherlands
https://www.wur.nl/en/persons/dr-im-ingrid-van-der-meer

Applications to host ICDRA-2028

With pleasure, we announce that the ISCDRA received four applications to host the ICDRA-2028.

Following are the four contesting places for the ICDRA-2028:

1. Shenzhen, China
2. Ghent, Belgium
3. Adelaide, Australia
4. Da Lat, Vietnam

Voting for the venue of ICDRA-2028 will take place during the upcoming conference in Italy. In the following, the applicants have presented details for the community to have a first hand information about the contesting candidate venue to host the ICDRA-2028.

1. Shenzhen, China

Host Institution: Shenzhen University of Advanced Technology (SUAT), Shenzhen, China

Proposed Conference Date: Any time in 2028 (Exact dates to be confirmed)

Submitted to: The 6th International Steering Committee on Duckweed Research and Applications

Shenzhen University of Advanced Technology (SUAT) is honored to submit this application to host the **9th International Conference on Duckweed Research and Applications (ICDRA 2028)** in Shenzhen, China. As a globally recognized platform for exchanging cutting-edge research on duckweed (Lemnaceae) – a versatile aquatic plant with enormous potential as a new model plant and in food security, environmental remediation, carbon capture, and bio-manufacturing – ICDRA aligns perfectly with SUAT's academic mission and Shenzhen's strategic focus on the innovation in basic plant biology and emerging agricultural technologies. We believe that with SUAT's emerging strength in duckweed science and synthetic biology, offers an outstanding setting for ICDRA 2028, combining academic excellence, international accessibility, and strong institutional support.

1. Shenzhen as an Ideal Venue for ICDRA 2028

Shenzhen, China's first special economic zone and the forefront of reform and opening up, occupies an irreplaceable strategic position in China's integration into the market economy. Internationally recognized as one of the most dynamic innovation-driven cities in the world, it provides several unique advantages for hosting ICDRA 2028:

- **Leadership in Biotechnology Innovation:** Shenzhen is China's foremost hub for biotechnology, synthetic biology, and sustainable agriculture innovation, supported by an exceptionally strong research-to-industry ecosystem. Shenzhen's "20+8" industrial clusters feature a world-leading synthetic biology and biomanufacturing sector, ideal for advancing duckweed-based bio-manufacturing, which is highly relevant to ICDRA's mission. Meeting participants will enjoy touring the beautiful futuristic SUAT campus and the largest and most comprehensive synthetic biology foundry in the world.
- **Favorable Conference Environment**: Shenzhen is also highly accessible internationally, served by major airports in Shenzhen, Guangzhou, and Hong Kong, ensuring convenient

global connectivity. With modern infrastructure, multilingual services, and a mild climate, the city provides a safe, welcoming, and highly suitable setting for international delegates. Shenzhen is an exciting coastal city with modern infrastructure and futuristic architecture, and is surrounded regions with diverse cultures and beautiful nature such as Macao, Hong Kong, Guangzhou, and Shaoguan and Chaoshan.

- **A rising hub for plant biology with an expanding duckweed research community:** Shenzhen is becoming a major hub in plant biology and agricultural biotechnology with excellent research programs in the fields located in Southern University of Science and Technology, Shenzhen University, Zhongshan University, SUAT and Agricultural Genomics Institute of Shenzhen. In addition to the major push for duckweed research at SUAT (see below), all these institutions are expanding their research into duckweed.

2. SUAT's Scientific Strength and Commitment to Duckweed Research

SUAT is a young, dynamic research university, located in Guangming Science Park, dedicated to addressing global challenges through interdisciplinary science and technology. Our strengths in duckweed research and academic governance are particularly relevant to ICDRA:

- **Dedicated Duckweed Research Program**: Duckweed research is a key pillar of SUAT's Institute of Emerging Agricultural Technology (iEAT), with research program spanning germplasm resources, molecular breeding, synthetic biology, and scalable cultivation technologies. We have established a major duckweed germplasm repository supported by genomics and multi-omics resources, and are advancing breeding breakthroughs through hybridization, haploid-based approaches, and genome editing. Leveraging Shenzhen's national synthetic biology infrastructure, we have developed high-throughput quantitative phenotyping pipelines, an efficient transformation system, and an AI-enabled codon optimization platform to enhance recombinant protein expression. In parallel, we are developing enabling cultivation technologies integrating automated production, intelligent monitoring, yield-promoting microbial inputs, and animal feed evaluation studies with partner institutions, thereby establishing a closed-loop research-to-application pipeline.

- **A World-Class Hub for Duckweed Research:** SUAT's iEAT is home to a preeminent research team positioned at the forefront of duckweed science. Under the leadership of eminent plant biologist Professor Zhenbiao Yang and through the unremitting efforts of experts including Professor Hongwei Hou, the team is relentlessly advancing duckweed's

potential as a transformative solution for global challenges. By hosting ICDRA 2028, this world-class team will not only share its latest breakthroughs but also actively foster the international collaborations essential to unlocking duckweed's full potential for global sustainability.

3. Conference Facilities and Organizational Capacity

SUAT is fully equipped to deliver a high-quality ICDRA 2028 with comprehensive support in infrastructure, funding, and event management:

- **State-of-the-Art Facilities**: Our campus features modern conference venues, including a 1,000-seat auditorium, multiple modular meeting rooms, and advanced video-conferencing systems for hybrid participation. The university's core laboratories – equipped with high-throughput sequencers, plant growth chambers, and analytical instruments, as well as surrounded by major scientific infrastructures, including the Synthetic Biology Facility and the National Pilot Center for Bio-manufacturing, are offering unparalleled resources for hands-on workshops and technical tours. We also offer on-campus accommodation, catering services, and transportation logistics to ensure a seamless experience for delegates.
- **Adequate Funding Support**: SUAT will cover accommodation, domestic transportation, registration fees, and honoraria or international flights for invited international speakers. Travel grants will be available for graduate students and early-career researchers who contribute poster presentations. The publication support for conference proceedings (e.g., in Wiley's journals) will also be provided.
- **Professional Event Management**: Our experienced team has a proven track record in organizing international conferences, such as the International Symposium on Agricultural and Plant Synthetic Biology, which attracted nearly 400 participants and more than 20 international speakers. We provide end-to-end logistics support, including visa assistance, transportation coordination, cultural activities, and hybrid participation options.

In conclusion, hosting ICDRA 2028 at Shenzhen will bring distinct value to the international duckweed community by fostering global collaboration, empowering young researchers, and highlighting duckweed's growing role in global sustainability, food security, and bio-manufacturing. We are confident in hosting ICDRA 2028 as a landmark event for the international community. We sincerely welcome the opportunity to work with the ICDRA

Steering Committee to deliver a successful and impactful conference in Shenzhen. We look forward to your positive response.

Zhengbiao Yang, Ph.D.

Professor & Dean

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Date: March 29, 2026

2. Ghent, Belgium

Dear Members of the ISCDRA,

The founding partners of the European Duckweed Network (EDN) are pleased to submit their application to host the next International Conference on Duckweed Research and Applications (ICDRA) in Ghent, Belgium.

The organizers

The European Duckweed Network (EDN) is a research network funded by the Research Foundation – Flanders (FWO) that promotes duckweed research through collaboration and knowledge exchange. As our funding is specifically intended to support conferences and workshops, ICDRA 2028 represents an excellent opportunity to make effective use of these resources.

With three active research groups focusing on duckweed, Ghent University represents the central hub of duckweed research in Flanders. The conference will therefore be organized in Ghent by these three groups:

- RE-Source Lab, led by Prof. Dr. Ir. Erik Meers
- Spatial Ecology and Evolution Group, led by Prof. Dr. Dries Bonte
- Bioinformatics and Evolutionary Genomics Group, led by Prof. Dr. Yves Van de Peer

Together, they will form the local organizing committee. Responsibilities will be shared among senior researchers, postdoctoral fellows, and PhD students, providing valuable opportunities for early-career researchers to develop organizational and leadership skills.

All three groups have extensive experience in organizing scientific events, including the European Duckweed Network Workshop (2025), the International Conference on Polyploidy, EVENET symposia, the European Sustainable Nutrient Initiative (ESNI) conference, and Biorefine Cluster Europe (www.biorefine.eu).

In addition, we will benefit from professional logistical support:

- Impact VZW (https://research-impact.eu/), a Ghent University–affiliated non-profit organization specializing in science communication and stakeholder engagement, will assist in coordination and in securing additional industry funding.
- VIB Conference Series, part of the Flemish Institute for Biotechnology (VIB), will provide comprehensive conference support, including website development (registration, abstract submission, and payments), marketing materials, event promotion, conference app development, sponsor invoicing, and on-site coordination.

The scientific program

The scientific program—including abstract selection and invited speakers—will be coordinated by a scientific organizing committee composed of EDN members representing key domains in duckweed research:

1. Diversity, Ecology, and Evolution (Prof. Dr. Dries Bonte & Dr. Quinten Bafort)
2. Genomics (Prof. Dr. Yves Van de Peer & Prof. Dr. Nele Horemans)
3. Physiology and Metabolism (Prof. Dr. Els Prinsen & Prof. Dr. Ann Cuypers)
4. Applications (Dr. Ir. Reindert Devlamynck, Prof. Dr. Ir. Erik Meers, Dr. Marcella Fernandes De Souza & Prof. Dr. Marcel Jansen)

Should submissions fall outside the available expertise within EDN, we will collaborate closely with ISCDRA and external experts to ensure high-quality scientific evaluation.

The Venue

Ghent is a vibrant and historic city, once described by Lonely Planet as "Belgium's best-kept secret." It is renowned for its rich cultural heritage, including the medieval Castle of the Counts, the world-famous Ghent Altarpiece, and its iconic skyline of three towers. The Belfry of Ghent and the city's beguinages are recognized as UNESCO World Heritage sites.

The city offers numerous opportunities for social and cultural activities, such as guided boat tours, beer tastings, and visits to the Botanical Garden.

Ghent is highly accessible, with excellent connections to the international airports of Brussels, Amsterdam, and Paris. A wide range of accommodation options is available to suit different budgets.

We are currently considering several high-quality venues:

1. UGent Aula Congress Center (reopening in 2027 following renovation)
2. Auditorium Oehoe and foyer (Campus Coupure, UGent)
3. Bijloke Conference Center (https://bijlokeheritagevenue.be/), a unique historic site that once served as one of Europe's largest medieval hospitals

All venues are centrally located or easily accessible by public transport and are well equipped to host international conferences.

Some pictures from Ghent (courtesy of visit Ghent)

3. Adelaide, Australia

Submitted by: Professor Jenny Mortimer, Professor of Plant Synthetic Biology, School of Agriculture, Food and Wine, and Deputy Director, Waite Research Institute, Adelaide University, South Australia, Australia

Email for correspondence: jenny.mortimer@adelaide.edu.au

Figure 1: Hikinbotham Terrace (above) and Hickinbotham Hall (below), National Wine Centre (Credit: Adelaide University)

Overview: We would like to host the 9th ICDRA in Adelaide, South Australia, in 2028. Adelaide is home to an active group of duckweed researchers, with international collaborations across the globe. Both the university and state have an active focus on novel foods and biomanufacturing, and the city sits in the middle of Australia's wine and food country. It is a compact, affordable city in which to run a meeting, with easy access to beaches, wildlife and the outback. This bid sets out the proposed venue, how to get here, what the city and region offer, and why Adelaide is a strong fit for the duckweed community.

The venue: Adelaide is a frequent host to conferences of all sizes, and so there are several venue options. Our preferred would be the National Wine Centre of Australia (Figure 1). The Centre is operated by Adelaide University and sits on the edge of the Adelaide Botanic Garden at the eastern end of North Terrace, a short walk from the city center. It can host a meeting of our size comfortably (100 to 200 participants), and can hold the talks, a poster session and catering under one roof, with outdoor terraces looking onto the gardens. Hotels across all price ranges are within walking distance.

Excursions and tours: Adelaide University's Waite campus, the largest concentration of agricultural research in the southern hemisphere, is about 20 minutes from the city and is where most of the local duckweed work is done. The campus also includes our winery, food innovation lab, plant synthetic biology foundry, vertical farms, and large-scale automated plant phenotyping platform. The Adelaide Botanic Garden, next door to the Wine Centre, is an easy on-foot excursion in its own right. PIRSA-SARDI, our state aquaculture and fisheries research facilities (where scaled up duckweed research has been carried out) is about 15 minutes from the city, and visits to our thriving horticulture and altprotein industry can be arranged.

Figure 2: Stargazing in Flinders Ranges (Credit: reddit, U _sohaib

Getting here: Adelaide has direct flights from Singapore, Kuala Lumpur, Doha, Dubai, Shanghai, Guangzhou and San Francisco, with one-stop connections covering the rest of the world; domestic links run through Melbourne (1h 20m), Sydney, Brisbane and Perth, and the airport is about 15 minutes from the city.

Adelaide and South Australia: Adelaide is laid out on a grid, ringed by parklands, and easy to walk or get around by tram. South Australia is the country's main wine state: the Barossa Valley, McLaren Vale, the Adelaide Hills and the Clare Valley are all within about an hour of the city. Adelaide hosts Tasting Australia, a national food and wine festival, as well as other art, food and cultural festivals throughout the year. Wildlife abounds (the Waite campus is visited daily by kangaroos, koalas [see picture on left], parrots and cockatoos), but it can also be seen up close at Cleland Wildlife Park (where you can arrange to cuddle a koala) or during hikes in the Belair National Park (see below), both about twenty minutes by car/train/bus from the city center. Sidetours can be organized to local wildlife parks to see the unique native wildlife, and to the local premium wine districts.

Figure 3: Rescue koala at Cleland Wildlife Park (Credit: Jenny Mortimer)

Figure 4: Kangaroos in Belair National Park (Credit: Jenny Mortimer)

For those who want to extend their stay, destinations such as Kangaroo Island and the Flinders Ranges offer native wildlife, fossils, and landscapes that are hard to find elsewhere, and the outback begins a few hours north.

Relevance to duckweed research and applications: Australia has a growing stake in duckweed, on both the research and applied sides. For example:

The Australian Collection of Duckweed Clones (ACDC). A national collection of Australian duckweeds, housed at the Waite campus and run jointly by the Mortimer and Gilliham laboratories, with clones collected from across South Australia, including from the River Torrens, which runs through the city. The collection is available to the community alongside the Rutgers and Landolt collections (with a manuscript forthcoming).

Food applications. Adelaide groups are active in duckweed as a future food, including, protein quality assessments, nutritional and aroma work, and collaborations with chefs at the Tasting Australia festival. Active research is also establishing how best to cultivate in indoor environments both on Earth but also in Space (see below!).

Plants for Space. Adelaide University leads the ARC Centre of Excellence in Plants for Space (P4S), which has identified duckweed as a candidate crop for closed-environment and off-Earth food and biomanufacturing. Recent work has included flying *W. australiana* on a sounding rocket to test the early effects of microgravity on growth, and, with NASA, assessing its suitability as a crop for the Artemis lunar landing. P4S brings together universities, government agencies (NASA, DLR and the Australian Space Agency) and industry partners in controlled-environment agriculture, a useful bridge between the duckweed community's public and private sectors.

Figure 5: Stokes Bay, Australia (Credit: Tourism Australia)

Proposed timing: We suggest the Australian spring (November to mid December) or Autumn (March to May), perhaps to align with ongoing events (e.g. Adelaide Fringe Festival, Tasting Australia), which would avoid the worst of the summer heat (or the gloomier days of our midwinter). However, we are happy to work with the committee to choose the appropriate dates.

Organising team: The bid is led by Prof. Jenny Mortimer, along with Prof. Matthew Gilliham and Prof. Matthew Tucker (Adelaide University). We will form a national organizing committee drawn from Australia's duckweed and plant-synthetic-biology groups, including partners in the ARC Centre of Excellence in Plants for Space. Adelaide University has the venue, the laboratories and the events support to run a meeting of this kind well.

We would be glad to welcome the duckweed community to Adelaide in 2028.

4. Da Lat, Vietnam

TRƯỜNG ĐẠI HỌC ĐÀ LẠT
DALAT UNIVERSITY

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Proposal to Host the 9th International Conference on Duckweed Research and Applications (ICDRA)

Dalat University, Vietnam, is honored to submit its application to host the **9th International Conference on Duckweed Research and Applications (ICDRA).**

Located in Da Lat City in Vietnam's Central Highlands, Dalat University offers an attractive venue for an international scientific conference. Da Lat is well known for its year-round mild climate, beautiful natural landscapes, rich biodiversity, and thriving agricultural sector. The city is easily accessible via Lien Khuong International Airport and provides a wide range of accommodation and tourism opportunities for international visitors.

In addition, Dalat University is one of Vietnam's leading universities in agricultural, biological, and environmental sciences. The University provides modern conference facilities, well-equipped laboratories, and an excellent academic environment that supports scientific exchange and international collaboration.

For more than a decade, Dalat University has been one of the leading institutions in Vietnam conducting duckweed research. The University's research has addressed duckweed biodiversity, taxonomy, conservation, biotechnology, wastewater treatment, biomass production, and sustainable agricultural applications. The research team has published 12 international peer-reviewed papers and maintains active collaborations with research laboratories and universities in Germany, the Czech Republic, the United States, Thailand, and other countries. These achievements reflect Dalat University's strong commitment to advancing duckweed research and promoting international scientific collaboration.

Furthermore, the proposed organizing committee consists of experienced faculty members and researchers from the Faculties of Biology, Agriculture, and Environmental Sciences, with extensive experience in managing international research projects, scientific collaborations, and academic events.

DLU DALAT UNIVERSITY

TRƯỜNG ĐẠI HỌC ĐÀ LẠT
DALAT UNIVERSITY

Add: 01 Phu Dong Thien Vuong Street, Lam Vien – Dalat Ward, Lamdong province Tel: (+84) 0263 3823 380 F: (+84) 0263 3822 246 E: daihocdalat@dlu.edu.vn W: www.dlu.edu.vn

Dalat University also has a proven record of successfully organizing international conferences, including the 17th International Conference on Knowledge and Systems Engineering (KSE 2025), the International Scientific Conference "Global Perspectives on the Anthropology of Vietnam" (2026), and the International Conference on "Conservation Action Planning for Vietnam's Threatened Fagaceae" (2026). These events have strengthened the University's expertise in conference organization, scientific program management, and international participant support.

In summary, by combining an active duckweed research community, experienced organizers, modern facilities, and the unique setting of Da Lat City, Dalat University is well positioned to host the 9th ICDRA. We are committed to delivering a scientifically outstanding, professionally organized, and welcoming conference that will strengthen international collaboration and promote future advances in duckweed research and applications.

We would be honored to welcome the global duckweed research community to Vietnam for the 9th ICDRA.

On Behalf of Dalat University
Vice Rector

TRƯỜNG ĐẠI HỌC ĐÀ LẠT

Trần Hữu Duy

DLU DALAT UNIVERSITY

Student spotlight:

Duckweed as a Molecular Farming Platform: From Silk Proteins to Egg Proteins

Two research summaries from the Aharoni Lab, Weizmann Institute of Science, exploring duckweed (*Lemna japonica*) as a platform for recombinant protein production.

Julia Eva Fortmueller

PhD Candidate, Weizmann Institute of Science, Israel

Email: julia.fortmueller@gmail.com

I completed my Bachelor and Master degrees in Biotechnology at the Technical University of Graz, Austria, where I developed a strong interest in protein science that later extended to biopolymers from both natural and synthetic systems. Joining Professor Asaph Aharoni's group at the Weizmann Institute of Science for my PhD opened a new field of research for me: plant science, with a particular focus on the duckweed species *Lemna japonica*. My research interests center on aquatic plant molecular farming (Figure 1) using plants as biological factories to express unique proteins that function as polymers.

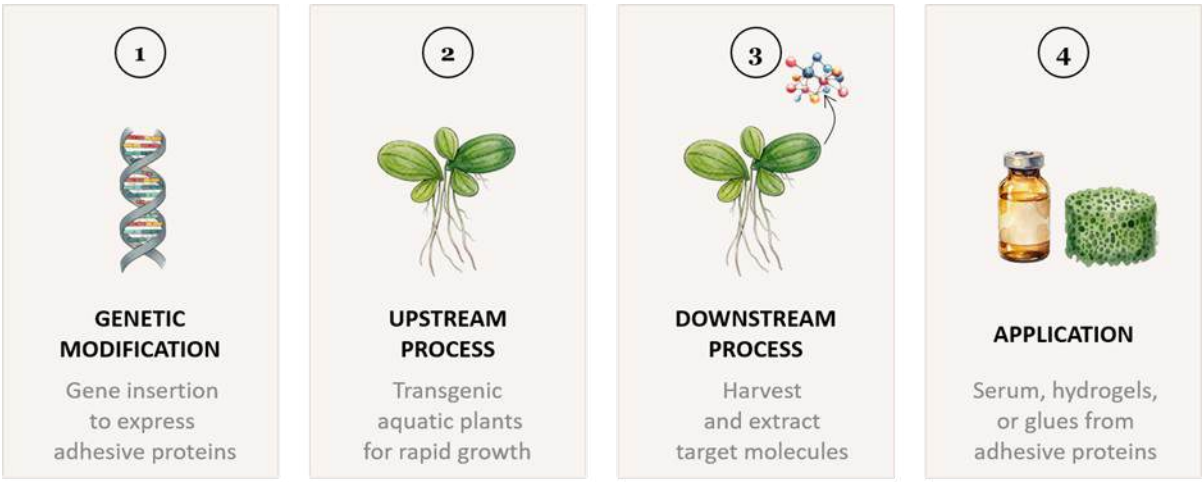

Figure 1: Aquatic plant molecular farming workflow: genetic modification, upstream growth, downstream processing, and application.

My project focuses on a protein derived from the silkworm, *Bombyx mori,* called silk sericin, which acts as a glue coating the silk fibers, shaping the cocoon and protecting the pupa inside from physical damage, pathogens, and environmental stress during metamorphosis. Sericin has attracted interest in biomedical applications ranging from cell-culture supplementation to tissue-engineering scaffolds and hydrogels. Through heterologous expression of sericin in *Lemna*, this work aims first

to establish the feasibility of duckweed as a recombinant protein expression platform, and second to evaluate the added functional value of combining duckweed-derived components with sericin for downstream biomedical applications.

We have generated stable transgenic duckweed lines expressing sericin and are currently evaluating expression levels ahead of downstream functional characterization.

Priel Siman-Tov

MSc Student, Weizmann Institute of Science, Israel

Email: priel.siman-tov@weizmann.ac.il

My research interests lie at the intersection of food science, biotechnology, and plant sciences. A background in pastry, paired with an interest in plant-based alternatives, motivated me to explore the biochemical mechanisms behind replacing complex animal-derived ingredients, specifically, how to substitute components like eggs. This led me to pursue a BSc in Food Science and Biochemistry at the Hebrew University of Jerusalem, followed by an MSc at the Weizmann Institute of Science. There, I joined Julia's project as a rotation student before establishing my own MSc project, in Prof. Asaph Aharoni's lab, focused on the sustainable production of egg proteins through recombinant expression in duckweed.

As a bioreactor platform, duckweed offers distinct benefits over both microbial and traditional plant hosts: unlike microbial systems, it carries no endotoxin risk and supports complex post-translational modifications, while its exceptionally rapid growth and high biomass yield give it an advantage over conventional terrestrial plants. My project builds on this platform to address the sustainability challenges posed by global population growth and the ecological limitations of traditional animal agriculture. Specifically, we aim to produce recombinant egg white proteins, which are highly valued in the food industry both for their nutritional profile and for essential techno-functional properties such as emulsification, gelation, and foaming.

Our experimental approach relies on molecular cloning and plant biotechnology workflows. So far, we have constructed expression plasmids containing different egg protein genes and successfully transformed *Lemna japonica* via *Agrobacterium tumefaciens*. We are now identifying the best-performing stable lines to carry forward for protein purification and functional assays.

Together, these projects highlight duckweed's potential as a recombinant expression platform for applications spanning biomedicine and sustainable food production, and we look forward to advancing both fields by leveraging this platform to deliver functional, real-world proteins.

From the Database

We checked this time in almost all publications whether the species identity of the investigated duckweeds 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

A cooperative cobalamide biosynthesis guild in the endosphere of the edible aquatic plant *Wolffia globosa* Mankai

Dutta, R; Obayomi, O; Yosef, AF; Ghazaryan, L; Chalifa-Caspi, V; Lapidot, M; Gillor, O. (2026) Environmental Microbiome DOI10.1186/s40793-026-00917-4.

Cobalamin (vitamin B_{12}) is synthesized only by certain bacteria and archaea and is rarely found in plant-derived foods because plants neither synthesize nor require this cofactor. The edible duckweed *Wolffia globosa* Mankai is unusual in containing bioavailable cobalamin, suggesting a microbial origin. However, how cobalamin biosynthetic capacity is organized within angiosperm-associated microbiomes remains largely unresolved. Here, we investigated bacterial community structure and cobamide biosynthetic potential across the cultivation medium, plant surface, and internal tissues of Mankai to determine how cobalamin production is maintained in this aquatic plant microbiome. Bacterial communities differed significantly among compartments, with the endosphere forming a low-diversity, host-filtered microbiome enriched in specialized taxa. Genome-resolved metagenomics showed that only a minority of endophytic bacteria encoded near-complete cobamide biosynthesis pathways consistent with de novo synthesis. In contrast, many co-occurring taxa lacked multiple biosynthetic steps but were enriched in genes associated with cobamide precursor salvage and remodeling. Network analysis identified putative producer taxa as highly connected hubs linked to salvager populations, consistent with metabolite cross-feeding. Comparative genomic analysis demonstrated reduced cobamide biosynthetic gene complements in endophytic genomes relative to closely related free-living strains, supporting adaptive pathway reduction in the host-associated niche. Cobalamin production in the Mankai endosphere appears to arise from a metabolically interdependent bacterial consortium rather than from single autonomous producers. These findings identify cooperative micronutrient biosynthesis as an organizing principle in plant-associated microbiomes and position Mankai as a tractable model for studying cobamide-mediated microbial cooperation in aquatic crops. Understanding these interactions may support microbiome-informed strategies to stabilize micronutrient production and functional resilience in controlled aquatic plant cultivation systems.

Targeted multiplex gene knockouts in *Lemna minor* using CRISPR/Cas9

Baghini, SS; Esfahani, K; Rad, N; Arezoumandi, M; Taghipour, E; Salmanian, AH (2026) Transgenic Research 35: 16.

Lemna minor (commonly known as duckweed) is a fast-growing aquatic plant recognized as a promising green bioreactor for recombinant protein production. Its rapid proliferation, high protein yield, environmental adaptability, and edibility make it highly attractive for biotechnological applications. It is essential to develop and expand genetic tools tailored to this species to maximize these advantages and further unlock its biotechnological potential. A key strategy for achieving this goal is the implementation of advanced genome editing technologies, such as the CRISPR/Cas9 system. Although multiplex CRISPR/Cas9 gene editing has previously been successfully applied in *Lemna aequinoctialis*, the capability of the endogenous plant tRNA processing system for multiplex editing in *L. minor* using the polycistronic tRNA-sgRNA (PTG)/Cas9 system has not yet been explored. In this study, a PTG construct was engineered to include four sgRNAs designed to simultaneously target two plant-specific glycosyltransferase genes: alpha-1,3-fucosyltransferase (FucT) and beta-1,2-xylosyltransferase (XylT). As anticipated, the PTG-Cas9 system successfully induced frameshift mutations, characterized by insertions and deletions (indels), in regenerated *L. minor* plants derived from

transformed calli. Validation via PCR and RT-PCR analysis, followed by sequencing of the target loci, confirmed the presence of indels at the target sites. Furthermore, western blot analyses utilizing antibodies specific to XylT and FucT in two homozygous lines (lines 44 and 217) revealed truncated XylT proteins in both lines. Moreover, an in-frame FucT protein was detected in line 217, whereas FucT expression was absent in line 44. This study marked the first successful demonstration of PTG-Cas9 system for multiplex genome editing in *L. minor*, paving the way for advanced genetic engineering in this species.

DF comment: The plant material comes from a stock collection but nothing is written whether and how species were identified.

Aquaculture/ Agriculture

Duckweed as a sustainable protein source: Effects of fertilizer regimes on growth, protein yield, and functional properties

Hiththatiyage, R; Ariyasena, P; Fernando, D; Abeywickrama, L; Sasindi, N; Wijesundara, S; Jayawardana, B; Madhujith, T; Liyanage, R; Jin, LH; Zhou, X (2026) Advanced Agrochem 5: 206-229.

Duckweed offers potential as a sustainable protein source in Sri Lanka. This study optimized growth, protein content, and safety in four wild varieties; *Spirodela polyrhiza* (SP), *Lemna minor* (LM), *Lemna perpusilla* (LP), and *Landoltia punctata* (LaP) using organic (OM; poultry manure at 1:30, 1:25, 1:20), inorganic (IM; Albert fertilizer at 0.5, 1.0, 2.0 g L^{-1}), and combined manure (CM; 1:30 OM + 0.5 g L^{-1} IM). Relative growth rates (RGR) was monitored over 40 days, followed by evaluation of protein content, heavy metals, extraction efficiency (alkali AE vs. salt SE), structural, and techno-functional properties. LP and LM showed the highest ($p < 0.05$) RGR under IM, while LP also recorded the highest RGR under OM (0.0937-0.0950 d^{-1}). Under CM, LM showed the highest RGR (0.0144 ± 0.0097 d^{-1}). Overall, IM promoted superior growth, with LP showing the highest ($p < 0.05$) RGR (0.24-0.25 d^{-1}), indicating strong adaptability. Wild duckweed initially contained higher ($p < 0.05$) heavy metals than post-fertilizer treatments. Protein content rose from baselines (17.3% LM to 26.6% LaP) to peaks of 33.1% (LaP, IM 2 g L^{-1}), 31.5% (LM, CM), and 27.6% (LM, OM 1:20). SE outperformed AE across all fertilizer treatments, yielding higher protein recoveries. Inorganic manure (IM) treatments delivered the highest extraction yields ($p < 0.05$), followed by combined manure (CM) and organic manure (OM). FTIR and Raman spectroscopy confirmed characteristic protein amide bands and structural features (1200-1700 cm^{-1}), while amino acid profiling indicated a balanced composition. Optimizing fertilizer regimes and extraction strategies can improve the nutritional quality and functional properties of duckweed, thereby strengthening its potential as a sustainable protein source for Sri Lanka's emerging bio-protein market.

Chlorella vulgaris lipid extraction side-stream enhances growth and protein enrichment in novel food *Lemna minor* (duckweed)

Fais, G; Castelli, S; Dessì, D; Perra, G; Lai, NCL; Cao, GCM; Concas, A (2026) Frontiers in Nutrition 13: 1822150.

The transition toward sustainable food systems requires strategies that simultaneously reduce bioprocess residues and enhance nutritional quality. Microalgal biorefineries generate substantial polar side-streams after lipid extraction, which remain largely underutilized despite their high nutrient content. Here, we evaluated the polar side-stream generated during lipid extraction of *Chlorella vulgaris*, representing the non-lipid and water-soluble fraction enriched in soluble metabolites and nutrients, as a bio-based fertilizing input for *Lemna minor* (duckweed), a Novel Food. The Chlorella Extraction Waste (CEW) was applied in hydroponic cultivation across a defined concentration window, and growth performance was quantified through image-based frond area analysis and kinetic modeling. Within a non-inhibiting range, the side-stream significantly stimulated duckweed growth, with 0.2 g L^{-1} nearly doubling biomass at day 7 compared to water control. Bench-scale validation against Hoagland solution and a commercial fertilizer demonstrated enhanced protein accumulation (27.1% DW), exceeding the commercial fertilizer treatment and accompanied by reduced carbohydrate allocation, while maintaining balanced pigment profiles, antioxidant activity, and stable lipid levels. The absence of carotenoid overaccumulation and major shifts in antioxidant activity indicates nutrient-enabled protein enrichment without detectable stress-associated metabolic reprogramming. These findings demonstrate that microalgal lipid extraction side-streams can function as nutrient-regime modulators capable of redirecting

carbon-nitrogen allocation toward protein-rich biomass, coupling circular bioeconomy valorization with nutritional upgrading of a fast-growing aquatic crop. This work provides a proof-of-concept for integrating biorefinery side-streams into sustainable micro-scale photosynthetic food systems.

DF comment: The identity of *Lemna* was evidently not investigated.

Ammonium-directed strategy shift in duckweed (*Spirodela polyrhiza*) synchronizes tunable wastewater treatment with industrial biomass valorization

Chen, Y; Long, SY; Shi, XC; He, Y; Li, SY; Cai, YX; Xiong, XQ; Liu, M; Zhang, LY; Sun, ZL (2026) Industrial Crops and Products 247: 123479.

Ammonium pollution, a major threat to aquatic ecosystems, requires efficient and sustainable remediation approaches. However, most previous studies have focused on either toxicity thresholds or single-level treatments, leaving the integrated physiological and metabolic responses across a continuous wide concentration gradient largely unexplored. Here, we systematically examined the physiological and metabolic responses of duckweed (*Spirodela polyrhiza*) across a 0-2062.5 mg/L $(NH_4)_2SO_4$ gradient to elucidate its adaptive strategies for ammonium remediation and industrial biomass valorization. Results indicate that ammonium concentration fundamentally shapes duckweed's physiological priorities. At low concentrations (0-82.5 mg/L), duckweed exhibits a growth-remediation mode characterized by high nutrient removal efficiency, with total nitrogen and phosphorus removal at 69.23%-78.25% and 83.91%-86.83%, respectively. At high concentrations (412.5-2062.5 mg/L), duckweed shifts to a storage-tolerance mode, suppressing growth and redirecting carbon flux to valuable energyrich compounds, accumulating starch (to 217.39 mg/g dry weight) and triglycerides (to 21.27 nmol/g dry weight) while maintaining cellular integrity. Importantly, low ammonium concentrations cause more severe membrane damage than high ammonium concentrations, challenging a simple linear dose-response assumption. Mineral analysis reveals adaptive nutrient management with enhanced phosphorus and zinc retention under stress. These findings demonstrate that the ammonium-dominated treatment conditions act as a bioprocess switch, directing metabolism toward wastewater treatment or starch- and lipid-rich biomass accumulation. This metabolic plasticity positions S. polyrhiza as a promising industrial crop and provides the first systematic study of a concentration-dependent strategy shift, offering a tunable framework that links wastewater nutrient recovery with directed feedstock production for the circular bioeconomy.

Process Simulation and Techno-Economic Analysis of *Wolffia*-Integrated Recirculating Aquaculture Systems for Nutrient Recovery and CO_2 Utilization

Motlagh, SR; Chalermthai, B; Khezri, R; Etesami, M; Chee, CY; Nootong, K. (2026) Sustainability 18: 4104.

Recirculating aquaculture systems (RASs) improve water-use efficiency in fish production but generate nutrient-rich effluents requiring management. Integrating aquatic biomass cultivation into RASs offers a promising approach to nutrient recovery, CO_2 utilization, and biomass production. This study evaluates the technical and economic feasibility of integrating *Wolffia globosa* cultivation with RASs through process simulation and techno-economic analysis (TEA). A pilot-scale system in Thailand was modeled using SuperPro Designer, comparing static and suspended aeration cultivation. The suspended configuration required only similar to 10-12 m^2 for 28.80 m^3, whereas static cultivation required 131 m^2 for 32.80 m^3, corresponding to about a 12-fold reduction in land area. The suspended system achieved higher annual biomass production (1056 kg dry weight (DW) yr^{-1}) than the static system (690 kg DW yr^{-1}), corresponding to CO_2 fixation of similar to 1.50 and similar to 0.98 t CO2 yr^{-1}, respectively. The static system achieved higher nutrient removal efficiencies (97% N and 99.66% P), while the suspended system showed lower removal (64% N and 65.30% P) but higher productivity. Economic analysis confirmed feasibility, with the suspended system achieving higher return on investment (17.56% vs. 12.89%) and a shorter payback period (5.70 vs. 7.76 years). These results demonstrate the potential of RAS-*Wolffia* integration as a circular approach for resource recovery and sustainable aquaculture.

Biochemistry

Structure elucidation of duckweed polysaccharide from *Spirodela oligorrhiza* and its anti-inflammatory activity

Wei, HL; Li, J; Sun, ZY; Ji, JS; Tao, J; Yin, YY; Deng, YC; Liu, WZ (2026) Carbohydrate Polymers 385: 125374.

Duckweed polysaccharides constitute an underexplored yet functionally important class of biomolecules within these rapidly proliferating aquatic plants. Although abundant in cell walls and implicated in diverse bioactivities, their structural elucidation beyond pectic fractions has remained scarce. In this study, the first comprehensive structural analysis of a purified polysaccharide (WHL-N1) derived from *Spirodela oligorrhiza* duckweed is reported. Monosaccharide composition analysis demonstrated galactose (46.15 mol%) and arabinose (39.44 mol%) as the predominant constituents. Structural investigation revealed a highly branched Type II arabinogalactan architecture with a weight-average molecular weight of 200.868 (±4.392) kDa and a narrow polydispersity index (1.147 ± 0.076), comprising a backbone of -> 3,6)-beta-D-Galp-(1 ->, -> 3)-beta-D-Galp-(1 ->, and -> 6)-beta-D-Galp(1 -> residues, decorated with side chains at the O-6 position of -> 3)-beta-D-Galp-(1 -> units. WHL-N1 displayed marked anti-inflammatory properties in a zebrafish model, significantly attenuating neutrophil migration and regulating pivotal inflammatory cytokines. Furthermore, in a dextran sulfate sodium (DSS)-induced colitis mice model, WHL-N1 reduced inflammatory cytokine levels, restored intestinal barrier integrity, and modulated gut microbiota composition, underscoring its promise potential for colonic inflammation. These findings position WHL-N1 as a promising candidate for nutraceutical and therapeutic development targeting colonic inflammation and microbiota dysbiosis, whilst offering a fundamental basis for future investigations into the structure-activity relationships of duckweed polysaccharides.

DF comment: The valid name of this species is *Landoltia punctata*.

Structural and functional characterization of duckweed protein-maltodextrin conjugates and their application in oleogels for low-fat mimetic food materials

Jang, Y; Said, NS; Lee, W (2026) European Food Researc and Technology 252: 209.

This study explores the functional and structural characteristics of duckweed protein-maltodextrin conjugates (DPM) as potential fat substitutes through oleogel application. Conjugates were prepared at protein-to-maltodextrin ratios of 1:1, 1:2, and 1:3, labeled DPM1, DPM2, and DPM3. Among these, DPM3 exhibited the highest degree of grafting (59.48%), solubility (61.42%), and antioxidant activity (22.70%), suggesting a more extensive Maillard reaction and superior functional properties. Circular dichroism spectroscopy revealed that conjugation disrupted alpha-helix structures while promoting beta-sheet formation, indicating enhanced intermolecular interactions and protein unfolding. Structural analyses using Fourier transform infrared spectroscopy (FT-IR) and fluorescence spectroscopy showed that conjugation altered protein secondary and tertiary structures, further supporting the improved functional performance. As maltodextrin content increased, foaming capacity decreased, with DPM3 exhibiting the lowest at 17.82%. Nevertheless, DPM3 demonstrated improved emulsion stability, likely due to thicker, viscoelastic interfacial layers. DPM-based oleogels outperformed CON counterparts in texture and oxidative stability. DPM2 formulations (O-DPM2) exhibited superior textural properties, including elasticity, chewiness, and hardness. By day 14, lipid oxidation levels were lowest in O-DPM2 (1.212 mg MDA/kg) and O-DPM3 (1.319 mg MDA/kg), with no significant difference between them. PCA analysis revealed that most of the texture attributes of oleogels were positively correlated with the degree of lipid oxidation. The findings highlight the potential of Maillard reaction-mediated conjugation to enhance functional attributes of duckweed protein, supporting its broader application in food systems.

DF comment: The authors used *Spirodela polyrhiza*.

Biotechnology

Fast-growing flowering aquatic plant, *Wolffia*-derived porous biochar as a sustainable anode material for lithium-ion batteries

Kapanya, T; Thanachayanont, C; Sarakonsri, T (2026) Biomass and Bioenergy 212: 109325.

Wolffia (referred to as "Khai-phum" in Thailand) is a high-biomass-yield resource that offers advantages for energy production and future human food supplies. In this study, *Wolffia* was evaluated as an alternative sustainable biomass-based carbon precursor for the fabrication of anode materials in lithium-ion batteries (LIBs). Compared with the non-activated material, $ZnCl_2$ activation markedly eroded the WDPB surface, exposing micropores, mesopores, and macropores. At 800°C, the specific surface area (SSA) values displayed negligible variation across all WDPBs with different $ZnCl_2$ ratios, owing to pore expansion and structural collapse. Notably, although WDPB1-800 exhibited the lowest Rct due to its prevalence of micropores and absence of macropores, it did not demonstrate superior electrochemical performance. While micropore structures are advantageous as they provide additional active sites for lithium storage, they also impede lithium-ion and electrolyte diffusion compared to mesopores and macropores, consequently impairing overall ion transport and electrochemical performance. Owing to its highly disordered carbon structure and hierarchical pore architecture, WDPB2-800 exhibited the best electrochemical performance as an anode in LIBs, by optimizing kinetic reactions whilst ensuring rapid ion diffusion. It achieved a remarkable specific capacity of 394.4 mAh/g, surpassing the 249.1 mAh/g recorded for WDPB1-800 after 100 cycles at 100 mA/g. This research clearly demonstrates that the integration of a highly disordered structure, elevated SSA, and hierarchical pores, coupled with a low micropore/ mesopore fraction, represents one of the most promising strategies for developing high-capacity, carbon-based LIB anodes derived from sustainable biomass.

Ultrasound-assisted extraction improves yield, functional properties, and structural characteristics of duckweed, a sustainable plant-based protein source

Habeeb, F; Bhat, N; Makroo, HA; Akhter, N; Majid, D; Dar, BN (2026) Innovative Food Science & Emerging Technologies 111: 104596.

Duckweed (*Lemna minor*), a fast-growing aquatic plant, represents a promising sustainable and nutrient dense alternative protein source for plant-based foods and feed. This study compares alkaline acid extraction (AAE) and ultrasound-assisted alkaline extraction (UAAE) for protein recovery, structural characteristics, and functional performance. The fresh biomass exhibited high moisture (90.18 ± 0.18%) and protein content (25% dry weight), with RuBisCo as the dominant protein. UAAE significantly enhanced protein yield (34%) and recovery (18%) at 70% amplitude for 20 min, outperforming conventional AAE. Ultrasound treatment improved solubility, water-holding capacity (3.4 g/g), foaming (65%), emulsifying activity (60.3 m2/g), and colloidal stability, accompanied by reduced particle size (744 nm) and increased zeta potential (-22.5 mV). Structural analyses (SDS-PAGE, FTIR, XRD, SEM) revealed ultrasonication-induced protein unfolding, reduced diffraction intensity indicative of structural loosening in the amorphous protein matrix, and enhanced exposure of polar amino acids, including norvaline, glutamic acid, aspartic acid, and arginine. These molecular and functional enhancements position duckweed protein as a technologically versatile, sustainable plant-based ingredient with potential applications in food formulations, protein-enriched products, and biotechnological applications. This work provides quantitative structure function insights, supporting the scale-up and utilization of duckweed as a novel alternative protein.

DF comment: Identification of the species was not even tried by the authors.

Understanding drying processes of steam-blanched *Wolffia globosa* under microwave vacuum drying through data-driven and semi-theoretical modeling

Pambudi, S; Churat, C; Nitinarot, M; Buntreekanok, W; Saechua, W; Jongyingcharoen, JS (2026) LWT – Food Science and Technology 250: 119471.

Steam blanching and microwave vacuum drying (MVD) are increasingly applied to preserve heat-sensitive, protein-rich biomaterials such as *Wolffia globosa*. However, accurately modeling moisture removal under MVD remains challenging due to the highly nonlinear and stage-dependent nature of microwave-induced drying. This study investigates the combined effects of steam blanching pretreatment and microwave power (540, 720, and 900 W at 5 kPa) on the drying kinetics of *W. globosa* and evaluates the capability of machine-learning models to predict moisture evolution in comparison with conventional semi-theoretical models. The results indicated that steam-blanched samples consistently exhibited higher maximum drying rates across all power levels, increasing from 1.088 to 1.298 $g_w/(g_{dm} \times min)$ at 540 W and from 1.974 to 2.126 $g_w/(g_{dm} \times min)$ at 900 W. The pretreatment also reduced total drying time from 19.5 to 18.0 min at 540 W, while drying time remained unchanged at higher microwave powers. Among the evaluated semi-theoretical models, the Midilli equation provided the best fit to condition-specific experimental data (R^2 = 0.9981-0.9994). However, the generalized Midilli model showed systematic underestimation at low moisture ratios across operating conditions (R^2 = 0.9865, RMSE = 0.9990). In contrast, the k-nearest neighbors (k-NN) model demonstrated strong generalized predictive capability across multiple drying conditions, achieving R^2 = 0.9798 and RMSE = 0.0481 on the testing dataset. Beyond improved predictive accuracy, the machine-learning framework effectively captured stage-dependent drying behavior patterns that conventional generalized semi-theoretical models failed to represent. These findings highlight the potential of data-driven approaches for modeling complex drying behavior under microwave vacuum conditions. The accurate prediction of moisture ratio may further support the optimization of MVD processes for *W. globosa* based food ingredients.

DF comment: Confirming that this is really *W. globosa* would have improved the quality of the publication.

Directional preparation of duckweed-based porous carbon and adsorptive removal of volatile organic compounds

Xiong, JB; Dan, JM; Duan, GH; An, XJ; Jiang, W; Yu, CL (2026) ACS Omega 11: 35789-35803.

In this reported study, a porous biochar adsorbent based on duckweed biomass was prepared by KOH impregnation activation and pyrolysis. The preparation process parameters were optimized through single-factor and orthogonal experimentation. The experimental results showed that the biochar AC-1.0-740-90, using 1.0 mol/L KOH and a pyrolysis temperature of 740°C, with a pyrolysis time of 90 min, exhibited the best adsorption performance for toluene, with a saturated adsorption capacity of 910.97 mg/g. Characterization test results indicated that the biochar AC-1.0-740-90 had a high specific surface area (SSA, 2202.6 m^2/g), a high proportion of micropores to the total pore volume (74%), abundant oxygen-containing functional groups, and highly dispersed heteroatoms N, P, and Fe. All these properties contributed to its excellent toluene adsorption performance. The multicomponent adsorption results showed that this sample had excellent adsorption performance for the ternary mixture of benzene, toluene, and chlorobenzene, with an adsorption capacity of 863.20 mg/g. During four toluene adsorption-desorption cycle tests, the adsorption capacity remained at around 90%. The toluene adsorption performance of sample AC-1.0-740-90 mainly involved pore filling and surface adsorption (such as n-pi interaction, pi-pi interaction, and the complexation of metal iron with toluene).

Intelligent process simulation framework for bio-oil production from *Wolffia globosa* fast pyrolysis via Aquila Optimizer

Piyachon, A; Aungkulanon, P; Prasartkaew, B; Sukpancharoen, S (2026) Energy Nexus 22: 100713.

Optimizing fast pyrolysis for eco-efficiency-simultaneously maximizing bio-oil yield while minimizing carbon emissions and electricity cost-remains intractable for conventional Response Surface Methodology (RSM), whose polynomial surrogates cannot escape the interpolation grid of a fixed experimental design. Here we

introduce an Intelligent Process Simulation Framework (IPSF) that couples a validated Aspen Plus steady-state process model directly with the Aquila Optimizer (AO), a population-based metaheuristic, through automated MATLAB-COM integration, enabling gradient-free exploration of the full multi-dimensional decision space. Using non-food-grade *Wolffia globosa* biomass-sourced from wastewater phytoremediation systems rather than dedicated cultivation-as a compositionally demanding case-study feedstock, we optimize three progressively complex scenarios (3, 4, and 5 decision variables encompassing pyrolysis temperature, vapor residence time, N2 carrier-gas flow rate, separator temperature, and separator pressure) under a composite desirability index that aggregates bio-oil mass flow, CO_2-equivalent emissions, and electricity cost. The AO consistently outperforms RSM numerical optimization, improving composite desirability by 3.7-10.5% across all scenarios and converging within 6-8 iterations to a non-intuitive operating regime: moderate pyrolysis temperatures (467-499°C), reduced N_2 flow (906-1137 kg/h), and elevated separator pressure (2.0 bar). This regime embodies a favorable trade-off in which less than 1.8% bio-oil sacrifice yields simultaneous reductions in CO2e (up to 1.9%) and electricity cost (up to 5.0%). The framework's modular structure-in which only the Aspen Plus process model requires reconfiguration for different feedstocks-suggests potential applicability to other biorefinery optimization problems, although such transferability remains to be demonstrated in future work.

Ecology

Competition enables rapid adaptation to a warming range edge in a model plant community

Usui, T; Angert, AL (2026) Science 392: eads4664.

Most predictions of whether populations will adapt to warming range edges ignore species interactions. We experimentally tested whether range-edge populations can adapt to warming within a competitive, model plant community (*Lemna* spp. and *Spirodela polyrhiza* duckweeds). Notably, we found that adaptation to warming range edges was possible only when populations evolved with interspecific competitors. Moreover, competitors enabled the evolution of both high-temperature tolerance and increased thermal performance breadth at the range edge, but not at the cooler range core. Our results reveal that competitors accelerate thermal adaptation when there is a shared evolutionary response to both competition and warming. Overall, we highlight the urgency of including community context in predicting future range shifts, showing that antagonistic interactions do not necessarily hinder adaptation to climate deterioration.

Enhanced net carbon dioxide sink in rice-duckweed system: Evidence from the diurnal flux variation

Liu, WX; Gao, N; Li, YW; Liu, XY; Zhou, X; Peng, YH; Zhu, LL; Meng, J; Xu, JZ (2026) Agricultura Ecosystems & Environment 405: 110379.

Rice, a staple food for nearly half of the world's population, has significant carbon sequestration potential. Duckweed, common in flooded paddies, is known to improve rice yield and nitrogen use efficiency, but its role in carbon sequestration remains unclear. A three-year field experiment (2021-2023) examined diurnal CO_2 flux and its components (soil and plant) dynamics in a rice-duckweed system under flooding irrigation versus alternate wetting and drying (AWD) irrigation. Results showed a distinct "U-shaped" diurnal pattern in net ecosystem carbon dioxide exchange, with peak uptake between 08:00 and 14:00. The introduction of duckweed enhanced this peak uptake capacity under both flooding irrigation and AWD conditions, attributable to duckweed's strong photosynthetic potential. The net primary productivity of duckweed (NPPD)was estimated at 18.2-345.2 mmol m^{-2} d^{-1}, contributing 3-75% of the interplant carbon exchange and 2-46% of the total ecosystem carbon dioxide exchange. Consequently, the daily cumulative ecosystem carbon dioxide uptake was higher by 43.1-306.2 mmol m^{-2} d^{-1} in duckweed treatments compared to the non-duckweed control. In paddies without duckweed, AWD increased daily soil respiration by 34.2%-92.9% relative to flooding irrigation, with the majority of this increase occurring during the mid-tillering and flowering stages. However, duckweed with a biomass density exceeding 200 g/m^2 was able to offset half to two-thirds of the AWD-induced carbon emissions. An integrated assessment of these dynamics identifies flooding irrigation with

duckweed (IF+D) as the most efficient practice for seasonal atmospheric CO_2 sequestration. Meanwhile, the system combining AWD with duckweed (IAWD+D) emerged as a balanced strategy that mitigates AWD-induced carbon loss while maximizing carbon storage in rice biomass. These findings suggest that irrigation management with duckweed phenology and rice growth stages can optimize carbon-water exchange in paddies.

Warming effects on dynamic interactions of three duckweed species

Zhou, T; Liu, H; Cai, GJ; Tan, XY; Liao, Y; Zhu, Q; Yang, CY; Wu, AP; Li, YZ; Liu, HY; Yuan, GX; Fu, H. (2026) Freshwater Biology 71: e70228.

Plant interactions critically govern ecosystem structure and stability, with climate warming altering interaction dynamics. This study investigates how these warming-induced shifts manifest in duckweed communities by examining temperature effects (25°C vs. 35°C) on three species: *Spirodela polyrhiza, Lemna minor*, and *Wolffia arrhiza*. Drawing upon the stress gradient hypothesis, we quantified the temporal shifts in intra- and interspecific interaction types and strengths using empirical dynamic models to test hypotheses regarding competitive and facilitative responses to elevated temperatures. Our experimental design involved maintaining three duckweed species (*S. polyrhiza, L. minor*, and *W. arrhiza*) in monocultures and various mixed-species combinations under two distinct temperature conditions (25°C vs. 35°C) for 40 days. Results showed that facilitation dominated intra- and interspecific interactions at both temperature conditions. Warming did, however, reconfigure certain interactions. Specifically, intraspecific interactions in *W. arrhiza* shifted from competition to facilitation in bi-culture with *S. polyrhiza*; in the tri-culture, warming shifted *S. polyrhiza* <-> *W. arrhiza* interactions from neutral to facilitative, and *W. arrhiza* -> *L. minor* interactions from facilitation to competition-a shift that was also observed in the *L. minor* and *W. arrhiza* bi-culture. Facilitation strength in tri-mixtures increased at 35°C, while interspecific interactions weakened across time. Notably, population stability of *S. polyrhiza* increased and community stability decreased with stronger interspecific interactions, whereas no such pattern was observed for *L. minor* or *W. arrhiza*. The prevalence of facilitation, even under increased warming, indicates a robust underlying tendency for positive interactions within this duckweed community. Our findings highlight that warming restructures duckweed interaction networks via varying dynamic strength between facilitation and competition. 6 Identifying temperature thresholds triggering transitions between facilitative and competitive interaction regimes will be critical for anticipating and managing aquatic ecosystem resilience under projected climate warming scenarios.

DF comment: Nothing was reported about the identification of the species.

Feed & Food

Modulation of functional properties, oxidative stability, and aroma evolution in cream-style salad dressing by *Wolffia globosa* powder incorporation

Sinthusamran, S; Techavuthiporn, C; Supapvanich, S; Syukri, D; Atika, Y; Pomasa, P (2026) Food Chemsitry 519: 149644.

Wolffia globosa is a novel plant-derived protein rich in bioactive compounds with potential functionality in food products. This study evaluated dried *W. globosa* powder (WP) as a partial egg yolk substitute (0-20%) in cream-style salad dressing, focusing on physicochemical properties, oxidative stability, and volatile compounds during storage. WP exhibited high protein content (32.31%), abundant chlorophylls and phenolics, and strong antioxidant capacity. Sensory evaluation identified 15% WP as the most acceptable formulation. WP incorporation altered color, reduced viscosity, increased droplet size, and enhanced electrostatic stability through a more negative zeta-potential. Nutritional quality improved via higher protein, fiber, and antioxidant levels. During 28 days at 30°C, WP significantly suppressed lipid oxidation. GC-MS chemometric analysis revealed reduced oxidation-derived volatiles and preservation of freshness-related monoterpenes. These results demonstrate WP's effectiveness in regulating emulsion structure and oxidative pathways, highlighting its potential as a functional, plant-based ingredient for stable salad dressings.

Effect of green mediterranean diet on serum folate and its interaction with genetic variation in folate metabolism: The DIRECT PLUS 18-month dietary randomized controlled trial

Zelicha, RCTH; Keller, M; Meir, AY; Rinott, E; Tsaban, G; Kaplan, A; Hoffmann, A; Ceglarek, U; Isermann, B; Stumvoll, M; Shelef, I; Hu, FB; Stampfer, MJ; Kovacs, P; Shai, I (2026) Clinical Nutrition 63: 106701.

Folate metabolism can be regulated by diet and genetic variation, particularly Methylenetetrahydrofolate reductase (MTHFR) rs1801133 polymorphism. We explored the effect of plant-based green-Mediterranean (green-MED) diet on serum folate and whether this effect varies by genetic variation in folate metabolism over 18-months. In the DIRECT-PLUS trial, 294 participants were randomized to healthy dietary guidelines (HDG), MED, or plant-based green-MED diets. Both isocaloric MED groups consumed walnuts (28g/ day), while green-MED group also avoided red/processed meat, consumed *Wolffia globosa* Mankai green shake (500 ml/day), and green-tea (3-4 cups/day). MRI assessed visceral adipose tissue (VAT). MTHFR rs1801133 single-nucleotide-polymorphism (SNP), and folate-pathway mRNA expression were evaluated. Participants (age = 50.1years; 90.6% men; body-mass-index = 31.4 kg/m^2; serum folate = 7.6 ng/mL; rs1801133 frequencies: CC = 38.5%; CT = 49.8%; TT = 11.7%) had 89% 18-month retention rate. Folate deficiency at baseline was rare (similar to 3%, n = 8), indicating effects within the normal range. After 18-months of intervention, the green-MED diet significantly increased serum folate (+1.2 ng/mL) compared to MED (+0.41 ng/mL) and HDG diets (+0.1 ng/mL), $p < 0.05$ between groups, with higher Mankai intake strongly associated with folate elevation ($p = 0.003$). rs1801133 TT-genotype carriers had lower folate at baseline ($p = 0.037$) and post-intervention ($p = 0.04$; recessive mode-of-inheritance), while CC/CT-genotype carriers showed the highest folate increase following the green-MED intervention ($p = 0.03$). TT-genotype carriers showed lower baseline MTHFD2 mRNA expression ($p = 0.005$), but an enriched transcription, compared to CC/CT-carriers post-intervention ($p = 0.001$). Mankai intake interacted with rs1801133 genotype on VAT ($p = 0.028$) and Framingham risk score ($p = 0.024$), with CC/CT-carriers showing dose-response benefits. The green-MED diet, enriched with Mankai, significantly increased serum folate compared with the MED and HDG diets. The response differed by MTHFR rs1801133-genotype, with CC/CT carriers showing greater increases in folate. These findings support a gene-diet interaction with potential implications for visceral adiposity and cardiometabolic risk.

Duckweed proteins in Neuro-Nutrition: Mechanistic insights and translational relevance for military and extreme environments

Bandyopadhyay, A; Sharma, N; Patial, PK; Sati, MM; Pal, T (2026) Plant Foods for Human Nutrition 81: 89.

Modern military performance increasingly depends on cognitive resilience and neurological health, making nutrition a strategic determinant of combat readiness. This review integrates nutritional neuroscience and military medicine to explore duckweed as a next-generation neuro-nutritional resource. Duckweed species possess protein content (25-45% dry weight) and a complete essential almost amino acid (EAA) profile that may contribute to pathways involved in neurotransmitter synthesis, neuroplasticity, and stress adaptation. Beyond amino acids, duckweed delivers neuroprotective compounds such as lutein, beta-carotene, polyphenols, and vitamin B_{12} (pseudo-cobalamins) that mitigate oxidative stress, inflammation, and cognitive decline induced by fatigue or trauma. Its growth rate, minimal land and water requirements make it a sustainable, field-deployable protein source suitable for remote bases or disaster zones. This review synthesizes evidence on duckweed's biochemical composition, digestibility, and safety, highlighting its potential as a sustainable substitute for soy or *Spirulina* in military rations. The convergence of plant biotechnology, cognitive nutrition, and defense health positions duckweed as a strategic component of future military and space nutrition systems, supporting nutritional strategies relevant to brain health in demanding operational contexts.

Dietary *Wolffia globosa* extract alleviates winter thermal stress in *Labeo rohita*: Evidence from growth performance, digestive enzymes, hematology, and IGF-I gene expression

Yadav, NK; Patel, AB; Rajesh, SD; Kumar, P; Kumar, S (2026) Animal Feed Science and Technology 340: 116826.

A feeding trial was conducted to evaluate the potential of *Wolffia globosa* extract-supplemented diets in mitigating low-temperature stress in *Labeo rohita* fingerlings. Fish (initial weight 8.35 ± 0.50 g) were fed four isonitrogenous (25% crude protein) and isocaloric (16.5 MJ kg^{-1} gross energy) diets containing graded levels of *Wolffia* extract (WE: 0, 0.5, 1.5, and 3.0 g kg^{-1}) for 75 days under winter thermal conditions (18-22°C). Growth performance, including final body weight, weight gain, thermal growth coefficient (TGC), and net yield, was significantly enhanced in fish fed 1.5 g kg^{-1} WE compared with other treatments ($p < 0.05$), while feed conversion ratio (FCR) was significantly reduced. Survival, hepatosomatic index (HSI), relative gut length (RGL), and condition factor were not affected by dietary treatments. Digestive enzyme activities (amylase and lipase) and in vitro protein digestibility were significantly higher in WE-fed fish than in the control group. Hematological indices, including RBC, WBC, and Hb levels, increased significantly with increasing WE inclusion ($p < 0.05$). Moreover, the relative expression of insulin-like growth factor-I (IGF-I) was markedly upregulated, showing a 4.7-fold increase over the control in fish fed 3.0 g kg^{-1} WE. Overall, dietary supplementation with WE at 1.5-3.0 g kg^{-1} effectively improved growth, digestive efficiency, and hematological status of *L. rohita* under winter thermal stress.

DF comment: The species of *Wolffia* was evidently not identified.

Unlocking the potential of a novel integrated multi-trophic aquaculture (IMTA) approach for producing resilient foods at scale in a post nuclear war world: A perspective exploiting freshwater fish, duckweed and microalgae

Rowan, NJ; Jansen, MAK; Klimek, P (2026) Resources Environment and Sustainability 26: 100354.

The global agriculture system governing food security is strongly reliant upon environmental conditions including temperature, precipitation and sunlight, all of which could be significantly disrupted by a catastrophe such as a nuclear war. In addition to affecting primary agricultural productivity, a nuclear war would compromise critical supply chains and infrastructure. This perspective paper addresses a potentially new food production approach post catastrophe using integrated multitrophic aquaculture (IMTA) systems operating at scale. Developing a rapidly deployable IMTA system that (a) does not rely on using fertilizers, pesticides, pollination nor seed, (b) does not require arable land, (c) uses wastewater, (c), produces different secondary biomass (food) options rich in protein based on using fish waste produced onsite, (d) uses renewable wind energy, and (e) operates to zero waste principles underscore the rationale for its development and deployment post catastrophe. This regional approach may be rapidly deployed (within 4 to 6 months) to help offset reduced yields achieved from staple agricultural crops affected by abrupt sunlight reduction (ASR) and climatic cooling post catastrophe. By combining IMTA with onsite renewable wind-power, the system could potentially continue to operate, albeit at reduced levels of efficiency based, on previously modelled nuclear winter scenarios. Options for different resilient foods produced and dried at IMTA sites from same fish waste stream are described based on important factors for disaster preparedness and recovery. Despite the efficient demonstration and production of fish, duckweed and microalgae, it would be very challenging to replicate this same approach elsewhere in an appropriate timeframe to offset short term loss of food reserves given current operational challenges ranging from planning permission to meeting supply chain expectations. This paper provides a valuable insight into tangible issues to be addressed for the scaling of IMTA systems to support dietary needs of local and potentially regional populations. Given increasing geopolitical uncertainties globally, this paper highlights the potential need for developing resilient food processes that do not compete with staple foods for water, soil, fertilizer nor seed. However, there is a pressing need to strategically invest, share knowledge and prepare IMTA sites ahead of a catastrophe so as to appropriately meet societal needs.

Dietary *Wolffia globosa* extract enhances innate immunity, serum biochemical responses, antioxidant defense, and expression of related gene in *Labeo rohita* under winter thermal stress

Yadav, NK; Patel, AB; Kumar, P; S, DR; Priyadarshi, H (2026) Frontiers in Immunology 17: 1864774.

Temperature is a key environmental factor regulating physiological, biochemical, and immunological processes in ectothermic fish such as *Labeo rohita*. Exposure to low-temperature conditions during winter

disrupts metabolism, suppresses immunity, and induces oxidative stress, thereby compromising fish health and productivity. *Labeo rohita* fingerlings (initial weight: 8.35 ± 0.50 g) were randomly distributed into four dietary treatments with three replicates each and fed isonitrogenous (25% crude protein) and isocaloric (16.5 MJ kg^{-1} gross energy) diets supplemented with graded levels of *Wolffia* extract (WGE; 0, 0.5, 1.5, and 3.0 g kg^{-1}) for 75 days. The experiment was conducted in outdoor cemented tanks during the winter season (December-March) under natural low-temperature conditions (18-22°C). Dietary WGE significantly enhanced (P< 0.05) non-specific immune responses, with anti-protease activity increasing by 8.22-12.32%, myeloperoxidase by similar to 200-300%, respiratory burst by 76.92%, and lysozyme by up to 146.94% compared to the control. Carcass composition improved significantly, with crude protein reaching 18.01% and lipid 4.63%. Serum glucose decreased (15.78-49.26%), while total protein and albumin increased. Liver function markers, including SGOT, SGPT, ALP, and CK-MB, declined significantly, indicating improved hepatic integrity and reduced physiological stress. Antioxidant status improved, with elevated SOD and CAT activities and reduced MDA levels by up to 43.97% (liver) and 46.69% (serum). Gene expression analysis revealed downregulation of stress-related genes (hsp70: 0.042-fold; hsp90: 0.147-fold) and upregulation of immune-related genes (c3: 5.51-fold; tnf-alpha: 3.37-fold; ifn-gamma: 7.73-fold). Conclusion: This study evaluated the protective effects of *Wolffia globosa* extract on innate immunity, antioxidant defense, and stress- and immune-related gene expression in *Labeo rohita*. Dietary WGE effectively mitigated cold-induced stress by improving immune function, antioxidant capacity, and metabolic performance. Among the tested levels, 1.5 g kg^{-1} WGE provided the most balanced physiological benefits, while higher inclusion levels induced stronger immune and antioxidant responses. These findings highlight the potential of *Wolffia globosa* as a sustainable functional feed additive for enhancing fish resilience under low-temperature conditions.

Evaluating co-ensiling strategies to valorise duckweed as a sustainable feed ingredient

Lambert, M; Wambacq, E; Devlamynck, R; de Souza, MF; Vermeir, P; Raes, K; Eeckhout, M; Meers, E (2026) Plants 15: 1865.

Duckweed (Lemnaceae) is a promising alternative feed crop, particularly in regions with nutrient surpluses and protein deficits, as it grows efficiently on nutrient-rich agricultural wastewater and provides protein-rich biomass. However, its high moisture content and rapid post-harvest spoilage pose major storage challenges. This study evaluated (co-)ensiling as a cost-effective preservation strategy for duckweed. Three separate experiments were conducted to assess the ensilability of duckweed alone and in combination with various agricultural co-substrates and additives, including corn silage, beet pulp, grass silage, hemp shives, hay, molasses, sun-dried duckweed and $CaCO_3$. Duckweed alone could not be successfully ensiled due to excessive moisture, resulting in poor acidification and high levels of undesirable fermentation products. During the long-term co-ensiling test, a duckweed-corn silage mixture containing 29% fresh duckweed and 71% corn silage showed the most stable fermentation profile, with low pH, limited fermentation losses, and no detectable butyric acid. A duckweed-grass silage mixture containing 51% fresh duckweed and 49% grass silage allowed higher duckweed inclusion and retained the highest level of apparent pepsin-digestible protein after storage, but showed elevated acetic acid and ethanol concentrations. A duckweed-beet pulp mixture containing 74% fresh duckweed and 26% beet pulp enabled the highest duckweed inclusion rate, but showed signs of clostridial fermentation, likely due to excess moisture. Microbiological analysis of this beet pulp mixture showed reduced Enterobacteriaceae after ensiling, but also increased clostridial counts. Oxalic acid concentrations were low in all duckweed-based silages, with the largest reduction observed in the duckweed-grass mixture. Overall, the results show that duckweed co-ensiling is feasible but highly dependent on co-substrate selection and moisture control. Further formulation optimisation is required, particularly for high-duckweed mixtures, to reduce the risk of clostridial fermentation and improve practical applicability as a storable feed ingredient.

The effect of high-pressure processing on the quality of functional drinks from kale and *Wolffia*

Chotyakul, N; Rungpichayapichet, P; Siriwongwilaichat, P; Mahayothee, B (2026) Journal of Food Science and Technology-Mysore DOI10.1007/s13197-026-06723-7

This study investigated the effects of High-Pressure Processing (HPP) on the quality and shelf life of a functional drink formulated from kale juice and *Wolffia globosa*. HPP treatments were conducted at 200, 400, or 600 MPa for durations of 5 and 10 min at 25°C, and compared against conventional thermal pasteurization (90°C for 1 min) during an 8-weeks storage period at 5 ± 1 degrees C. Quality evaluation included physical attributes (color, viscosity), chemical profiles [total soluble solids (TSS), pH, protein, vitamin C, total phenolic content (TPC), antioxidant activity], and microbiological stability [total mesophilic aerobic bacteria (TMAB)], yeasts and molds] analyses. HPP treated samples exhibited significantly higher retention of chlorophyll pigments compared to thermal pasteurization, while maintaining pH stability ($p > 0.05$). Increasing pressure levels tended to increase TSS and viscosity whereas prolonged holding times resulted in slight reductions of these parameters. HPP exhibited high retention of protein, vitamin C, and TPC compare to pasteurization. Increasing pressure intensities significantly enhanced the inhibition of microbial growth, with 600 MPA providing optimal quality retention and microbiological safety throughout the storage duration. These findings suggest that HPP at 600 MPa serves as a better alternative to thermal processing for the preservation of nutrient functional beverages.

Lemnaceae as a poultry feed supplement: a review on the nutritional and economic potential for long term feed sustainability

Salian, A; Dhanorkar, M; Tongaonkar, S; Dey, D; Ali, S; Kumar, P; Singh, P (2026) Journal of Animal Science and Biotechnology 17: 94.

Soybean is a conventional and widely used protein source in the poultry feed. Fluctuating soybean prices linked to changing climatic and agroeconomic condition brings in multiple sustainability and food security concerns in the poultry feed sector. Additionally, competition for agricultural land, coupled to rising feed costs has put economic strain on the consumers. There is a need to promote alternative low-cost protein sources to bring in circularity in production systems, reduce environmental footprints, and improve long-term resilience of the poultry feed sector in the face of climate change. This work explores duckweed as a promising and economical alternative to soybean for use in the poultry feed sector and evaluates the limitations in their widespread use. Rich protein (around 35%-40%), high lysine content and diverse nutritional profile of duckweed confirm its suitability as an attractive component in the poultry feed, particularly relevant in the era of climate change and feed security crisis. Large scale production is however limited by processing and storage concerns that need to be further addressed to increase the economic viability of duckweeds. Production systems based on recycled wastewater makes the process sustainable. Reduction in antinutritive factors by enzymes or microbial fermentation will further enhance the acceptability of these plants as feed components. This study highlights opportunities to reduce the dependency on climate-sensitive soybean while promoting a circular bioeconomy approach in the poultry feed sector. Use of duckweed offers a promising approach to ensure a climate resilient and sustainable poultry sector, with potential to enhance food security.

Dietary supplementation of *Lemna minor* improves glycemic regulation in grass carp (*Ctenopharyngodon idella*)

Banik, A; Kumar, S; Srivastava, PP; Dubey, MK; Kumar, N; Brahmchari, RK; Nayak, SK. (2026) Scientific Reports DOI10.1038/s41598-026-50948-7.

Efficient carbohydrate utilization remains a major challenge in fish nutrition, particularly for fish fed high-carbohydrate diets. *Lemna minor* (duckweed), a plant rich in bioactive compounds including flavonoids and polyphenols, has potential as a functional feed ingredient to enhance glucose metabolism. In this study, grass carp were fed diets supplemented with *L. minor* at 2%, 6%, and 10% inclusion levels, alongside positive and negative control diets. Postprandial responses were monitored over 12h, assessing digestive enzyme activities (amylase, glucokinase, pyruvate kinase), glucose transporter expression (GLUT2), plasma metabolites (glucose, insulin), and hepatic metabolic markers, including glycolytic (Glucokinase and Pyruvate kinase) and gluconeogenic enzymes (G6Pase, FBPase, PEPCK) and glycogen content. Dietary *L. minor* significantly accelerated digestive enzymes, with amylase activity peaking at 3h post-feeding compared to delayed 6-h peaks in controls. GLUT2 expression upregulated in parallel, supporting enhanced glucose absorption. Hepatic glucokinase and pyruvate kinase activities were elevated in LM-fed groups, with LM10% demonstrating the highest responses. Correspondingly, plasma glucose was faster, with normoglycemia achieved by 6h in the

LM10% group, whereas controls exhibited prolonged hyperglycemia. Insulin secretion was robust and sustained in *Lemna*-fed groups (3-9h), contrasting with inadequate responses in controls. Hepatic G6Pase activity and gene expression were downregulated by LM-fed fish, indicating reduced gluconeogenesis, while glycogen storage was enhanced. These results indicate that *L. minor* improves postprandial carbohydrate metabolism in grass carp through coordinated modulation of digestive enzyme activity, glucose transport, insulin response, and hepatic metabolic pathways. The present study concluded that 10% inclusion level provided optimal metabolic benefits, highlighting the potential of *L. minor* as a sustainable, bioactive feed additive to enhance carbohydrate utilization in herbivorous aquaculture species.

Characterization of metabolites in plant-based milk yogurt enriched with *Wolffia globosa* to improve bionutritional and functional properties

Punyauppa-Path, S; Taesuk, N; Maneerat, S; Najomtien, P; Kiatprasert, P; Kanchanarach, W; Kanpipit, N; Phupaboon, S. (2026) Internatonal Jounal of Molecular Sciences 27: 4256.

Riceberry rice milk (RBRM) is rich in phytochemicals, particularly anthocyanins, which are known for their potential in managing type 2 diabetes (T2D). This study aimed to develop a novel RBRM-based yogurt derived from its polysaccharide and protein components and to evaluate the effects of supplementation with *W. globosa* powder (WGP) at 0% (F1, control), 5% (F2), 10% (F3), and 15% (F4) on nutritional and functional properties. Among all formulations, F4 exhibited the highest nutritional values, including dietary fiber (41.25%), curd protein (21.34%), and carbohydrate (starch) content (25.25%), with a lower fat content (2.13%) compared to other groups. In terms of antioxidant activity, F4 showed high total phenolic content (33.70 mg GAE/g) and total flavonoid content (25.2 mg QUE/g), along with strong radical scavenging activities, with DPPH and ABTS inhibition values of 41.52% and 78.18%, respectively. Furthermore, F4 demonstrated notable antidiabetic potential through alpha-amylase and alpha-glucosidase inhibition, with IC_{50} values of 0.89 and 1.32 mg/mL, respectively. Widely targeted metabolomics analysis identified 88 differential metabolites between F4 (potent condition) and F1 (control group). Twelve selected compounds from RBRM-WGP yogurt contributed to increased levels of amino acids, peptide derivatives, saccharides, organic acids, polyphenols, and flavonoids. Molecular docking analysis revealed that key metabolites, including vignatic acid B, glimepiride, and indoramin, exhibited strong binding affinities with the active sites of alpha-amylase (PDB: 2GVY, *Aspergillus niger*) and alpha-glucosidase (PDB: 3A4A, *Saccharomyces cerevisiae*). These findings indicate that phytonutrient compounds, particularly indoramin, play a significant role in enhancing the nutritional composition and functional properties of RBRM-WGP yogurt for potential applications in food processing.

In vivo safety evaluation of diet containing *Lemna minor* protein isolate & hydrolysate in mice

Sharma, S; Joshi, AKR (2026) Journal of Advanced Scientific Research 8: 1-19.

The safety and biological effect of protein isolate and protein hydrolysate were compared with casein based standard diet in Swiss albino mice in the present study. Animals were fed a 14% protein casein-based AIN-93M diet (control), isolate 14%, or hydrolysate 14% for 28 days and daily weight changes were recorded. After mild anesthesia, blood samples were collected from the retro-orbital area for biochemical and haematological analyses. Mice were sacrificed and the major organ tissues were examined. Serum biomarkers such ascreatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, blood urea, serum creatinine, lipid profile parameters, and random blood glucose levels, were analyzed. Statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test. All observed values remained within normal physiological ranges, indicating normal organ function and metabolic status. Overall, the findings suggest that dietary supplementation with *Lemna minor* protein isolate and hydrolysate does not elicit adverse biochemical or haematological effects in Swiss albino mice, thereby supporting their safety for potential nutritional and functional food applications.

Refining the moderate inclusion range of dried Asian watermeal (*Wolffia globosa*) in the diets of Two-Spotted Crickets (*Gryllus bimaculatus*): Integrating segmented regression and nutritional self-Selection

Mitchaothai, J; Lertpatarakomol, R; Lukkananukool, A; Trairatapiwan, T; Kaewsiri, N; Grabowski, NT (2026) Insects 17: 420.

The integration of rapidly renewable biomass into insect production systems has been proposed as a strategy to improve resource-use efficiency in insect production. This study evaluated the graded inclusion levels of dried watermeal (*Wolffia globosa*) in diets of two-spotted crickets (*Gryllus bimaculatus*) and assessed voluntary nutrient regulation under free-choice feeding. Four fixed-inclusion diets (0%, 25%, 35%, and 45% watermeal) and one self-selection treatment were tested over 28 days. Growth performance, feed conversion ratio (FCR), survival rate (Surv), production index (PI), and whole-body composition were determined. Repeated-measures analysis using linear mixed-effects models indicated that treatment, week, and their interaction were statistically significant ($p \leq 0.024$). However, partial R^2 analysis showed that the independent contributions of treatment and week were negligible, whereas the treatment x week interaction explained measurable variance, indicating that dietary effects were primarily expressed through time-dependent responses. Segmented regression identified a breakpoint at 35% watermeal inclusion (95% CI: 24.93-45.07), indicating that PI was the highest within a moderate supplementation range under the present fixed-diet conditions rather than at a precise single optimum. Inclusion levels beyond this threshold reduced performance. Under free-choice conditions, crickets progressively increased watermeal intake with age and maintained stable nitrogen-free extract (NFE):crude protein (CP) and gross energy (GE):CP intake ratios, selecting an average of 25-35% watermeal over the experimental period. This supplementation range improved feed efficiency and protein deposition while limiting lipid accumulation, suggesting improved energy-protein balance and nutrient partitioning. The self-selection result is interpreted as evidence of behavioral intake regulation under choice conditions and not as direct validation of the segmented-regression breakpoint. Collectively, these findings provide complementary statistical and behavioral evidence supporting a biologically relevant moderate inclusion range (approximately 30-35%) of dried watermeal.

Digestibility, energy value, and performance of *Lemna minor* as a novel protein source in broiler chicken diets

Niermeyer, J; Demann, J; Petersen, F; Dusel, G; Devlamynck, R; Ulbrich, A; Olfs, HW; Westendarp, H (2026) Poultry 5: 24.

Global population growth is expected to increase poultry meat demand, intensifying the need for sustainable protein sources. Soybean meal, the primary protein feed for poultry, has negative associations with deforestation and long transport distances. Duckweed has emerged as a possible, more sustainable alternative due to its high growth rate and protein yield. The nutrient digestibility and performance effects of the duckweed species *Lemna minor* (*L. minor*) in broiler diets were investigated in two experiments. Experiment 1 determined the ileal digestibility of crude protein, amino acids, phosphorus, and metabolizable energy in *L. minor*. The digestibility of most amino acids in *L. minor* ranged from 70% to 96%, with lysine and methionine at 87% and 86%, respectively. At 48%, the digestibility of cysteine was markedly lower than that of the other amino acids. However, the digestibility of P exceeded 90%. The energy values of dry matter were 7.05 MJ AME and 6.13 MJ. Experiment 2 tested the inclusion of *L. minor* (up to 10%) in isoenergetic and isonitrogenous diets. No significant effects on nutrient digestibility, weight gain, feed intake, or feed conversion ratio were observed. Both experiments demonstrate that *L. minor* cultivated under controlled conditions is a highly digestible, reliable feed source. Its inclusion in broiler diets is feasible, as it does not impair performance, yet provides amino acid balance whilst ensuring biomass quality. These findings support *L. minor* as a novel protein alternative and warrant further research on higher inclusion rates.

General Topics and Conferences

Duckweed: Research Meets Applications-2nd Edition

Sree, KS; Appenroth, KJ; Oláh, V (2026) Plants 12: DOI10.3390/plants15121846

This second edition of the Special Issue "Duckweed: Research Meets Applications" provides an update on the progress made in recent years in research on and the development of practical applications of duckweeds, tiny aquatic plants also known as water lentils (Lemnaceae Martinov). These monocots have emerged as model plants for research in various areas of plant biology. This edition comprises 12 original research articles, one review article, one perspective, and one conference report, several of which showcase national and international scientific and research cooperation amongst various groups of researchers worldwide. The 7th International Conference on Duckweed Research and Applications (7th ICDRA) held in Thailand from 12 to 16 November 2024 not only highlighted the progress made in the duckweed research arena but also the boom in the practical applications of duckweeds, especially regarding their use for human nutrition. A glimpse of the advances presented there is reported in the conference report (7th International Conference on Duckweed Research and Applications: Depicting an Era of Advancing Research Translation Toward Practical Applications).

Beyond tiny aquatic plants: how duckweeds serve the world as revolutionary crops for alleviating resource and energy crises

Yang, JJ; Islam, MF; Kazmi, A; Zhao, XY; Hu, HK; Sun, GH; Ishizawa, H; Hou, HW (2026) Food Chemsitry 516: 149330.

As an aquatic plant with outstanding growth and application potential, duckweed shows great value in ensuring food security, developing energy substitution, promoting biomanufacturing, and advancing ecological sustain-ability. This paper reviews its core advantages including fast growth and rich nutritional profiles (high protein, balanced amino acid composition, and abundant starch accumulation), as well as its diverse applications in alternative protein, sustainable biofuels, synthetic biology chassis, and space life support systems. It also addresses key technologies for large-scale production, such as cultivation optimization, high-density industrial cultivation systems, and food processing. Through life cycle and techno-economic assessments, this review clarifies the environmental and economic feasibility of duckweed utilization, providing a comprehensive reference for its future research and industrial translation.

Duckweeds: from fundamental biology to a sustainable plant chassis for biotechnology

Yin, GM; Yang, L; Li, S; Zhang, Y (2026) Advanced Biotechnology 4: 16.

Duckweeds (Lemnaceae), the smallest and fastest-growing flowering plants, have emerged as a transformative platform for sustainable biotechnology. This review synthesizes recent advances that underpin their potential as a next-generation plant chassis. We discuss duckweed's unique biology, characterized by reductive evolution, extreme phenotypic plasticity, and a simplified epigenome that favors transgene expression. The decoding of its minimalist genome, along with the establishment of efficient genetic tools including optimized transformation and CRISPR-Cas9 editing, enables precise genetic and metabolic engineering. While traditional uses in phytoremediation and animal feed validate its utility, duckweed's rapid growth in contained, soil-free culture and its edibility offer distinct advantages for molecular farming over established systems like tobacco. We highlight progress in engineering duckweeds to produce vaccines, therapeutic proteins, and high-value metabolites. To transition from proof-of-concept to an industrial workhorse, future efforts must focus on integrated omics databases, universal genetic toolkits, and scalable cultivation. Converging fundamental insights with synthetic biology principles positions duckweed as a versatile and powerful chassis for the bioeconomy.

Growth & Development

Root plasticity and elemental stoichiometry are associated with competitive shifts between *Azolla* and *Lemna* under different nitrogen levels

Liu, S; Liang, XY; Chen, YC; Li, MJ; Li, WJ; Zhang, JE; Li, RH (2026) Plants 15: 1853.

Nitrogen (N) availability fundamentally shapes the community structure and competitive dynamics of floating macrophytes in paddy ecosystems. This study investigated the competitive interactions between *Azolla* and *Lemna* by applying a gradient of N concentrations (0-12 mg L^{-1}) across two experimental periods (November-January and March-May). Our results demonstrate a clear divergence in resource-use strategies between the two species: *Azolla* exhibited stronger stoichiometric homeostasis and a more conservative growth profile, retaining a competitive advantage under N-limiting conditions. Conversely, Lemna displayed a more opportunistic strategy, gaining a competitive advantage in N-rich environments through greater morphological plasticity and luxury nutrient uptake. This nitrogen-driven shift in competitive balance was associated with differences in root traits and stoichiometric flexibility. Stoichiometrically, *Lemna* exhibited greater flexibility in nutrient balance, including higher phosphorus accumulation under N-rich conditions, which may support rapid biomass expansion. Differences between the two experimental periods were also associated with variation in trait expression, suggesting that temporal context influenced how the two species responded to N enrichment. These findings highlight the importance of nitrogen management in steering floating-plant communities in paddy ecosystems: low-N inputs may help maintain *Azolla*-dominated communities with biofertilizer potential, whereas high-N conditions may favor *Lemna* and its rapid nutrient uptake.

Duckweeds as a model for minimal floral development

Eu, CS; Chakraborty, A; Xu, SQ (2026) Trends in Genetics 42: 555-564.

While most frameworks of floral evolution are based on complex, multi-whorled flowers, duckweeds represent the opposite extreme, with highly reduced angiosperm reproductive structures comprising a single gynoecium and one or two stamens embedded within the frond. We review current work on the genetic regulation and evolution of duckweed flowers. Morphological evidence indicates duckweed flowers arose by progressive miniaturisation from a typical aroid spadix-spathe inflorescence. We outline how single-cell and multiomic atlases, integrated with comparative and population genomics, can reconstruct developmental trajectories across lineages. Treating duckweeds as a 'minimal flower' model could shed new insights into the minimal cellular and genetic programme required for flower development, and open opportunities to rationally engineer reproductive traits in crops and other plant systems.

Temperature-Dependent Clonal and Species-Level Growth Variation in *Spirodela*, *Landoltia*, *Lemna*, and Interspecific *Lemna* Hybrids

Mascheretti, I; Mallardi, A; Liberatore, C; Martinelli, T; Lauria, M (2026) Plants 15: 1649.

Duckweeds are minute, fast-growing monocot aquatic plants that propagate clonally and combine high biomass productivity with a valuable biochemical composition (high-quality proteins, a favorable polyunsaturated fatty acid profile, and starch-rich tissues) and efficient nutrient uptake, making them attractive for feed/food, bioenergy, and wastewater-based phyto-bioremediation. Temperature is a key factor shaping duckweed growth, and selecting clones that perform well within specific thermal ranges can improve cultivation across different applications. Here, we screened 97 clones from the genera *Spirodela, Landoltia*, and *Lemna*, including the hybrids *Lemna* x *japonica* and *Lemna* x *mediterranea*, under warm (WC; 30/25°C) and relative cool (CC; 20/16°C) conditions. Relative growth rate (RGR) ranged from 0.150 to 0.338 day^{-1} under WC and from 0.113 to 0.318 day^{-1} under CC, revealing strong interspecific and intraspecific variation. While WC generally promoted higher growth than CC, notable exceptions occurred at both interspecific and intraspecific levels. Tests under more extreme regimes (EWC; 35/30°C; ECC; 16/12°C) confirmed strong clone-specific responses, with some clones maintaining or improving growth under EWC relative to WC, whereas ECC generally reduced growth relative to CC. Climatic provenance was a weak predictor of performance, showing limited correspondence between RGR and mean annual temperature at the site of origin. Overall, these results highlight the value of within-species phenotyping across relevant temperature regimes to identify high-performing duckweed material for applied use.

History

First authentic fossil evidence of a great duckweed (Araceae: *Lemnoideae*) from the Eocene of India

Ali, A; de Almeida, RF; Patel, R; Rana, RS; Khan, MA (2026) Geobios 95-96: 1-11.

Spirodela Schleid. is one of the most abundant and widespread giant duckweeds (Araceae: *Lemnoideae*), although its fossil record remains relatively unexplored. We present a well-preserved, impressed and compressed fossil rosette bearing four leaves similar to this extant free-floating great duckweed from the early Eocene (Palana Formation) sedimentary sequences of Gurha Lignite Mine, Rajasthan, western India. We infer its taxonomic position based on phylogenetic analysis using macromorphological traits and compare it with other modern and fossil species of Lemnoideae. The fossil specimen is characterized by four peltate sessile leaves (one mother, two daughters, and one granddaughter) arranged spirally in a rosette, leaves with campylodromous venation, and the presence of stolons and adventitious roots. After a thorough comparison, we described our Eocene specimen as a new genus and species, namely Eospirodela indica Ali, Almeida et Khan gen. et sp. nov. Together with other fossils reviewed from the literature, this new taxon helps to elucidate the evolution of duckweeds. Our finding strongly supports the existence of a freshwater ecosystem complementing the earlier published data on the Rajasthan Basin during the early Eocene (55 ± 10 Ma), in contrast to the present desert vegetation of the region. It also throws light on the paleoecological and paleobiogeographical history of duckweeds.

DF comment: Most duckweed researchers consider duckweed as a plant family, Lemnaceae (Tippery et al. Plants (2021) 10, 2639).

Interaction with other organisms

Microbial community restructuring and transcriptional responses to acute stress in duckweed enhance lead phytoremediation

Dong, YH; Meng, B; Huang, JH; Mao, K; Liu, YT; Geng, HY; Tan, AJ; Yang, GL; Feng, XB (2026) Journal of Hazardous Materials 514: 142796.

Lead (Pb) contamination in aquatic ecosystems poses a persistent threat to environmental quality and human health. Duckweed-mediated phytoremediation serves as a valuable model for exploring the short-term physiological endurance of aquatic macrophytes under extreme Pb stress in highly contaminated aqueous environments. However, the integrated mechanisms underlying Pb hyperaccumulation in this system remain insufficiently understood. In this study, we employed a combined physiological, microbiomic, and transcriptomic approach to investigate the acute stress responses of the hyperaccumulating duckweed *Landoltia punctata*. Noninvasive micro-test technology (NMT) demonstrated that this hyperaccumulation was driven by enhanced, rootspecific Pb^{2+} uptake, with the Pb-hyperaccumulating genotype exhibiting a 36.08% higher net influx than the non-hyperaccumulating genotype. Meanwhile, Pb exposure induced pronounced kingdom-specific restructuring in the root-associated microbiome, characterized by bacterial specialization for potential detoxification and fungal transitions toward opportunistic saprotrophy. Transcriptomic profiling further revealed a transcriptional shift favoring defense pathways in the host, marked by the upregulation of core stress signaling and the repression of energy-intensive lipid metabolism to sustain essential structural barriers. Collectively, our findings indicate that this short-term physiological endurance involves a complex host-microbiome response, with causal relationships requiring further functional validation. These results provide mechanistic insights and a theoretical framework for future optimization of phytoremediation systems.

Selective feeding of the duckweed weevil on three common species of duckweed in Japan

Isoda, M; Takahashi, K; Yagi, H; Muranaka, T; Oyama, T; Kanaoka, MM (2026) Plant Species Biology 41: e70065.

Duckweed is a floating aquatic plant that inhabits freshwater ecosystems and has attracted attention for its potential applications in animal feed and biofuel production. To facilitate such practical applications, an efficient and cost-effective cultivation system is required. Duckweed weevils are among the most common and widely distributed insect herbivores that feed on duckweed. In this study, we investigated the duckweed weevils (*Tanysphyrus brevipennis*) behaviors and feeding preferences for three common duckweed species: *Spirodela polyrhiza* (Sp), *Landoltia punctata* (Lp), and *Lemna aequinoctialis* (La). Fasted duckweed weevils were placed with individual duckweed species, and the Lp consumed significantly smaller areas than Sp and La because of fewer duckweed weevil feeding events and less damage per feeding bout. In the mixed-species settings, duckweed weevils showed a clear feeding preference for Sp and La over Lp; the preference in descending order was Sp, La, and Lp. We further examined the effect of anthocyanins on preference using stress-induced anthocyanin accumulation in Sp. Plants with anthocyanins had smaller areas of consumption than those without anthocyanins, suggesting that anthocyanins serve as a deterrent to duckweed weevil feeding. In conclusion, our findings regarding the feeding preferences of duckweed weevils and the defensive role of anthocyanins provide valuable insights for the dynamics of the community composition of coexisting duckweed species, contributing to more robust and efficient duckweed cultivation systems.

Metabolomics responses of *Microcystis aeruginosa* to UV-B radiation and TiO_2 nanoparticles in monoculture and co-culture with *Lemna minor*

Abdulmalik, E; Ahmed, ZP; Otogo, RA; Samuel, SA; Daniel, BA; Abdullahi, AK; Adamu, I; Cataldi, TR; Labate, CA; Chia, MA (2026) Algal Research 96: 104764.

As anthropogenic activities have increased, stratospheric depletion has also increased, resulting in intensified ultraviolet (UV) radiation, especially UV-B, reaching the Earth's surface. Concurrently, the expansion of the manufacturing sector has increased the release of titanium dioxide nanoparticles (TiO_2 NPs) into freshwater ecosystems. Both UV-B radiation and TiO_2 NPs are known to increase reactive oxygen species (ROS) production; however, their combined effects on the metabolomic profile of cyanobacteria during interactions with aquatic macrophytes, remain poorly understood. This study investigated the effects of high (2 W m^{-2}) and low (0.8 W m^{-2}) UV-B radiation, individually and in combination with TiO_2nanoparticles (48 mg/L), on the growth and metabolomic profile of *Microcystis aeruginosa* in monoculture and co-culture with *Lemna minor*. The experiment was conducted for seven days under laboratory conditions designed to simulate acute stress, and growth was monitored on days 1, 3, 5, and 7. Metabolomic profiling was performed using two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC x GC-TOFMS). The results showed that the growth of *M. aeruginosa* was significantly reduced across all treatments but not in the co-culture experiment, revealing the regulatory role of biological interactions. Metabolomic analysis revealed treatment-specific changes in amino acid, lipid, organic acid, carbohydrate, and secondary metabolic pathways under UV-B and TiO_2 NPs exposure, highlighting oxidative stress and defense mechanisms. The findings show that abiotic and biotic stressors interact to redefine metabolic pathways and oxidative stress responses in *M. aeruginosa*, highlighting the importance of macrophyte interactions and chemical stressors in predicting bloom formation in freshwater ecosystems.

DF comment: Identification on morphological basis alone does not identify *Lemna minor* reliably.

Molecular Biology and Genomics

Chromosome-scale genome assemblies of duckweeds provide insights into genomic plasticity, aquatic adaptation and morphological reduction

Tek, MI; Boyle, B; Normandeau, E; Lecomte, L; Sabri, M; Torkamaneh, D. (2026) Genome Biology and Evolution DOI10.1093/gbe/evag163.

Duckweeds (Lemnaceae) present a striking example of convergent genome evolution following the return from land to water. As the smallest and fastest-growing angiosperms, they exhibit extreme morphological reduction yet retain remarkable genomic plasticity through recurrent interspecific hybridisation, chromosomal rearrangements, and selective gene-family remodelling. The genomic mechanisms that distinguish this secondarily aquatic lifestyle from terrestrial ancestors, and whether these changes are convergent with other

aquatic lineages such as seagrasses, have remained incompletely resolved. Here we report chromosome-scale genome assemblies for four duckweed species, *Spirodela polyrhiza*, *Lemna minuta*, *Lemna japonica*, and *Lemna aequinoctialis*, generated with PacBio HiFi long reads and Omni-C chromatin conformation capture. These assemblies include the first genomic characterisation of an unresolved hybrid lineage (*L. aequinoctialis* *) that harbours a previously uncharacterised 3.5 Mb reciprocal translocation between subgenomes, as well as confirmation of the allodiploid origin of *L. japonica*. Comparative phylogenomics with land plants and the seagrass *Zostera marina* reveals a coherent, non-random programme of gene loss: effector-triggered immunity (ETI) components (EDS1 and PAD4) and the high-affinity nitrate transporter NRT2 are convergently absent across duckweeds and *Z. marina*, consistent with relaxed pathogen pressure and abundant dissolved nutrients in aquatic habitats. In contrast, secondary-metabolite biosynthesis pathways for flavonoids, anthocyanins, flavones and flavonols are retained or expanded despite overall genome compaction. These findings illustrate how the return to aquatic environments following terrestrialisation shaped duckweed genome evolution through convergent gene loss and selective pathway retention, and provide high-quality genomic resources to support future research in plant evolutionary biology and biotechnology.

Triploidy is prominent in the duckweed Lemna minor complex

Michael, TP; Hoang, PTN; Abramson, BW; Mamerto, A; Pasaribu, B; Fuchs, J; Ernst, E; Allsing, N; Braglia, L; Petrus, S; Schubert, V; Hartwick, N; Wang, M; Chen, Y-F; Lensink, M; Duong, T; Colt, K; Bog, M; Sree KS; Morello, L; Appenroth, KJ; Schubert, I; Martienssen, RA; Lam, E (2026) Communications Biology DOI10.1038/s42003-026-10294-w.

Duckweeds (Lemnaceae Martinov) are aquatic monocotyledonous plants known to be the smallest and fastest-growing flowering plants on Earth. Many species are morphologically indistinguishable due to their reduced structures, yet molecular evidence suggests that visually similar clones could be distinct species or hybrids. Using a comprehensive suite of genomic, molecular and cytogenetic methods, we analyzed 58 presumed *Lemna minor* clones to resolve their relationships. Our study revealed extensive genome plasticity within the "*Lemna minor* complex," identifying diploid and triploid *L. minor* clones, as well as allodiploid and allotriploid interspecific hybrids, in addition to a novel African-clade *L. minor* lineage. Triploidy in the *L. minor* complex is prevalent in nature, occurring in 33% of the clones previously identified as *L. minor* in our collection, and is associated with enhanced growth metrics under optimal conditions. For asexually propagative species such as duckweeds, where the triploid block is not a deterrent, triploid progenies may become the majority as has been observed in numerous wild *Lemna* populations. Our study shows that polyploidy and hybridization in these primarily asexually propagating plants could play important roles in their population dynamics and evolution under changing environments.

Duckweed biomanufacturing: longstanding promise, decade-long lag, recent renaissance

Tek, MIbrahim; Villot, S; Michaud, D; Torkamaneh, D. (2026) Trends in Biotechnology DOI10.1016/j.tibtech.2026.05.006.

Molecular farming has become a mature, cost-competitive route to recombinant protein, vaccine, and phytochemical production. *Nicotiana benthamiana* dominates the landscape, while aquatic plants of the family Lemnaceae (duckweeds) continue to be the perennial 'next big thing' that never truly arrives. These tiny floating plants offer the fastest biomass doubling time of any angiosperm, up to 45% crude protein, human-compatible N-glycosylation, and the unique ability to grow on wastewater. Monoclonal antibodies and edible vaccines have been expressed at research scale for 2 decades. Still, there is no duckweed-derived biomolecule manufactured commercially. In this opinion article, we examine the reasons for this paradox and ask whether explosive progress in the last few years finally turns duckweed from perennial hype into a genuine industrial platform.

Repeatability of phenotypic consequences due to whole-genome duplication in *Spirodela polyrhiza*

Mortier, F; Bafort, Q; Bonte, D; van de Peer, Y (2026) New Phytologist DOI10.1111/nph.71256.

Whole-genome duplication (WGD) is widespread in plants, yet the extent to which it yields predictable phenotypic outcomes remains unclear. Here, we show that the phenotypic consequences of genome doubling in a duckweed model system, *Spirodela polyrhiza*, are highly repeatable and largely deterministic. We previously generated three independent colchicine-induced autotetraploids from each of nine globally distributed diploid genotypes and now quantified growth and morphology across a salt gradient. In benign conditions, diploids grew faster, whereas tetraploids had larger, thicker fronds. As salinity increased, the diploid growth advantage diminished, and tetraploids frequently matched or exceeded diploid growth rates. By partitioning the components of variance in growth in our experimental design, we found that ploidy per se explained a comparable amount of phenotypic variation in growth and substantially more variation in salt tolerance than the genotypic background, with evidence of rare within-genotype stochastic differences between tetraploids. These results indicate that the shifts in morphology and stress tolerance from genome doubling are predictable and can match the phenotypic effect from genetic sequence diversity.

Newly unveiled meiosis elucidates the unreduced gamete frequency and its impact on the evolution of the *Lemna minor* complex

Lee, Y; Schubert, V; Stepanenko, A; Kim, G; Braglia, L; Schubert, I; Morello, L. (2026) Journal of Experimental Botany DOI10.1093/jxb/erag215

Fusion of gametes possessing meiotically reduced (haploid) chromosome complements is the main pathway of propagation among eukaryotes. However, duckweeds, the smallest angiosperms, propagate mainly vegetatively, and meiosis has not yet been documented in detail for this plant family. The more surprising was the recent evidence of rather frequent interspecific hybrids and triploid clonal accessions, which became obvious by genome size measurements, genomic in situ hybridization (GISH), and combined plastid and nuclear DNA markers. These observations indicated sexual propagation involving reduced as well as unreduced male and female gametes in *Lemna minor* and *L. turionifera*, leading to allodiploid and allotriploid hybrids (MT, MMT, MTT) and autotriploid *L. minor* (MMM) accessions. Here, we i) documented the meiotic stages of *Lemna* species for the first time photographically; ii) provided evidence of unreduced male gametes through fluorescent in situ hybridization (FISH) with single locus probes; iii) determined their abundance in different individuals and iv) discussed possible reasons for unreduced male gamete formation. These findings open new insights into the modes of sexual reproduction and evolution of duckweeds, which may be useful for future breeding efforts in this emerging crop.

Physiology & Stress

Multiple stressors may pose greater risks to macrophyte community dynamics by driving complex and unanticipated higher-order interactions

Mao, HZ; Tian, YQ; He, GR; Yang, H; Zhu, L; Cun, ZJ; Li, ZQ (2026) Environmental Pollution 401: 128286.

Freshwater ecosystems play a pivotal global role in water security and ecosystem services, but are increasingly threatened by multiple stressors, particularly emerging contaminants. These contaminants are in proximity to human activities and tend to accumulate in sediments, which further amplifies their ecological impacts on freshwater ecosystems. Nevertheless, most studies have focused on the effects of single or pairwise stressors, leaving the effects of complex higher-order interactions on macrophyte communities scant. Here, we conducted a full-factorial experiment using 96 glasshouse mesocosms in 10 L systems containing two macrophyte species (*Lemna minor* and *Spirodela polyrhiza*) maintained in a controlled glasshouse environment to investigate the single and higher-order interactive effects of the three emerging contaminants, including microplastics, antibiotics, and antiviral drugs, on two common free-floating macrophytes and their interactions. Our results show that individual and combined stressors cause species-specific responses in free-floating macrophytes, thereby directly or indirectly altering community dominance patterns. Multiple stressors mainly showed antagonistic interactions, triggering negative feedback regulation that alleviated macrophyte growth inhibition. And the feedback is context-dependent, as changes in environmental conditions and stressor combinations may reverse these feedback patterns, potentially enhancing ecological toxicity. Our findings highlight that the inherent complexity and unpredictability of higher-order interactions among multiple stressors can heighten risks to macrophyte communities. We advocate that

evaluations of multiple stressors in freshwater ecosystems should integrate context-dependent responses, stressor interactions, and feedback due to redundancy and compensatory dynamics. Future studies should incorporate both biotic and abiotic stressors across multiple gradients and higher ecosystem levels, accounting for non-linear and context-dependent responses.

DF comment: *Lemna minor* was not identified. Thus, the paper described the effects on *S. polyrhiza* and an unknown *Lemna*.

High-value biochar from sunflower husk pyrolysis enhances growth and physiological performance of *Spirodela polyrhiza*

Osmanska, J; Szufa, S; Romanowska-Duda, Z; Mohammad, O; Unyay, H; Wozniak, C; Piersa, P; Czerwinska, J; Kazimierski, P; Regkouzas, P; Stefanakis, A; Panek, B; Onwudili, JA (2026) Scientific Reports DOI10.1038/s41598-026-58255-x.

Rising populations intensify food demand and place increasing pressure on already degraded soils, driving the need for sustainable, waste-derived fertilizers that can enhance plant growth. This research aimed to assess the potential use of biochar as a soil amendment to support plant growth. The biochar used in this study was produced from sunflower husk and examined in three variants obtained at pyrolysis temperatures of 300, 400, and 500°C, applied at different dosage levels. *Spirodela polyrhiza* served as the model plant species. Assessment was based on changes in chlorophyll index, fluorescence, frond number, and overall plant condition. Temperature-dependent changes in biochar properties were further analysed using SEM, FTIR, BET, and GC-MS. The results indicate that the pyrolysis products used in this study did not exhibit toxic effects on *S. polyrhiza* growth. Instead, all analysed variants showed a generally positive influence on plant development, reflected in a clear stimulatory response. The findings demonstrate that the impact of biochar on plant growth was strongly dependent on both the pyrolysis temperature and the applied dose, with the most favourable outcomes observed for biochar produced at 400-500°C. Overall, the study identifies sunflower husk as a duckweed growth-stimulating material and supports its further consideration as an environmentally safe, waste-derived fertilizer component.

Developing carbon assimilation methods in duckweed for insights into photosynthesis and growth mechanisms

Rintoul, RC; Gill, AR; McAusland, L; Murchie, EH; Gilliham, M; Mortimer, JC (2026) Plant Physiology DOI10.1093/plphys/kiag311

Infrared gas analysis (IRGA) is the primary technique for quantifying CO_2 uptake in plants, but technical obstacles have limited its application to aquatic species, inhibiting exploration of their photosynthetic mechanisms. Using an IRGA with a customised aquatic chamber, we developed a methodology for measuring CO_2 responses in duckweed, a group of fast-growing, floating angiosperms. We compared three morphologically contrasting species which have commercial or environmental significance: *Wolffia australiana*, *Spirodela polyrhiza*, and *Lemna minuta*. Light response curves showed species variation in light saturation intensity and light saturated rates of photosynthesis, with *W. australiana* generally showing the highest rates of exchange per unit area under CO_2 limited and CO_2 saturated conditions. We estimated biochemical parameters (V_{cmax}, J_{max}) from CO_2 response curves, though direct measurement of conductance and intercellular CO_2 (Ci) was not possible. Using estimated C_i, predicted Vcmax and Jmax values fell within ranges consistent with other angiosperms, and again *W. australiana* had the highest values. Temperature response curves suggested an optimum of 35°C. Combining gas exchange and chlorophyll fluorescence further revealed a higher photosynthetic performance in *W. australiana* grown at lower plant densities. The suite of methodologies presented here will enable future mechanistic studies of duckweed carbon assimilation, growth dynamics and environmental responses, advancing understanding to levels comparable with that of many other terrestrial plant species.

DF comment: The clones come from an established stock collection but nothing was reported concerning the species identification.

Trophic diversity in duckweed: Mixotrophy, more than the sum of its extremes

Sun, ZL; Chen, Y; Li, FJ; Zhao, XY; Han, QX; Hou, HW (2026) Advanced Science DOI10.1002/advs.75665

The duckweed family Lemnaceae, aquatic plants within the early-diverging monocot order Alismatales, are model organisms in plant biology. Owing to their exceptionally rapid growth rates, high protein content, notable capacity for starch accumulation, and demonstrated utility in phytoremediation, duckweeds are increasingly recognized as promising candidates for diverse biotechnological applications spanning environmental remediation, bioenergy production, and sustainable biomass generation. Mixotrophy - the capacity of autotrophic plants to utilize exogenous organic carbon - is increasingly recognized as widespread in aquatic ecosystems, yet it has been less studied in duckweeds compared to microalgae and cyanobacteria. Here, we compile evidence from physiological, structural, and environmental perspectives supporting mixotrophy and heterotrophy in duckweeds, a clonal, genomically tractable angiosperm with rapid vegetative growth and direct environmental exchange, offering advantages beyond microalgal models. We emphasize mechanisms that enable mixotrophy to achieve synergistic outcomes beyond the additive effects of autotrophy and heterotrophy. Additionally, we explore the ecological and biotechnological significance of these trophic strategies and propose that duckweeds represent a valuable model for understanding mixotrophy across aquatic plants and for linking microalgal findings with higher-plant physiology.

Heritable transgenerational fitness variation correlates with copper resistance in the clonal duckweed *Spirodela polyrhiza*

Chávez, A; Schafer, M; Finkemeier, I; Xu, SQ; Huber, M (2026) Proceedings of the Royal Society B – Biological Sciences 293: 20253304.

Transgenerational effects can vary among genotypes of a given species; however, we cannot yet predict the direction and magnitude of the transgenerational effects. Here, we explored whether a genotype's transgenerational fitness effects correlate with plant resistance and the production of specialized metabolites. Thus, we exposed monoclonal, single-descendant lineages from 56 globally distributed genotypes of the clonal duckweed *Spirodela polyrhiza* to five generations of control conditions or copper excess, followed by five generations without stress, and then measured plant fitness and phenotypes in both environments. Previous copper excess reduced fitness variation within genotypes, while generating heritable and repeatable differences in transgenerational fitness effects among genotypes: genotypes in which previous stress increased fitness under recurring conditions in comparison to controls tended to show reduced fitness when the stress was absent, and vice versa. The transgenerational fitness effects correlated with copper resistance: the more susceptible a genotype, the more its offspring suffered from ancestral stress in the absence of recurrent stress; however, these offspring tended to be more resistant to copper excess, likely by protecting photosystem II. Together, these findings show that transgenerational fitness effects are heritable, variable and can be anticipated from stress resistance.

Phytomedicine

Fish immunization by duckweed biomass accumulating recombinant cyprinid herpesvirus 3 antigens induces specific immune response

Kishchenko, O; Peterson, A; Adamek, M; Schroers, VJ; Piackova, V; Korytar, T; Cuacos, M; Giritch, A; Kuhlmann, M; Schubert, I. (2026) New Biotechnology 94: 272-282.

Cyprinid herpesvirus-3 (CyHV-3) is highly contagious and lethal to cyprinid fish, necessitating development of sustainable vaccination strategies. Our study explores duckweed as platform for vaccine production and as vehicle for oral administration which is considered most suitable for mass vaccination of fish. Using a deconstructed potato virus X-based transient expression system, serologically recognizable recombinant CyHV-3 antigens (ORF25, ORF81, ORF136, ORF72) and fusion proteins (ORF136::T2A::ORF72, ORF25::T2A::ORF81) were accumulated in the duckweed *Landoltia punctata*. The specific immune response of fish on immunization by the freeze-dried antigen-expressing duckweed biomass was proved by the serum

neutralization test. Uptake of green fluorescent protein fused to the adjuvant cholera non-toxic subunit B (CTB::GFP) from duckweed biomass by fish intestinal cells was demonstrated after intubation of fish with CTB::GFP-expressing biomass. Therefore, transient expression of recombinant proteins in duckweed is a promising tool for antigen production and can facilitate the development of oral vaccines for veterinary application.

Wolffia globosa ethanolic extract protects against bisphenol A-induced osteoblast dysfunction via antioxidant defense, apoptosis inhibition, and β-catenin modulation

Wudtiwai, B; Pitchakarn, P; Temviriyanukul, P; Sittiju, P; Inthachat, W; Karinchai, J; Phunsanit, N; Kongtawelert, P; Pothacharoen, P (2026) International Journal of Molecular Sciences 27: 5352.

The prevalent endocrine disruptor bisphenol A (BPA) is associated with aging-related conditions, including metabolic disorders. It has been shown that BPA promotes bone fragility through oxidative stress-induced apoptosis and impaired osteoblast differentiation. The identification of sustainable bioactive substances that alleviate BPA-induced bone toxicity is thus of biomedical and environmental significance. Wolffia globosa (WG), the world's smallest flowering aquatic plant, has recently gained attention as a high-protein, antioxidant-rich nutraceutical, yet its impact on BPA-induced osteoblast dysfunction has not been systematically investigated. This study presents a comprehensive assessment of WG ethanolic extract (WGE) in MC3T3-E1 pre-osteoblasts, incorporating thorough phytochemical characterization, acute high-dose and chronic low-dose BPA exposure models, and multi-faceted mechanistic analysis. LC-MS/MS profiling identified luteolin (116.17 ± 0.69 μg/g), rosmarinic acid (54.80 ± 2.12 μg/g), and apigenin (48.77 ± 0.61 μg/g) as the predominant bioactive compounds. WGE exhibited potent antioxidant capacity across DPPH and ABTS radical scavenging assays, complemented by high ORAC and FRAP values, reflecting broad-spectrum antioxidant mechanisms. Treatment with WGE (25 and 50 μg/mL) resulted in significant alleviation of BPA-induced cytotoxicity, decreased intracellular ROS levels, and inhibited apoptosis. WGE (12.5 μg/mL) also modulated autophagy-related markers (LC3-II, Beclin-1, and p62), suggesting potential autophagic participation, although flux verification was not conducted. Treatment with WGE (12.5 μg/mL) also restored BPA-suppressed osteogenesis under chronic exposure, as evidenced by enhanced alkaline phosphatase activity, and increased both mineralization and upregulation of osteogenic genes including runt-related transcription factor2 (Runx2), collagen type I alpha 1 (Colla1), alkaline phosphatase (ALP), and osteocalcin (OCN). These effects were accompanied by partial reactivation of Wnt/beta-catenin signaling. This study is the first to demonstrate that WGE protects osteoblasts from BPA toxicity by concurrently strengthening antioxidant defenses, limiting apoptosis, modulating autophagy-related markers, and supporting beta-catenin-mediated osteogenesis, highlighting WG as a promising sustainable nutraceutical candidate for the prevention of environmental toxin-related bone fragility.

DF comment: Also in Thailand *Wolffia* should be confirmed to be *W. globosa*.

Phytoremediation

Morphophysiological characteristics of two duckweed species grown in gold mine tailings and HAuCl for phytomining purpose

Yusuf, RM; Chandra, AK; Miftahudin, M; Dorly, D; Satya, A; Susanti, E; Hamim, H (2026) Plant and Soil DOI10.1007/s11104-026-08815-x

Phytomining can be used to extract gold from low-concentration sources, such as gold mine tailings. Duckweeds (Lemnaceae) are known as heavy-metal hyperaccumulators. This research aimed to observe the morphological, anatomical, and physiological characteristics of two duckweed species (*Landoltia punctata* and *Lemna aequinoctialis*) grown in media containing tailings and gold solution (HAuCl4) and to assess their gold accumulating capability. The experiment was carried out by exposing *L. punctata* and *L. aequinoctialis* to 2.5 L of Hoagland's solution treated by 0 g (0%), 100 g (4%), 250 g (10%), 500 g (20%) of tailings suspended by aqua regia, or by 0.7 mM of HAuCl4 (Au 138 ppm). The treatments and observation were carried out in greenhouse conditions for 7 days. The duckweeds were then harvested for morphological, anatomical, and

physiological measurement, and gold accumulation analysis. A high content of tailing or Au treatment caused stunting growth in both duckweeds. Anatomical and physiological observations revealed that both duckweeds experienced heavy metal stress, indicated by increasing reactive oxygen species (ROS) and thinner fronds. *L. punctata* had superior ability to tolerate the stress based on the ability to increase ROS scavengers to countermeasure ROS production. In addition, under the highest tailing content (20%), *L. punctata* was still able to accumulate gold up to 1.7 μg g^{-1} and produced higher gold nanoparticles with gold content reached 2.3% in media containing 138 ppm Au. The gold phytomining process caused stress in duckweeds. Nevertheless, both species have great potential as gold phytomining agents.

DF comment: To distinguish *L. aequinoctialis* from other *Lemna* species on a morphological basis is very difficult.

Integrated effects of light intensity, spectral composition and photoperiod fragmentation on growth and nutrient removal of three duckweed species

Cichy, P; Kalka, J. (2026) Scientific Reports DOI10.1038/s41598-026-59786-z.

The performance of duckweed-based systems used for wastewater treatment and biomass production depends strongly on light conditions, yet the combined effects of light intensity, spectral composition, and photoperiod structure on plant growth and nutrient removal remain insufficiently understood. This study evaluated how these light parameters influence growth and nutrient removal in three widely distributed and commonly studied duckweed species (*Lemna minor*, *Landoltia punctata* and *Spirodela polyrhiza*) cultivated under controlled conditions. A range of sub-saturating to saturating and potentially supra-saturating light intensities (50-1220 μmol m^{-2} s^{-1}), spectral composition (blue, green, red, white and mixed spectra), and fragmented photoperiod regimes (12L:12D to 1L:1D) were examined. Spectral and photoperiod treatments were conducted at a constant daily light integral (~12.96mol m^{-2} d^{-1}), allowing comparison of light-quality and temporal effects under the same total photon input. Relative growth rate (RGR), dry biomass as well as nitrogen ($N\text{-}NO_3$) and phosphorus ($P\text{-}PO_4$) removal were assessed. Growth responses showed saturation with increasing light intensity, with RGR reaching saturation at lower levels (160-296 mol m^{-2} s^{-1}) than dry biomass (~343-440 μmol m^{-2} s^{-1}). Red-enriched white light generally enhanced growth and nutrient removal across species, whereas fragmented photoperiods reduced growth despite identical daily light integral.

DF comment: In contrast to *S. polyrhiza* and *L. punctata*, *L. minor* cannot be identified on a morphological basis only.

Transcriptomic insights into nitrogen-regulated cadmium absorption and accumulation in *Spirodela polyrhiza*: toward enhanced phytoremediation strategies for cadmium-polluted environments

Yang, J; Islam, MF; Sun, G; Hu, H; Heenatigala, PPM; Zhao, X; Hou, H. (2026) Environmental Pollution 406: 128621.

To reveal the molecular mechanism by which nitrogen (N) regulates cadmium (Cd) uptake and accumulation in *Spirodela polyrhiza*, transcriptome analysis was performed on *S. polyrhiza* 7498 under low nitrogen (0.25N) and high nitrogen (1N) conditions combined with 50 μM $CdCl_2$ stress. We systematically investigated the effects of N on metabolic pathways and key regulatory factors in *S. polyrhiza* under Cd stress. A total of 19,325 genes were identified, and the number of differentially expressed genes (DEGs) increased significantly with prolonged treatment time. These DEGs were mainly enriched in pathways related to oxidative stress, metal ion binding, photosynthesis, and sugar metabolism. N enhanced Cd transport and detoxification by upregulating the expression of heavy metal transporter genes including IRT, MTP, HMA, and HIPP26. Meanwhile, it increased the expression of genes associated with chlorophyll synthesis, photosystems, and ATP synthesis to alleviate Cd-induced inhibition of photosynthesis, and regulated sugar metabolism to promote starch degradation and sucrose transport, thereby providing carbon skeletons to support Cd stress responses. In addition, transcription factors such as bHLH56, bHLH47 and WRKY4 played crucial regulatory roles in N-Cd crosstalk. Among them, GATA22, as a hub gene in the co-expression network, co-ordinately regulated genes involved in N metabolism, photosynthesis, and Cd tolerance. This study provides a theoretical basis for illustrating the

molecular mechanism of N-Cd interaction in aquatic plants, and offers genetic resources and technical insights for enhancing phytoremediation of Cd-contaminated water bodies using *S. polyrhiza* combined with N management.

DF comment: Evidently, 1N does not mean 1 normal solution but designates group 1, which has 10 mM ammonium nitrate.

Sustainable phosphorus recovery and starch-protein-rich biomass generation from wastewater via duckweed

Chen, Y; Cai, YX; Zhang, YH; Li, SY; Long, SY; Xiong, XQ; Liu, M; Chen, JY; Ma, SZ; Zhang, LY; Sun, ZL (2026) IScience 29: 116515.

Escalating phosphorus (P) pollution and resource scarcity underscore the urgent need for sustainable wastewater treatment and nutrient recovery systems. However, systematic research on P-removal efficiency and its recovery across concentrations under mixotrophic conditions is still lacking. In this study, *Spirodela polyrhiza* was used to investigate the physiological responses to P-containing simulated wastewater with respect to its efficacy for pollutant removal and the potential for simultaneous biomass recovery. Our results indicate that *S. polyrhiza* achieved a maximum total P-removal efficiency of 93.78% at 17 mg/L P, while exhibiting high adaptive plasticity. Under P deficiency, carbon was preferentially allocated to starch accumulation (up to 214 mg/g DW), while elevated P levels stimulated protein and lipid synthesis. The biomass was also enriched with essential minerals, including P, K, Mg, and Zn, supporting its potential use as animal feed, biofuel feedstock, or biofertilizer. This synergistic approach offers a practical pathway to advance the circular bioeconomy and address pressing environmental challenges related to nutrient pollution and resource scarcity.

Molecular, microbial, and ecological drivers of duckweed phytoremediation in aquatic environments

Thingujam, D; Malacrinò, A; Pajerowska-Mukhtar, KM; Mukhtar, MS (2026) Biology – Basel 15: 963.

Aquatic ecosystems are under severe stress from a diverse combination of contaminants, including heavy metals, pesticides, pharmaceuticals, and microplastics, driven by rapid industrialization, intensive agriculture, and urbanization. Globally, 80% of wastewater remains untreated, and conventional systems often fail to address emerging contaminants. Consequently, toxic heavy metals like lead and mercury can persist in water sources for decades. In response, phytoremediation has emerged as a scalable, eco-friendly, nature-based alternative. Among phytoremediation agents, duckweeds are increasingly recognized for their rapid growth, simple morphology, and continuous water-column contact. This review outlines the landscape of duckweed-based remediation, detailing molecular detoxification pathways and the synergistic role of associated microbiomes in enhancing environmental cleanup. Evidence indicates that contaminant removal is often supported by plant-microbe interactions. Despite extensive laboratory validation, field-scale implementation remains constrained by environmental complexity, pollutant mixtures, and variable climatic conditions. Furthermore, while duckweed systems hold promise within circular bioeconomy frameworks, converting wastewater into nutrient-rich biomass, contaminant accumulation in plant tissues raises concerns about biomass utilization and contaminant carryover. Addressing these challenges requires an integrative approach that links molecular detoxification, ecological interactions, and engineered system design to realize the full potential of duckweeds for sustainable aquatic pollution management.

Managing post-phytoremediation biomass within a circular economy framework: Multitrophic ecotoxicological assessment of biomass, derived biochar and their leachable fractions

Cichy, P; Kalka, J; Zabczynski, S; Wasik, P; Korus, A; Chabinski, M; Szlek, A (2026) Applied Sciences 16: 6104.

Phytoremediation is a sustainable approach for the remediation of heavy metal-contaminated soils; however, the management of contaminated biomass generated during this process remains an insufficiently addressed challenge. Such biomass constitutes a secondary waste stream that may release mobile pollutants and pose

environmental risks. In this study, an integrated ecotoxicological assessment framework was applied to evaluate phytoremediation-derived biomass and its transformation products obtained via pyrolysis. Two types of woody biomass with different heavy metal contents and their corresponding biochars produced at 700 °C were investigated. A multitrophic battery of bioassays combining direct exposure assays using terrestrial organisms (higher plants, *Eisenia fetida*, and soil microbial activity) with leachate-based assays using aquatic organisms (*Lemna minor*, *Daphnia magna*, and *Aliivibrio fischeri*) was applied. Untreated biomass exhibited high to extreme toxicity in aquatic systems (toxic units, TU >100) and significant phytotoxic effects. Pyrolysis substantially reduced contaminant mobility and ecotoxicity of leachates, resulting in lower toxicity (TU typically <15) and no significant effects on plant growth, earthworm survival, or soil microbial functional diversity. Residual toxicity was linked to elevated pH and trace amounts of thermally generated organic substances. These results demonstrate that pyrolysis effectively reduces the environmental risk of contaminated biomass and supports the use of multitrophic ecotoxicological testing for safe waste valorization within circular economy strategies.

Nitrogen removal mechanisms in duckweed-based stabilization tanks with and without aerobic zones and intermediate feeding stream

Páez, LEC; Bejarano, JRC; Carabalí, MMS (2026) Journal of Environmental Management 411: 130194.

This study evaluated the performance of duckweed-based systems (*Spirodela polyrhiza*) for enhancing nitrogen removal from anaerobically pretreated domestic wastewater. Three pilot-scale treatment lines, each consisting of seven tanks in series, were operated under tropical conditions to assess the effects of plant coverage and intermediate feeding. To stimulate nitrification, specific tanks 4 and 5 in Lines 2 and 3 were maintained without duckweed, creating aerobic zones with dissolved oxygen (DO) levels up to 6.4 mg O_2 L^{-1}. Line 3 also incorporated an intermediate feeding of 10% raw UASB reactor effluent to mitigate carbon limitation for denitrification. Results showed high system stability, with average TSS removal efficiencies reaching 89.9%. While TKN and NH_4^+-N removal showed no significant differences between lines, NO_3^- - N concentrations were significantly higher in Line 1, indicating that duckweed-covered units primarily facilitate balanced nitrogen transformation through microenvironment regulation rather than simple uptake. Nitrogen mass balance identified nitrification denitrification and duckweed uptake as the dominant removal pathways. Interestingly, the intermediate feeding strategy did not significantly enhance overall nitrogen removal, suggesting that supplemental carbon must be more precisely synchronized with active redox zones and microbial activity. These findings highlight that the strategic spatial configuration of vegetated and non-vegetated zones is a critical management tool. This research provides a baseline for transitioning from passive lagoons to precision-engineered nature-based solutions, emphasizing the importance of aligning carbon availability with oxygen-driven redox gradients to maximize nutrient mitigation in decentralized wastewater treatment.

Phytoremediation of paper mill effluent contaminated with lead using EDTA and citric acid supported aquatic plant species: *Eichhornia crassipes*, *Lemna minor*, and *Salvinia molesta*

Kaushal, J; Bhardwaj, P; Rani, L; Yadav, OP; Prabha, C; Bairagi, AK; Amin, MK (2026) Boresource Technology Reports 34: 102733.

In the current study aquatic plant species - *Pontederia crassipes*, *Lemna minor*, and *Salvinia molesta* - have been employed along with ethylenediamine tetra-acetic acid (EDTA) and citric acid as chelating agents for the removal of lead (Pb) from effluent wastewater collected from a pulp and paper mill at Yamuna Nagar (30.133 degrees N 77.288 degrees E) in India. The removal of Pb from the wastewater was more in both the case of EDTA and citric acid-assisted phytoremediation treatments compared to when no mediator ligand was used. It was revealed that the chelate-mediated *Pontederia crassipes* exhibited the highest uptake of lead among the studied plant species. Compared to citric acid, using EDTA as a mediator for the studied plant species proved more efficient for eliminating Pb from wastewater. The EDTA (0.1 g L^{-1}) assisted *Pontederia crassipes* as a phytoremediation system enabled as high as 85% removal of lead from the studied effluent wastewater. This promises the use of this system for efficiently treating the effluent wastewater contaminated with lead.

Tentative mechanisms of chelator-mediated metal ion transport and their detoxification within the plant cells are discussed.

DF comment: The identity of *Lemna minor* was simply assumed although *L. aequinoctialis* is more common in Haryana, India.

Synergistic removal of morpholine fungicides and cadmium from agricultural water by a biochar-immobilized bacterial-duckweed system: Quantifying roles of biodegradation, adsorption, and phyto-uptake

Han, L; Smagghe, G; Yang, J; Yu, JX; Zheng, HDH; Chen, XS; Wu, XM (2026) Journal of Hazardous Materials 510: 142125.

The co-contamination of agricultural water by morpholine fungicides (e.g., flumorph and dimethomorph) and cadmium (Cd) poses significant ecological threats, challenging conventional treatment approaches. This study developed an innovative bioremediation system integrating biochar-immobilized microbial consortia with phytoremediation, and quantified the individual contributions of biodegradation, biosorption, phyto-uptake, and biochar-adsorption to the synergistic removal of pesticide and Cd co-contaminants. A novel Cd-tolerant and flumorph-degrading bacterium, *Alcaligenes faecalis* X4, was combined with a dimethomorph-degrading strain (*Bacillus cereus* WL08) to form a stable consortium. This consortium was capable of simultaneously metabolizing both fungicides into less toxic products and adsorbing cadmium. The consortium was immobilized on bamboo charcoal to produce a biocomposite (BCI-X4 + WL08), which achieved high removal efficiencies under optimized conditions: 97.65% for flumorph (50 mg/L), 94.23% for dimethomorph (50 mg/L), and 82.68% for cadmium (10 mg/L). Subsequent introduction of duckweed (*Lemna minor*) contributed an additional 15.40-28.00% removal via phyto-accumulation. Partitioning analysis confirmed true synergistic interactions - rather than merely additive effects-enhancing overall removal by up to 3.27-fold while alleviating oxidative stress in the plants. A compound ecological filter bed incorporating both BCI-X4 + WL08 and duckweed demonstrated practical applicability under outdoor conditions, achieving average simultaneous removal rates of 94.96% (flumorph), 91.43% (dimethomorph), and 85.42% (Cd) across three consecutive seasons, along with improved water quality parameters. This work presents a scalable, eco-friendly strategy for the in situ remediation of surface waters co-contaminated with pesticides and heavy metals, and provides a quantitative assessment of the distinct microbial, plant, and biochar contributions to the synergistic remediation process.

Phytoremediation of emerging and traditional contaminants in environmentally relevant co-exposure settings: a tool to reduce environmental hazards?

Chowdhury, KF; Nightingale, JH; Carter, LJ (2026) Frontiers in Water 8: 1812220.

Water bodies are routinely exposed to a mixture of contaminants via various anthropogenic exposure mechanisms. Phytoremediation offers a low-cost nature-based solution to remove contaminants from polluted water bodies. The ability of the aquatic macrophyte, *Lemna minor* to accumulate both organic [antibiotics, plant protection products (PPPs)] and inorganic contaminants (metals), individually and in co-contaminant mixtures, was evaluated to offer new insights into its potential for phytoremediation in realistic exposure scenarios. When exposed individually, diazinon accumulated to the greatest extent in *L. minor*, (95.51 ± 2.13%), followed by ciprofloxacin (31.91 ± 6.07%), atrazine (13.04 ± 3.44%), trimethoprim (11.43 ± 5.77%), chromium (4.5 ± 2.74%), and cadmium (3.08 ± 1.36%) These findings highlight that accumulation by *L. minor* is highly compound specific. Further results revealed that contaminant co-exposure significantly ($p = 0.002$-0.029) influenced the uptake of selected contaminants. The presence of chromium increased cadmium uptake by a factor of 6.34, while the combination of multiple contaminants (e.g., metals, PPPs, and antibiotics) reduced uptake. Changes in uptake capacity have potential knock-on effects for environmental risk assessments, including an assessment of the potential for antibiotics to contribute to the development of antibiotic resistance. For ciprofloxacin and diazinon, phytoremediation for 72 h was sufficient to reduce the concentration of a single antibiotic below concentrations predicted to result in the development of resistance (PNEC-R) or effects to *Daphnia magna*. In co-contaminant mixtures a risk was identified with RQ values being

a factor of 17 greater than the toxicity threshold for *D. magna*. Our results support the need for a more comprehensive assessment of the impact of co-contaminant mixtures on *L. minor* uptake capacity to optimise phytoremediation strategies for improved water quality and reduced environmental risks.

DF comment: The species was not identified.

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.

Comment DF: The authors were evidently not interested in the identity of their plants.

A rapid *Lemna* bioassay empowered by open-source image analysis

Azak, KS; Kotancilar, SK; Dindar, M; Basaran, N; Genc, S; Bilen, Ö; Yilmaz, M (2026) Environmental Monitoring and Assessment 198: 766.

Aquatic ecosystems are increasingly affected by complex combinations of pollutants, underscoring the need for rapid, economical, and efficient phytotoxicity assays. Despite *Lemna minor* being a standardized test organism, traditional bioassays predominantly depend on frond counting and visual assessment, which are time-consuming and subjective. This study enhanced a smaller, plate-based *Lemna* bioassay through quantitative, open-source image analysis, creating an efficient digital ecotoxicology procedure that requires minimal test volume. Potassium dichromate ($K_2Cr_2O_7$) served as a reference toxicant for assay optimization. An image-based concentration-response study (0-160 ppm) demonstrated a distinct, dose-dependent inhibition of growth. The negative control exhibited a significant increase in frond area (from 20.56 ± 5.01 to 52.03 ± 1.78 px), while exposure to 40 ppm $K_2Cr_2O_7$ led to nearly total growth inhibition and net tissue loss (growth rate: - 0.66 ± 1.78; growth inhibition: 104.97 ± 13.40%). Forty parts per million (ppm) was identified as the appropriate positive control based on biological significance and statistical validity. In the optimized time-course assay, control plants demonstrated sustained exponential growth over six days, whereas chromium-treated plants showed negligible or adverse effects on frond area, with a noticeable difference between treatments by day six. The frond area was measured using available software (GIMP and ImageJ), yielding objective, reproducible results with interobserver variability not exceeding 5%. The suggested small-volume, image-based *Lemna* assay improves sensitivity, repeatability, and throughput, while minimizing reagent consumption and experimental costs and providing a reliable instrument for regular aquatic toxicity assessment.

DF comment: The species was not identified.

Understanding plastic leachate fate and impact in wet clay-containing media - An attempt via montmorillonite catalyzed ozonation of polypropylene and toxicity on *Lemna minor*

Loumaizia, A; Cuche, A; Dewez, D; Azzouz, A (2026) Journal of Environmental Chemical Engineering 14: 123680.

The natural degradation of polypropylene (PP) in aquatic media was investigated in accelerated mode through ozonation in different clay-containing waters. The toxicity of the resulting leachate on biodiversity was assessed using the plant *Lemna minor* as a reliable bioindicator. It appears that polymer degradation unavoidably triggers generating mainly C16-C18 nanoplastics even at short exposure to oxidation conditions, inducing early toxicity in aquatic media. PP showed less stability as commercially supposed, even under short oxidation exposure and may exhibit harmful effect in contact with foods, drugs or living species. Both polypropylene oxidation and derivative toxicity were found to specifically evolve in time according to the type of water and clay suspension, the exchangeable cation, water composition and pH. The latter was found to play a key role in the clay dispersion and contact surface which, in turn, appear to strongly influence the catalytic activity, PP degradation and derivative distribution. These findings allow correlating polymer oxidation to the ecological impact of microplastics, thus providing the basis for a better understanding of their fate in aquatic media and, probably, for a suitable management of plastic-containing wastewaters.

DF comment: *Lemna minor* can act only as "reliable bioindicator" if it is reliably identified – which is not the case in the present publication.

Cadmium stress triggers dopamine signaling in duckweed (*Lemna turionifera* 5511) revealed by a fluorescent biosensor

Hao, BS; Qu, ZY; Yang, YW; Wang, WQ; Xing, Y; Han, YJ; Yang, L (2026) Frontiers in Plant Science 17: 1833157.

Cadmium (Cd) contamination poses a serious threat to aquatic ecosystems and human health. Duckweed has been recognized as a promising plant for phytoremediation of heavy metal-polluted water, yet the signaling mechanisms underlying its response to Cd stress remain poorly understood. Dopamine (DA), a catecholamine known as a neurotransmitter in animals, has recently been identified as a potential regulatory molecule in plants, but its dynamic response under heavy metal stress has not been clearly characterized. In this study, dopamine signaling dynamics in duckweed (*Lemna turionifera* 5511) were investigated using the genetically encoded fluorescent dopamine sensor GRAB-DA2h. Stable transgenic duckweed lines expressing the sensor were generated via Agrobacterium-mediated transformation, enabling real-time visualization of intracellular dopamine changes. With Cd treatment, GRAB-DA2h fluorescence in root tissues increased within 1 minute and remained elevated during the imaging period, indicating a rapid early signaling response. Following a 24-hour exposure to 50 mM CdCl2, the protoplasts isolated from fronds exhibited an increase in fluorescence intensity from 345.18 to 391.14, while those derived from roots showed an increase from 112.75 to 140.68. These findings provide direct evidence of dopamine signaling dynamics in duckweed under cadmium stress and demonstrate the applicability of genetically encoded biosensors for studying plant stress signaling. This work offers new insights into plant responses to heavy metal stress and may contribute to improving phytoremediation strategies.

Silver-form-driven nanotoxicity reveals *Spirodela intermedia* W. Koch as a potential bioindicator for aquatic systems

Cruz, MS; Petri, M; Terraciano, L; Lagorio, MG; Gonzalez, GA; Diz, V (2026) Chemical Research in Toxicology 39: 1102-1110.

Silver nanoparticles (AgNPs) and silver sulfide nanoparticles (Ag_2SNPs) are increasingly being used in diverse industries due to their unique physicochemical properties. However, their release into aquatic environments raises concerns regarding their potential ecological impact, making them an excellent model system for evaluating potential biomonitors. In this study, we evaluated and compared the phytotoxic effects of AgNPs and Ag_2SNPs suspensions on the free-floating macrophyte *Spirodela intermedia* W. Koch, a species with broad distribution from Mexico to South America and promising potential as a bioindicator. The designed protocol

involved both types of nanoparticles, which were synthesized and characterized via UV-Vis spectrophotometry and other techniques. In addition, morphometric parameters (frond number, frond abscission, and chlorosis), as well as enzymatic activity of ascorbate oxidase (AO) and chlorophyll-a variable fluorescence, were assessed after exposure to environmentally relevant concentrations. Results indicated that AgNPs induced significantly higher toxic effects than Ag_2SNPs, with marked frond abscission, chlorosis, AO activity reduction, and damage to the photosynthetic apparatus. These findings indicate that, as expected, the chemical form of silver nanoparticles plays a key role in phytotoxicity and that *S. intermedia* exhibits adequate sensitivity to be proposed as a bioindicator candidate in aquatic ecosystems, thereby expanding the global portfolio of bioindicator species and highlighting the ecological value of native flora for sustainable water quality monitoring.

Exploring the impact of pH on the toxicological response of *Lemna minor* to pharmaceuticals - insights from sulfamethoxazole exposure

Bethke, K; Caban, M. (2026) Environmental Toxicology and Chemistry DOI10.1093/etojnl/vgag136.

Aquatic acidification driven by increasing atmospheric CO_2 is expected to modify the environmental fate and effects of ionizable pharmaceuticals; yet, pH-dependent toxicity is rarely addressed in standard ecotoxicological assessments. Over half of pharmaceuticals are ionizable within environmentally relevant pH ranges, meaning shifts in pH can affect their speciation, bioavailability, and ecotoxicity. Here, we investigated, for the first time, the influence of pH on sulfamethoxazole (SMX) toxicity toward the aquatic macrophyte *Lemna minor*, following OECD 221 guidelines. Experiments in Steinberg medium (pH 5.0-5.5) revealed increasing SMX toxicity with decreasing pH, consistent with a higher fraction of neutral SMX. Photolysis was a major degradation pathway, while interpretation of pH effects was complicated by pH increases during the test, associated with *L. minor* growth and medium alkalinization. Our findings demonstrate that the standard Organisation for Economic Co-operation and Development (OECD) Test Guideline 221 is not well-suited for assessing pH-dependent toxicity of ionizable pharmaceuticals without pH control. We highlight the need for buffering strategies and systematic pH monitoring in plant-based ecotoxicity tests, as accounting for pH is crucial for accurate environmental risk assessment of ionizable pharmaceuticals under ongoing aquatic acidification.

Ecotoxicological effects of psychoactive pharmaceuticals in *Lemna minor*: Phytoremediation potential and mixture risk assessment

Witt, NGDM; Barros, DC; Oliveira, BFD; Guimaraes, BLS; Sudul, DD; Juneau, P; Gomes, MP (2026) Toxics 14: 420.

The increasing consumption of psychoactive pharmaceuticals has led to their continuous release into aquatic environments. This study assessed the ecotoxicological responses, phytoremediation capacity, and ecological risk of seven psychoactive pharmaceuticals-citalopram (CIT), sertraline (SER), fluoxetine (FLU), alprazolam (ALP), clonazepam (CLO), risperidone (RIS), and topiramate (TOP)-using *Lemna minor* under controlled exposure conditions. Plants were exposed to a concentration gradient, and physiological endpoints, including relative growth rate, chlorophyll content, and maximum photosystem II efficiency (Fv/Fm), were evaluated alongside compound removal and abiotic degradation. Dose-response modeling revealed substantial variability in toxicity, with TOP (EC_{50} = 74.11 ng L^{-1}), CLO (104.8 ng L^{-1}), and RIS (138.5 ng L^{-1}) exhibiting the highest potency, whereas FLU (1751 ng L^{-1}), CIT (89,941 ng L^{-1}), and ALP (465,351 ng L^{-1}) were less toxic. Relative growth rate was the most sensitive endpoint. Mixture exposure did not result in additional toxicity compared to the most responsive individual compounds. Abiotic degradation was negligible for most compounds (<3%), except for SER (similar to 42%) and FLU (similar to 22%). In contrast, *L. minor* achieved net removal efficiencies of up to 81%, although reductions occurred under mixed conditions. Probabilistic risk assessment indicated a high ecological risk (msPAFtotal = 1.0), with RIS as the dominant contributor.

Environmental toxicity of selected aminoglycoside antibiotics to *Lemna minor*

Vake, T; Krulec, L; Kubelj, P; Snoj, T (2026) Journal of Hazardous Materals Advances 22: 101186.

Aminoglycoside antibiotics (AGs) are widely used in human and veterinary medicine. After administration, AGs undergo minimal metabolism and are excreted in their active form, contributing to their presence in the environment. The aim of our study was to determine the ecotoxicological characteristics of five AGs commonly used in human and veterinary medicine: gentamicin, neomycin, spectinomycin, streptomycin and dihydrostreptomycin. Their effects on growth and chlorophyll synthesis were investigated under laboratory conditions using the model plant organism *Lemna minor*. For each AG, logarithmic dilution was performed to yield test solutions with concentrations ranging from 0.001 to 100 mg/L. In the growth inhibition test, low concentrations of spectinomycin and streptomycin stimulated the growth of *L. minor*. The lowest EC_{50} values, indicating the greatest toxicity, were detected for gentamicin and dihydrostreptomycin, at 0.397 and 0.625 mg/L, respectively. The results of the chlorophyll synthesis test revealed that neomycin and gentamicin had the lowest EC_{50} values, at 45.33 and 56.01 mg/L, respectively. The other AGs tested did not notably affect chlorophyll synthesis.

DF comment: The species was not identified.

Beyond particle effects: Leachate-induced duckweed toxicity from PBAT and PLA under global warming

Wang, YH; Song, ZY; Guo, Q; He, LW; Li, HZ; Huang, YN; Dang, F (2026) Environmental Science and Technology 60: 12479-12488.

Mounting evidence confirms that microplastics (MPs) pose ecological risks. Despite the environmental relevance of aged MPs under warming, little is known about their particle versus leachate toxicity. Here, we mechanistically quantify these contributions in the duckweed *Spirodela polyrhiza* at concentrations of 10-500 mg L^{-1}. Our results reveal polymer- and end point-specific effects, indicating distinct modes of action. While particle effects dominated the toxicity of polyvinyl chloride (PVC, 55.2-86.2%) and polystyrene (PS, 50.5-67.1%), the toxicity of poly(butylene adipate-co-terephthalate) (PBAT) and polylactic acid (PLA) was primarily driven by their leachates (51.1-78.5% and 54.0-91.7%, respectively). High-risk chemical candidates, including docosanamide, pyrene, hexadecanamide, nonanoic acid, stearic acid, oxepanone, and adipic acid, were further identified and prioritized within the leachates from PLA and PBAT through nontarget screening via HPLC-MS and integration with the Toxicological Priority Index (ToxPi) framework. Although elevated temperatures enhanced chemical leaching, the resulting toxicity did not increase proportionally. By elucidating how particles and leachates differentially drive MP toxicity in a polymer-specific manner under warming, this work provides critical insights for developing mechanism-driven risk assessment frameworks.

Aquatic ecotoxicity risk assessment of difenoconazole and its transformation residues using experimental-in silico integrated approach

Vulpe, CB; Cosma, CA; Pujicic, A; Agachi, BV; Isvoran, A; Iachimov-Datcu, AD (2026) Agronomy 6: 774.

Difenoconazole is a triazole fungicide used to ensure sustainable agricultural, although it may also affect the aquatic environment. This study assessed the effects of this fungicide by both an experimental and a computational approach. The experimental assessment involved the acute exposure of *Lemna minor* to different concentrations of difenoconazole and the determination of main endpoints such as number of fronds and colony, as well as secondary endpoints represented by gravimetric, morphometrical and biochemical parameters. The in silico analysis consisted of the testing of difenoconazole and 14 of its transformation residues (TRs), using three computational tools (admetSAR, ADMETlab and T.E.S.T.) to assess either their toxicological endpoints (EC_{50}, IGC_{50}) or their probability of affecting a range of model aquatic organisms. The results highlighted a concentration-dependent effect of difenoconazole on both main and secondary endpoints. The calculated EC_{50} value was 2.47 mg/L (data validated by EC_{50} on *Lemna gibba* from Pesticides Properties DataBase), which categorizes difenoconazole as moderately toxic in the aquatic environment. The in silico assessment showed that two of the TRs showed lower toxicity, with these having only one aromatic ring compared to the others analyzed.

Lemna minor's accumulation and physiological response to common over-the-counter pharmaceuticals: paracetamol, pseudoephedrine, and their combination

Urbaniak, M; Tomczyk, P; Kózka, B; Giebultowicz, J (2026) International Journal of Phytoremediation DOI10.1080/15226514.2026.2653810.

Pharmaceutical contamination of aquatic environments is an emerging concern, particularly for over-the-counter drugs such as paracetamol (PA) and pseudoephedrine (PSE), which persist in surface waters due to incomplete wastewater treatment. This study investigated the bioaccumulation and physiological responses of *Lemna minor* exposed to PA, PSE, and their mixtures at environmentally relevant concentrations. Accumulation in fronds was quantified, and plant responses were assessed using biomass, chlorophyll content, and phenolic compounds. PSE showed strong, concentration-dependent accumulation, increasing significantly ($p < 0.05$) from 6.3 ± 1 µg/g d.w. at 0.001 mg/L to 219 ± 51 µg/g d.w. at 0.1 mg/L. In contrast, PA remained below detection limits at 0.01-0.1 mg/L and was detected only at 1 mg/L (12 ± 7 µg/g d.w.), suggesting limited uptake or rapid transformation. Under mixture exposure, PSE accumulation decreased by 39% at the lowest concentration but increased by 62% at the highest mixture level compared with single-compound treatments, whereas PA uptake remained largely unchanged. PA significantly stimulated biomass production, increasing fresh weight by up to 168% at 0.1 mg/L ($p < 0.05$), while PSE increased biomass by up to 119%. Low-dose exposures induced elevated phenolic levels, indicating activation of stress-related metabolic pathways, whereas higher PA concentrations enhanced chlorophyll a content, with the highest mixture treatment showing a 66% increase relative to control. PCA revealed a shift from photosynthetic investment toward secondary metabolism under pharmaceutical exposure. These asymmetric interactions demonstrate that mixture effects cannot be predicted from assays. *L. minor* exhibited sensitivity to pharmaceutical stress and a capacity to accumulate PSE, supporting its use as a bioindicator and phytoremediator. This study presents the first systematic assessment of pseudoephedrine (PSE) uptake and bioaccumulation in *L. minor*, demonstrating strong concentration-dependent accumulation and underscoring its environmental relevance. By examining paracetamol-PSE mixtures at a 10:1 ratio typical of over-the-counter formulations, it reveals asymmetric interactions that alter accumulation patterns and plant responses compared with single-compound exposures. Combining biochemical markers with multivariate analysis shows a shift from growth to secondary metabolism under pharmaceutical stress, providing new insights for mixture-aware environmental risk assessment and phytoremediation strategies.

Mitigating antibiotic phytotoxicity in duckweed through cerium oxide nanoparticles: A multidimensional assessment of physiological recovery, metabolic reprogramming, and nutritional homeostasis

Xu, LN; Qiu, Y; Zhang, P; Maqbool, F; Guo, YB; Wang, YJ; Shao, QH; Li, JH; Guo, X; Liu, Y (2026) Journal of Environmental Sciences 164: 875-886.

Antibiotics stress seriously threatens the aquatic environment and public health worldwide. In this work, the alleviation of cerium oxide nanoparticles (CeO2 NPs) on sulfamethoxazole (SMX) and tetracycline (TC) toxicity to duckweed (*Lemna turionifera*) was evaluated. We found that CeO_2 NPs (10 mg/L) alleviated the toxicity of both SMX (5.0 mg/L) and TC (1.0 mg/L), increasing fresh biomass by 129.1 %, and 206.9 %, respectively, relative to SMX and TC alone. CeO_2 NPs reduced oxidative stress in SMX and TC-stressed duckweed fronds. Specifically, SMX and TC disrupted the chloroplast ultrastructure, which was manifested by the blurrier chloroplast membrane, loosely structured thylakoids, and decreased starch particle size and number. CeO_2 NPs restored the structural damage to enhance photosynthetic activity. Furthermore, the starch, glucose, and total carbon contents were increased by CeO_2 NPs, indicating the alleviation of CeO_2 NPs on photosynthetic carbon fixation capacity. Metabolically, glycerate-3-phosphate, sucrose, alanine, serine, and malic acid were significantly up-regulated, as well as a significant decrease of ribulose 5-phosphate, and aspartic acid, demonstrates a positive regulation of CeO_2 NPs on the carbon metabolism in duckweed fronds under SMX and TC stress. Meanwhile, the nutritional quality of duckweed (including protein, lipid, and flavonoids) harvested from the stress was effectively enhanced by CeO_2 NPs. Collectively, these findings highlight the significant potential of CeO_2 NPs to alleviate the antibiotics-induced phytotoxicity, and provide a promising nanoenabled strategy to enhance the recycling of duckweed as feed.

Instructions to Contributors for the Duckweed Forum

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

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.

DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 3,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

Title:

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

References:

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

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

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

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

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

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

Authors:

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

Image resolution and submission:

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

Links for Further Reading

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

http://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

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

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