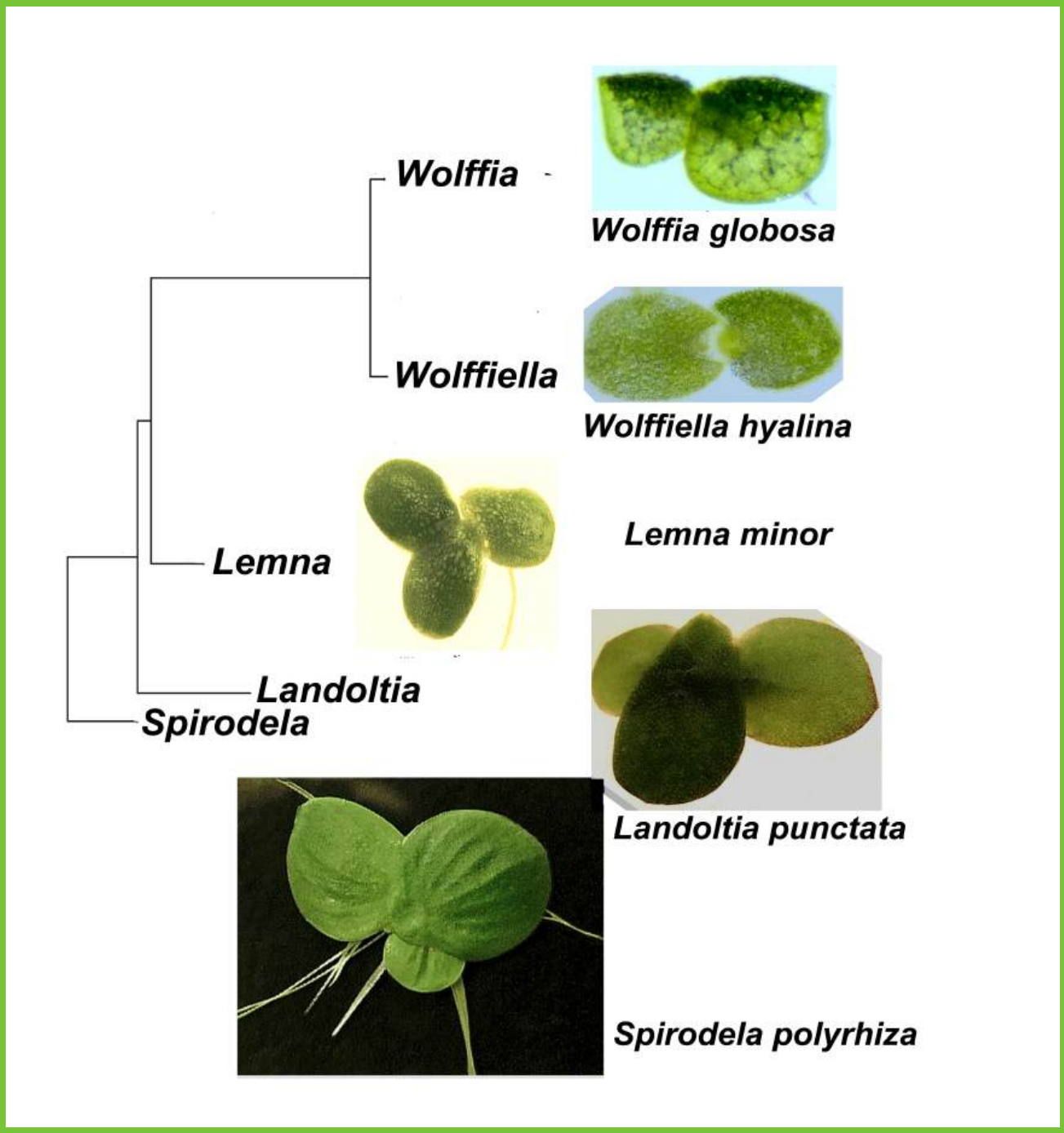
Newsletter for the Community of Duckweed Research and Applications, edited by the ISCDRA

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 14 (2), issue 53, pages 40 - 92 (2026)



The genera of Lemnaceae (Duckweeds)

Cover page

The genera of Lemnaceae (Duckweeds): The schematic representation of a tree of Lemnaceae with its five genera is related to the article by the 6th ISCDRA "Accurate species identity is an important prerequisite for duckweed research". This contribution stresses the requirement for species identification, to be in agreement with good scientific practices, addressing the authors, reviewers and editors of the scientific publications on duckweeds. (Photo credit: Klaus-J. Appenroth, FSU, Germany).

In this issue

Letter from the Editor:.....40

Accurate species identity is an important prerequisite for duckweed research.....41

ICDRA-8: Announcement.....43

Announcement: Call for Nominees as Candidates for the 7th ISCDRA.....44

Historical connection between the Indian Botanical Garden and three renowned duckweed researchers.....45

Request for applications to host ICDRA-2028.....49

History of duckweed transformation.....50

Student spotlight: Maria Spektor.....55

From the Database.....59

Instructions to Contributors for the Duckweed Forum.....90

Links for Further Reading.....92

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

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

Letter from the Editor:

April 30th, 2026

Dear Duckweed Community,

My greetings to all for a blossoming spring time and also summer time in certain parts of the world.

For a scientific community to prosper in the right direction, good scientific practices and standards are extremely important. This issue focuses on one such practice, accurate duckweed species identification. It has been observed that several scientific publications consider duckweed species identification as a superficial task whilst publishing their results. This has several implications not only to citing such a published article but also to other researchers who want to base their research on such a published work. This problem and the solution to it has been dealt with by the current 6th ISCDRA in a detailed opening article of this issue. It is also highlighted by dedicating a cover image representing the five genera of duckweeds. We would urge the scientific community to take special care in identification of duckweed species used in a scientific publication.

This DF issue also presents two historical articles, one of them about the connection of the duckweed researchers from the 18th and the 19th century to the Indian Botanical Garden in Kolkata, India and another article on the history of duckweed genetic transformation. I am sure you will enjoy the reads and will appreciate the efforts of the researchers who contributed to the growth of the field of duckweed research in the past. Student spotlight of this issue is Maria Spektor from Israel who has brought forward her interesting journey with duckweeds and their cultivation. The database section curated by Klaus Appenroth is an integral part of this issue as well.

We still have the application to the venue of the ICDRA in 2028 open. Interested candidates are encouraged to apply. In this issue, we have also announced for the nominations of the candidates for the next ISCDRA. We encourage eligible candidates to be nominated as per the announcement in this issue.

Registration for the 8th ICDRA in Naples, Italy is now open. A detailed announcement of the same is presented in this issue. We encourage you to register and to participate in the upcoming vibrant duckweed meeting in Italy. Please contact the organizers for any further details. Your active participation at the ICDRA in Italy will add to the scientific value of the meeting.

All best wishes from ISCDRA.

Enjoy reading the Duckweed Forum !!!

Sincerely,
K. Sowjanya Sree
Chair, ISCDRA

Accurate species identity is an important prerequisite for duckweed research

Klaus-J. Appenroth; Eric Lam; Asaph Aharoni; Marcel AK Jansen; Tsipi Shoham; K. Sowjanya Sree

6th International Steering Committee on Duckweed Research and Applications (ISCDRA)

Biological research deals with organisms or parts of organisms, from molecular, cellular level, to the level of organs or whole plants, in populations or ecosystems. In each case we need to know from which organism the new information has been deduced, which is critical for its independent validation as well as for the incorporation of new information into the body of knowledge in the scientific community. Research in the field of duckweed (or water lentils; family Lemnaceae Martinov; Tippery et al., 2021) is no exception.

Species identity has often been facilitated by the use of morphological markers and more so in the current times by molecular barcoding. For duckweeds, the use of morphological features could tell some species apart (Hegelmaier, 1868; Landolt, 1986; Bog et al., 2020). However, duckweeds being small in size and having reduced structural and developmental complexities, the efficient use of these morphological markers has been challenging even for a trained researcher. As Elias Landolt (Appenroth et al., 2023) once mentioned, often, common workers in the field tend to identify *Lemna* species as *Lemna minor* because it is small (the meaning of "minor" is "small") and *Wolffia* species as *Wolffia arrhiza* because the plants do not have roots (the meaning of "arrhiza" is "without roots"), although this is a general characteristic of the whole genus *Wolffia* (Personal communication to KJA).

What can be done to solve this problem? The first step is, of course, using a key of species determination which is based on morphological markers (Bog et al., 2020). With a good understanding of this key, the genera can be identified with accuracy. There are five genera: *Spirodela, Landoltia, Lemna, Wolffiella* and *Wolffia*. For more details, see Bog et al. (2020). The main morphological markers are the following:

1. Genus *Spirodela*: Fronds have 7 to 21 roots (take care not to count the roots per colony!), many but never all of them perforate (pierce through) the scale-like leaflet; fronds 1 to 2 cm large, 1 – 2 times as long as wide.
2. Genus *Landoltia*: Fronds have 2 to 7 roots (rarely more than 7), all of them perforate the scale-like leaflet; normally not larger than 1 cm, 1.5 – 2 time as long as wide; with prominent papillae along the median line of the frond surface (this marker gave the name of the single species *L. punctata*).
3. Genus *Lemna*: One single root per frond
4. Genus *Wolffiella*: No roots; fronds flat with air spaces; daughter fronds emerging from a terminal flat pouch at the base of the mother frond; shape depends on the species.
5. Genus *Wolffia*: No roots; fronds three-dimensional (globular, ellipsoid, cylindrical, conical, naviculate (boat-shaped) or semi-spherical); daughter fronds emerging from a single terminal conical cavity at the base of the mother frond; colonies normally consist only of one mother and one daughter frond.

What can be done for identification at the species level? In total there are presently 35 species of Lemnaceae (Bog et al., 2020; Appenroth et al., 2024), while new hybrid species are also increasingly being identified (Braglia et al., 2021). The method of choice is plastidic DNA barcoding (Bog et al., 2019): Fragments of the plastidic DNA can be amplified by PCR and sequenced to deduce the species identity through comparison to a previous database of known sequences generated for all known duckweed species (Borisjuk et al., 2015). Alternatively, higher resolution for species distinction can be obtained by the method of Tubulin-Based Polymorphism Fingerprinting, a nuclear DNA-based technique, which permits additionally the identification of interspecific hybrids from different fragment size and number after PCR amplification (Braglia et al., 2021). These methods are now routine in many labs with modern molecular biology equipment and training. Sequencing is usually carried out by commercial vendors or institutional core facilities for very reasonable fees. Laboratories that do not have the capacity to carry out these experiments may seek cooperation or could contact one of the duckweed stock collections (Appenroth, 2025) across the globe for help. These methods and resources should make it possible in most cases to identify the species, or at the least narrow down to a few possible candidates.

It is an essential requirement for the proper species identity and source of the duckweed used in the research to be known before the results can be accepted for publication. Otherwise, the reported observations cannot be compared with other findings. We ask not only authors to take this requirement seriously, but its enforcement by reviewers and editors will be especially critical. One criterion of good scientific practice is the opportunity that other researchers could reproduce the published results. Without knowing at least the species identity, this will be impossible. And reproducibility is a key determinant for the reliability of good science in general, and also critical for maintaining public confidence.

References

Appenroth, K.-J. (2025) Storage conditions of duckweeds in stock collections. Duckweed Forum 13: 98 – 102.

Appenroth, K.-J.; Jansen, MAK; Lam, E.; Shoham, T.; Sree, K. (2024) Important terms in duckweed research defined. Duckweed Forum 12: 90 – 94.

Appenroth, K.-J, Sree, K.S., Lam, E. (2023) Elias Landolt (1926– 2013), Father of modern duckweed research. Duckweed Forum 11: 47 - 54.

Bog, M.; Appenroth, K.J.; Sree, K.S. (2019) Duckweed (Lemnaceae): its molecular taxonomy. Frontiers in Sustainable Food Systems 3: 117.

Bog, M., Appenroth, K.J.; Sree, K.S. (2020) Key to the determination of taxa of Lemnaceae: an update. Nordic Journal of Botany 38: e02658.

Borisjuk N, Chu P, Gutierrez R, Zhang H, Acosta K, Friesen N, Sree KS, Garcia C, Appenroth KJ, Lam E. (2015) Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species. Plant Biol (Stuttg). 17 Suppl 1:42-9.

Braglia, L.; Lauria, M.; Appenroth, K.J.; Bog, M.; Breviario, D.; Grasso, A.; Gavazzi, F.; Morello, L. (2021) Duckweed species genotyping and interspecific hybrid discovery by Tubulin-Based Polymorphism Fingerprinting. Frontiers in Plant Science 12: 625670.

Hegelmaier, F. (1868) Die Lemnaceen. Eine monographische Untersuchung. Leipzig, Germany: Verlag Wilhelm Engelmann.

Landolt, E. (1986) Biosystematic investigations in the family of duckweeds (Lemnaceae). The family of Lemnaceae-a monographic study, vol. 1. Zurich, Switzerland: Veroeffentlichungen des Geobotanischen Institutes der ETH.

Tippery, N.P.; Les, D.H.; Appenroth, K.J.; Sree, K.S.; Crawford, D.J.; Bog, M. (2021) Lemnaceae and Orontiaceae are phylogenetically and morphologically distinct from Araceae. Plants 10: 2639.

ICDRA-8: Announcement

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

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

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

Key Dates

- ○ Registration & Abstracts Opening **27 April 2026**
- ○ Early bird deadline **27 June 2026**
- ○ Registration & Abstracts deadline **27 July 2026**
- ○ Oral presentation/poster confirmation **31 Aug. 2026**

Included in Registration Fees

- All lunches and coffee breaks
- Apericena
- Pizza Dinner
- Conference Dinner
- Royal Palace tour
- Guided Visit to Ercolano excavations (included only for students)

€ Registration Fees

Category	Early Bird (before 27 June)	Regular (after 27 June)
Students	350 €	400 €
Academics	450 €	500 €
Privates / Companies	650 €	700 €

Registration opens 27 April 2026.

Registration opens 27 April 2026:
www.8thicdra.info/registration

Patronships

Website: www.8thicdra.info | **Contact:** 8icdra@gmail.com

Announcement: Call for Nominees as Candidates for the 7th ISCDRA

The International Steering Committee on Duckweed Research and Applications (ISCDRA) was founded during the 2nd International Conference on Duckweed Research and Applications (ICDRA) at Rutgers, the State University of New Jersey, New Brunswick, NJ in 2013.

Members of the ISCDRA cooperate with each other in order to steer and promote duckweed research and applications for the benefit of our community. Assisting in the publication of the community newsletter, the Duckweed Forum, is one of the obligations, among others, that are expected of the committee members.

Procedure for the election of members to the 7th International Steering Committee on Duckweed Research and Applications

1) The ISCDRA should consist of 5 members who will be elected before the biennial ICDRA in a secret poll.

2) Anyone who has previously attended any of the ICDRA or will attend the upcoming 8th ICDRA in Naples, Italy can suggest potential candidates including themselves. Candidates should have attended at least one of the three previous ICDRA meetings. Suggestions may be sent to the Chair of the present ISCDRA – Dr. K. Sowjanya Sree, Email: ksowsree@gmail.com; kssree9@bhu.ac.in. The deadline for submission of candidate names is 25th June, 2026.

3) The voting procedure will be announced in the upcoming DF issue in July, 2026.

4) The five newly elected members will be notified by email and they will elect the head of the committee before the 8th ICDRA-2026 in Naples, Italy.

5) In case that by chance all elected members are either from the applied field or from the research field, the elected Chair will appoint one additional member from the missing field.

6) At the end of the ISCDRA meeting (General Assembly during the 8th ICDRA) the previous Committee will report shortly about the activities since the previous election and the duty will be transferred to the newly elected ISCDRA.

Historical connection between the Indian Botanical Garden and three renowned duckweed researchers

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

[1]School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India

[2]Matthias Schleiden Institute – Genetics I, Friedrich Schiller University Jena, 07743, Jena, Germany

The "Acharya Jagadish Chandra Bose Indian Botanic Garden", situated in Howrah, West Bengal, India is one of the largest and oldest Botanical Gardens in the world. It is spread across an area of approximately 1.1 square kilometre with 24 interconnected lakes, housing 1377 plant species. It was established in 1786, almost 50 years prior to the Key Botanical Garden near London. Following the tragic "Great Bengal Famine" in 1770 which caused ca. 10 Million deaths in India, Colonel Robert Kyd from the East India Company (which later went on to be the roots of British rule in India), as the first honorary superintendent of the garden, played a key role in establishing it in order to grow economically important plants. The intention, however, was also to tap the rich natural resources of India for the British Empire. Besides the introduction of tea, several other cash crops like Cardamom, Cinchona, Cinnamon, Coffee, Cotton, Indigo, Nutmeg, pepper, Clove, Sugarcane, potato, Sago and teak were cultivated (https://bsi.gov.in/garden-page/en?rcu=140,21; accessed 14/04/2026).

Figure 1: The Banyan tree at the Indian Botanical Graden, Kolkata

The largest plant of the Botanical Garden, a famous tourist attraction, is an almost 250 years old Banyan tree (*Ficus benghalensis*), spanning an area of 16000 square meters with 3618 prop roots supporting the expanding plant as pillars. In the current article, we are however, focused on the smallest flowering plants from the family Lemnaceae (duckweeds or water lentils) that are rather inconspicuous compared to the Banyan tree. Back then, in the 18th century, research on these tiny plants in India was closely connected with the Calcutta Botanical Garden, as it was called in that time. In the following we will journey through the initial years of the establishment of

the Botanical garden when three renowned researchers contributed to describing some of the duckweeds for the first time.

1. Dr. William Roxburgh (1751 – 1815)

Figure 2: Memorial of Roxburgh at the Indian Botanical Garden

William Roxburgh was born in Craigie in Ayrshire, Scotland. Although he studied medicine at the University of Edinburgh (1771), like several surgeons of the time, he was also interested in Botany. As a result, in 1781, he was appointed as the Superintendent of the Botanical Garden in Samalkot (currently in Andhra Pradesh, India) under the East India Company. Later, in 1793, he was appointed as the first official, salaried Superintendent of the Botanical Garden in Calcutta, following Col. Robert Kyd. During his tenure, he introduced a great diversity of plants to the garden and prepared a catalogue containing 3500 species growing in the garden, which was published as "Hortus Bengalensis" in 1814 by William Carey (https://bsi.gov.in/garden-page/en?rcu=140,21). A memorial stands tall in his honour in the Botanical garden today.

Interestingly, one of the several plant species described for the first time by Roxburgh was the very common Lemnaceae member *Wolffia globosa*, found in "Bengal", however under the name *Lemna globosa* Roxburgh which was published in Flora Indica (Roxburgh, 1832).

2. L. *globosa*. *R.*

Single, globular, rootless, minute, one, or at most two together, singly about the size of a grain of sand.

With L. *orbiculata*, found in very great abundance on tanks and pools of stagnant water in Bengal forming a compact green scum, over the surface.

Figure 3: Excerpt from (Roxburgh, 1832) p. 565

Daubs (1965) reviewed the species described by Roxburgh which was however, incomplete which induced Hartog and Plas (1970) to re-investigate the plant nomenclature. After it became clear that this species does not belong to the genus *Lemna* but to *Wolffia*, a new name had to be given as "nov. comb." (new combination), indicating formal renaming of the species after its transfer into another genus. Finally, this resulted in the currently accepted name of the species as *Wolffia globosa* (Roxb.) den Hartog & van der Plas. Hartog and Plas (1970) also published a key of species determination described as "synopsis", and the several synonyms of this species showcase its complicated taxonomic history.

2. Wolffia globosa (Roxb.) den Hartog & van der Plas, *nov. comb.* — *Lemna globosa* Roxb., Fl. Ind. 3 (1832) 565. — *Grantia globosa* Griff. *ex* Voigt, Hort. Suburb. Calc. (1845) 692; Not. 3 (1851) 229. — *Wolffia schleideni* Miq., Nederl. Kruidk. Arch. 3 (1855) 428; Fl. Ned. Ind. 3 (1855) 221. — *Wolffia delilii* var. *schleideni* Kurz, J. Linn. Soc. Bot. 9 (1866) 265. — *Wolffia cylindracea* Hegelm., Lemnac. (1868) 123.

Figure 4: Excerpt from Hartog & Plas (1970)

2. William Griffith (1810 – 1845)

In their Historical account, Sree and Maheshwari (2019) presented a detailed report of William Griffith and his connection with the Botanical Garden in Kolkata. In short, Griffith was a British Botanist, born in Ham Common, Greater London. He studied medicine, like Roxburgh, and also discovered his preference for Botany. He sailed to India in 1832, and thereafter stayed all his life in the "East Indies" and never returned to England. He started his professional life as an Assistant-Surgeon in Madras (now Chennai) in the service of the East India Company and was called to

Calcutta to take charge of the Botanical Garden in 1842. He played an important role in crafting the today's famous Assam tea. His botanical excursions spanned vast areas from Myanmar, Thailand, Afghanistan, Malaysia, Bhutan and many places in India, collecting and describing several new plant species. In 1845 William Griffith passed away in Malacca, Malaysia of a fatal illness.

In India, collected in Calcutta or Serampore (now Srirampur), Griffith described a species that he called *Grantia microscopica* sp. novo and genus novo in honour of James William Grant, Esq (Griffith, 1851a, b). He mentioned its similarity to "*Lemna globosa*", described by Roxburgh. Already in that time, Voigt (1845) discussed the possibility of this species belonging to the genus *Wolffia*. Currently, the valid name for this species is *Wolffia microscopica* (Griff.) Kurz.

Figure 5: Memorial in honour of William Griffith

226 LEMNACEÆ.

(1) *Grantia microscopica.*

Flos axilis e centro paginæ superior exserta, spatha o. Anth. unolcularis.

There is I think one other species of this genus which was known to Roxburgh as Lemna globosa (?) I have not hitherto met with it in flower.

Figure 6: Description of *Grantia microscopica* (Griffith, 1851a)

Also for William Griffith a memorial of honour stands tall in the Botanical Garden in Kolkata (Sree and Maheshwari, 2019) to recall his important work during his short tenure at the garden. The species *W. microscopica* was described further by Khurana and Maheshwari (1983) and recently by Sree et al. (2015).

3. Wilhelm Sulpiz Kurz (1834 – 1878)

Wilhelm Sulpiz Kurz was a German Botanist, born in Augsburg (Kurz, 1878). As a guest student he attended several courses, including Botany, at the University of Munich, Germany. He served for some time in the Colonial Power "Koninklijk Nederlandsch-Indisch Leger" (army). Later, he found a job as botanical assistant at the Botanical Garden Kebun Raya Bogor in Buitenzorg, Indonesia (https://en.wikipedia.org/wiki/Bogor). He was evidently quite successful with this work and he got an offer as Curator of the Herbarium of the Indian Botanical Garden. Here he had his most productive period, collecting plants and publishing his results for instance from Andaman Islands and from Burma (now Myanmar) (https://plants.jstor.org/stable/10.5555/al.ap.person.bm000152226). In 1877 he travelled to Burma but could not return to Calcutta as he passed away in Pulo-Penang in January 1878 of a fever. The friends of his erected a memorial in his honour at the Indian Botanical garden.

Figure 7: Memorial in honour of Wilhelm Sulpiz Kurz

Kurz investigated especially two Lemnaceae species (the family name was already defined by Martinov (1829)), *Grantia microscopica* (as per the then existing name of the species) and *Lemna tenera*. As a result, he reassigned *Grantia microscopica* Griff. Ex Voigt to the genus *Wolffia* (Kurz, 1866). Thus, the currently accepted name of

this species is *Wolffia microscopica* (Griff.) Kurz. This re-designation was confirmed by Christoph Friedrich Hegelmaier (1833–1906), the then expert on Lemnaceae (Hegelmaier, 1885, 1895).

During his excursions and travels to Pegu (now Bago) in Burma (now Myanmar) in 1870, Kurz collected and described another duckweed species for the first time, *Lemna tenera* Kurz. He published the description in 1871 (Kurz, 1871). Hegelmaier (1895) completed the description and Van Der Plas (1971) gave an accurate description with figures along with its further biogeography.

2. **Wolffia microscopica**, *Kurz.* **Frondiculæ lineam vix excedentes, cellulosæ, supra planiusculæ, subtus in radiculam (?) subcylindricam productæ ; pollen glabrum (sec. *Griffith*).**
Grantia microscopica, *Griff. Not. Monocot.* 226, t. 266–268.
***Hab.* Bengal, *Griffith*.**
I have not yet seen this species, which seems to be a rare one.

Figure 8: Excerpt describing *Wolffia microscopica* (Kurz, 1866)

LEMNACEÆ.

109. Lemna tenera, n. sp.

Frondiculæ cruciatæ, lanceolatæ ad lineari-lanceolatæ, sæpius subcurvulæ, acuminatæ, basi magis minusve rotundatæ, membranaceæ, subtus (in• viro) obsolete trinerves et reticulato-venosæ; radiculæ solitariæ.—Frondiculæ 3—4 lin. longæ, basi lineam circiter latæ ; radiculæ vix pollicares.

Pegu, in jungle-swamps of Pazwoon doung valley, rare.

Figure 9: Description of *Lemna tenera* Kurz

All the three renowned duckweed researchers of the 18th and 19th century are well remembered and honoured for their contributions to botany in India. Tourists visiting the Indian Botanical garden get acquainted with the surnames of these researchers as these monuments are on the tourist map for visitors of the Indian Botanical garden. Their dedication and meticulous work serves as an inspiration to several researchers in the field today.

References

Roxburgh, W. (1832) Flora Indica. Mission Press, Calcutta. 3, 565-566.

Daubs, E.H. (1965) A monograph of Lemnaceae. Univ. of Illinois Press, Urbana. Illinois Biol. Monogr. 34: 118 pp.

Den Hartog, C., v. d. Plas, F. (1970) A synopsis of the Lemnaceae. Blumea 18: 355-368; 367.

Den Hartog C. and Van Der Plas F. (1970) A synopsis of the Lemnaceae. Blumea 18: 355-368.

Griffith, W., Notulae ad plantas asiaticas. Bishop College Press, Calcutta, India, 1851a, 3, 223-229.

Griffith, W., Icones plantarum asiaticarum. Bishop College Press, Calcutta, India, 1851b, 3, plates CCLXVI-CCLXVIII.

Hegelmaier, F. (1885) *Wolffia microscopica*. *Bot. Z.* 43: 241–249.

Hegelmaier F., (1895) Systematische Übersicht der Lemnaceen. Botanisches Jahrbuch. 21: 268-305.

Kurz, S. (1878) Flora 61 (8) 113 – 119.

Kurz S. (1866) Enumeration of Indian Lemnaceae. J. Linn. Soc. Bot. 9, 264-268.

Kurz S. (1871) On some new or imperfectly known Indian plants. 109. *Lemna tenera*. Journal of the Asiatic Society of Bengal 40(2), 78.

Landolt, E. (1992) *Lemna tenera* Kurz, a little known species of Lemnaceae. Ber. Geobot. Inst. ETH, Stiftung Rubel, Zürich 58: 124-131.

Martinov, I. Techno-Botanical Dictionary; Pechashano v Imperatorskoi Tipografii: Saint Petersburg, Russia, 1820.

Sree, K. S., Maheshwari, S. C. (2019) William Griffith, Esq., F.L.S. (1810-1845) the discoverer of *Wolffia microscopica*, a unique duckweed species endemic to India. Duckweed Forum 7: 8-12.

Sree, K. S., Maheshwari, S. C., Boka, K., Khurana, J. P., Keresztes, A., Appenroth, K-J. (2015) The duckweed *Wolffia microscopica*: A unique aquatic monocot. Flora, 210: 31-39.

Voigt, J. O. (1845) Hortus Suburbanus Calcuttensis. Bishop College Press, Calcutta, India, pp. 692.

Van Der Plas F. (1971) Flora Malesiana Ser. 1. Spermatophyta 7, 219-237.

Request for applications to host ICDRA-2028

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

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

History of duckweed transformation

Naama Zioni, Marvin Edelman and Asaph Aharoni

Faculty of Biochemistry, Department of Plant and Environmental Sciences and Department of Immunology & Regenerative Biology, Weizmann Institute of Science, Rehovot, Israel



The way to stable transformation

In 1980, a short note appeared suggesting that intact *Lemna perpusilla* plants could take up heterologous DNA (Frey et al., 1980). This raised the possibility of rapid, whole-plant, stable transformation in duckweed. There was no follow up, however two years earlier to this, Wei-Chin Chang and collaborators had succeeded in regenerating *Lemna gibba* plants, and *Lemna perpusilla* frond-like structures, through callus culture in a 4-month tissue culture cycle (Chang and Chiu, 1978; Chang and Hsing, 1978). The two *Lemna* species responded quite differently to the added growth substances in callus inducing media, a feature to be found repeatedly in the coming years for other Lemnaceae species, and even different isolates within a species, as callus culture became the almost exclusive route to stable transformed duckweed.

Somewhat surprisingly, it took two full decades for callus culture to be taken up and applied to the transformation task in Lemnaceae. Along the way, an important study by Hugh Frick (1991) identified common carbohydrates in *Lemna minor* which supported callus growth but not plant growth (*viz.* galactose, sorbitol), and *vice versa* (*viz.* glucose, fructose, starch, mannitol). Subsequently, the effects of medium components and light intensity on callus induction, growth and frond regeneration were optimized for *L. gibba* (Moon and Stump,1997; Li et al., 2004), while carbohydrate and phytohormone requirements for callus induction, callus growth and frond regeneration were established for clones of *Landoltia punctata* 5562 (Li et al., 2004) (formerly named *Spirodela oligorrhiza* and *Spirodela punctata 8717* (erroneously appearing in the Rutgers database as *Lemna disperma* 8717 [Li et al., 2004]) and, more recently, for *Wolffia arrhiza* (Khvatkov et al., 2015).

In parallel with efforts to establish stable transformation, early work in the 1990s explored transient transformation systems as a means to study gene expression in duckweed. Elaine M. Tobin and collaborators pioneered a transient transformation system in *L. gibba* to examine phytochrome-regulated gene expression. They used the biolistics particle gun system developed a few years prior (Klein et al., 1987) for delivering nucleic acids into living cells.

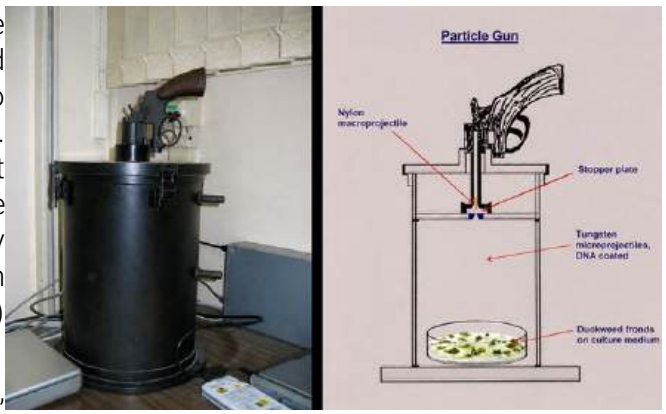


Figure 1: Early particle gun for duckweed transformation withDuckweed Plant illustration (Edelman group, 1990).

The *Lemna* Rubisco small subunit gene SSU5B, which was positively regulated by phytochrome in vivo, showed phytochrome regulation in the

transient assay system. The transient system was then used to show that a sequence upstream of SSU5B was necessary to confer phytochrome regulation on a reporter gene in response to a single, brief red-light treatment (Rolfe and Tobin, 1991). Refining their technique, the Tobin laboratory went on to define two 10-bp promoter regions that are critical for phytochrome regulation of the *L. gibba* light harvesting chlorophyll a/b protein of PSII (Kehoe et al., 1994). A decade later, conditions for transient transformation using Agrobacterium-mediated gene transfer were described for the duckweed *Wolffia columbiana* (Boehm et al., 2001). However, while these systems demonstrated that transient gene expression could be achieved, stable transformation remained a major unresolved challenge.

It wasn't until 1999 that two patent applications dealing with stable transformation, one titled "Genetically engineered duckweed" (Stomp and Rajbhandari 1999), the other, "Transgenic Lemnaceae" (Edelman et al., 1999), were published and entered the public domain. In the summer of 1995, an Israel - North Carolina Workshop was held at the North Carolina Biotechnology Center, USA at which Edelman presented his group's findings on *Agrobacterium*-mediated GUS transformation of *Landoltia punctata* (then known as *Spirodela oligorrhiza*), employing the tissue culture procedures published later by Li et al. (2004). After his talk, Stomp privately approached him with pictures of similar experiments being conducted by her with *L. minor*. Two start-up companies were formed based on the parallel technologies. Biolex, using the *L. minor* system, eventually bought out Lemnagene, which was using the *Landoltia punctata* system. However, Biolex never made it to the public offering it was planning in the financial crisis days of 2008 and eventually had to declare bankruptcy in 2012.

Biotech excitement aside, the science continued to advance. Yamamoto et al. (2001) published their work on the genetic transformation of *L. minor* and *L. gibba,* while Vunsh et al. (2007) described theirs on high expression of transgene protein in *Landoltia punctata 5562* (formerly, *Spirodela oligorrhiza*). Rapid follow ups from these groups described scientific (if not financial) success stories in the therapeutic protein (Gasdaska et al., 2003) and pharmaceutical (Rival et al., 2008) spheres.

Transition to the Genomic Era: Duckweed Transformation Between 2014 and 2018

The period between 2014 and 2018 represented a critical transitional phase in duckweed research, bridging earlier proof-of-concept transformation studies and the emergence of more systematic, genome-informed engineering approaches. While this interval did not produce a single transformative breakthrough comparable to later developments, it was characterized by a convergence of genomic, physiological, and methodological insights that collectively reshaped the trajectory of the field (Wang et al., 2014; Van Hoeck et al., 2015; Michael et al., 2017).

A defining milestone at the beginning of this period was the publication of the complete genome sequence of *Spirodela polyrhiza* in 2014 (Wang et al., 2014). This achievement provided a comprehensive molecular framework for a representative duckweed species. The genome was notable for its relatively small size and reduced gene redundancy, suggesting that duckweed could serve as a simplified model for studying gene function, and revealed signatures of evolutionary reduction associated with the aquatic lifestyle of Lemnaceae (Wang et al., 2014; Michael et al., 2017).

The availability of a reference genome altered how transformation was conceptualized in duckweed. Prior to 2014, approaches were largely empirical, relying on trial-and-error. With genomic information, researchers could design constructs more rationally and interpret transgene integration within a defined framework (Van Hoeck et al., 2015; Michael et al., 2017).

Despite these advances, transformation efficiency remained highly variable, and it became clear that the primary limitation was not DNA delivery but the ability to regenerate whole plants from transformed tissues. Regeneration, achieved through callus induction and organogenesis, showed strong dependence on species, genotype, and culture conditions, including media composition and

phytohormone balance (Li et al., 2004; Khvatkov et al., 2015; Yang et al., 2018). Even minor variations could lead to significant differences in transformation outcomes.

This realization led to a conceptual shift: transformation success was increasingly understood as a function of biological compatibility rather than purely technical optimization. As a result, protocols developed for one genotype or species were often not transferable to others, limiting reproducibility across laboratories (Yang et al., 2018; Yamamoto et al., 2001).

At the same time, duckweed research experienced a resurgence driven by advances in genomics and systems biology. Duckweed re-emerged as a valuable system due to its rapid clonal growth, minimal morphology, and suitability for controlled cultivation, while transcriptomic and proteomic approaches enabled broader functional analyses (Wang et al., 2014; Van Hoeck et al., 2015).

The combination of genomic resources and omics approaches created new opportunities for functional studies. However, the lack of robust and reproducible transformation systems limited the extent to which these possibilities could be fully realized (Yang et al., 2018; Vunsh et al., 2007).

A key milestone was the demonstration of stable Agrobacterium-mediated transformation in *S. polyrhiza* in 2018 (Yang et al., 2018). This confirmed that genetic manipulation of this species was feasible, although the system remained dependent on specific genotypes and optimized conditions and did not resolve broader challenges of reproducibility (Yang et al., 2018; Barragán-Borrero et al., 2026).

In parallel, early efforts began to explore alternative transformation strategies, including direct transformation of intact tissues and early transient expression systems (Boehm et al., 2001; Yang et al., 2018), laying the groundwork for the methodological diversification that followed.

From Feasibility to Reproducibility: Duckweed Transformation Between 2018 and 2026

Following the demonstration of stable Agrobacterium-mediated transformation in *S. polyrhiza* in 2018, the duckweed field entered a phase of rapid methodological diversification and conceptual refinement (Yang et al., 2018). This milestone established that stable genetic transformation could be achieved in a key duckweed species but did not resolve a central challenge: transformation systems remained highly dependent on genotype and regeneration capacity, limiting reproducibility across laboratories (Li et al., 2004; Yang et al., 2018; Barragán-Borrero et al., 2026).

In the years following 2018, research focused on expanding transformation strategies. Continued refinement of callus-based systems improved selection and regeneration but remained sensitive to biological variability, reinforcing regeneration as a primary bottleneck (Li et al., 2004; Khvatkov et al., 2015; Islam et al., 2026).

At the same time, alternative approaches gained prominence. Direct in planta transformation methods aimed to bypass tissue culture constraints, although efficiency remained variable and species-dependent (Yang GL et al., 2018; Wang et al., 2021). The expansion of transformation systems into *Lemna* species highlights the diversification of duckweed as a genetic platform.

A major advancement was the re-emergence of transient expression systems as a flexible alternative to stable transformation. Agrobacterium-mediated approaches enabled high levels of recombinant protein production in duckweed species (Peterson et al., 2021), while alternative delivery strategies such as DNA-wrapped carbon nanotubes further expanded the transformation toolkit (Islam et al., 2024).

In parallel, genome editing technologies introduced the possibility of precise genetic manipulation. CRISPR/Cas9-mediated editing was demonstrated in *Lemna aequinoctialis* (Liu et al., 2019; Wang et al., 2021), expanding the functional genomics toolkit. However, editing efficiency and stability remain limited, largely due to dependence on transformation and regeneration systems and challenges in maintaining stable transgene expression. Evidence of unstable expression suggests the involvement

of gene silencing mechanisms that restrict sustained activity (Peterson et al., 2021; Barragán-Borrero et al., 2026).

Transformation systems were also extended toward applied biotechnology. Efficient transformation in *L. minor* enabled recombinant protein production with potential applications such as vaccine delivery (Tan et al., 2022).

Despite these advances, reproducibility and standardization remained major limitations, as protocols were often genotype-specific and difficult to transfer between laboratories (Yang et al., 2018; Islam et al., 2026).

A major breakthrough was achieved in 2025–2026 with the development of reproducible transformation and genome editing systems in *S. polyrhiza* (Barragán-Borrero et al., 2026), complemented by systematic optimization approaches that significantly improved efficiency across transformation workflows (Islam et al., 2026).

Taken together, developments between 2018 and 2026 reflect a transition from feasibility to increasingly integrated and reproducible transformation platforms, positioning duckweed as a versatile system for functional genomics and applied plant biotechnology.

Conclusion and Future Perspectives

Looking forward, a central challenge will be the development of genotype-independent transformation systems. Achieving this goal will require a deeper understanding of the molecular mechanisms underlying regeneration and developmental plasticity, likely through integration of transcriptomic and epigenomic approaches. In parallel, continued advances in genome editing are expected to enable more precise and multiplexed genetic modifications (Acosta et al., 2021). From an applied perspective, duckweed holds considerable promise as a biotechnology platform. Its rapid growth, simple morphology, and compatibility with controlled cultivation systems make it particularly attractive for the production of recombinant proteins and other high-value compounds. In conclusion, while challenges remain, particularly in overcoming genotype dependence, recent advances indicate that duckweed is approaching a level of genetic accessibility comparable to established plant systems, positioning it as both a valuable model for plant biology and a versatile platform for future biotechnological applications (Barragán-Borrero et al., 2026).

References

Acosta, K., Appenroth, K. J., Borisjuk, L., Edelman, M., Heinig, U., Jansen, M. A. K., Oyama, T., Pasaribu, B., Schubert, I., Sorrels, S., Sowjanya Sree, K., Xu, S., Michael, T. P., & Lam, E. (2021). Return of the Lemnaceae: duckweed as a model plant system in the genomics and postgenomics era. *Plant Cell* (Vol. 33, Issue 10). https://doi.org/10.1093/plcell/koab189

Barragán-Borrero, V., De, A., Lopes, S., Diniz, E., Batista, R., Höfer, M., Elias, R., Chakraborty, A., Ponce-Mañe, A., Descombes, C., Diezma-Navas, L., Petraki, L., Huber, M., Xu, S., & Marí-Ordóñez, A. (2026). Strain, procedures, and tools for reproducible genetic transformation and genome editing of *Spirodela polyrhiza* (L.) Schleid. *New Phytologist* 250: 735-756. DOI10.1111/nph.70919.

Boehm, R., Kruse, C., Voeste, D., Barth, S., & Schnabl, H. (2001). A transient transformation system for duckweed (*Wolffia columbiana*) using Agrobacterium-mediated gene transfer. *Journal of Applied Botany*, *75*(3–4).

Chang, W. C., & Hsing, Y. ie. (1978). Callus formation and regeneration of frond-like structures in *Lemna perpusilla* 6746 on a defined medium. *Plant Science Letters*, *13*(2). https://doi.org/10.1016/0304-4211(78)90241-9

Chang, W.-C., & Chiu, P.-L. (1978). Regeneration of *Lemna gibba* G 3 through Callus Culture. *Zeitschrift Für Pflanzenphysiologie*, *89*(1). https://doi.org/10.1016/s0044-328x(78)80033-6

Edelman M; Perl A; Flaishman M; Blumenthal A (1999) Transgenic Lemnaceae. WO 99/19497

Frey, M., Rall, S., Roth, A., & Hemleben, V. (1980). Evidence for uptake of plamid DNA into intact plants (*Lemna perpusilla*) proved by an *E. coli* transformation assay. *Zeitschrift Für Naturforschung. Section C: Biosciences*, *35*(11–12). https://doi.org/10.1515/znc-1980-11-1243

Frick, H. (1991). Callogenesis and Carbohydrate Utilization in *Lemna minor*. *Journal of Plant Physiology*, *137*(4). https://doi.org/10.1016/S0176-1617(11)80307-X

Gasdaska, J., Spencer, D., & Dickey, L. (2003). Advantages of Therapeutic Protein Production in the Aquatic Plant *Lemna*. *BioProcessing Journal*, *2*(2). https://doi.org/10.12665/j22.gasdaska

Islam, T., Kalkar, S., Tinker-Kulberg, R., Ignatova, T., & Josephs, E. A. (2024). The "Duckweed Dip": Aquatic *Spirodela polyrhiza* Plants Can Efficiently Uptake Dissolved, DNA-Wrapped Carbon Nanotubes from Their Environment for Transient Gene Expression. *ACS Synthetic Biology*, *13*(2). https://doi.org/10.1021/acssynbio.3c00620

Islam, T., Ligaba-Osena, A., & Josephs, E. A. (2025). Systematic Optimization Enables Near-Perfect In Vitro Transformation Efficiencies for *Spirodela polyrhiza* (Greater Duckweed). *bioRxiv*. DOI10.1101/2025.09.10.675371.

Kehoe, D. M., Degenhardt, J., Winicov, I., & Tobin, E. M. (1994). Two 10-bp regions are critical for phytochrome regulation of a *Lemna gibba* Lhcb gene promoter. *Plant Cell*, *6*(8). https://doi.org/10.2307/3869890

Khvatkov, P., Chernobrovkina, M., Okuneva, A., Shvedova, A., Chaban, I., & Dolgov, S. (2015). Callus induction and regeneration in *Wolffia arrhiza* (L.) Horkel ex Wimm. *Plant Cell, Tissue and Organ Culture*, *120*(1). https://doi.org/10.1007/s11240-014-0603-4

Klein, T. M., Sanford, J. C., Wolf, E. D., & Wu, R. (1987). High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature*, *327*(6117). https://doi.org/10.1038/327070a0

Li, J., Jain, M., Vunsh, R., Vishnevetsky, J., Hanania, U., Flaishman, M., Perl, A., & Edelman, M. (2004). Callus induction and regeneration in *Spirodela* and *Lemna*. *Plant Cell Reports*, *22*(7). https://doi.org/10.1007/s00299-003-0724-4

Liu, Y., Wang, Y., Xu, S., Tang, X., Zhao, J., Yu, C., He, G., Xu, H., Wang, S., Tang, Y., Fu, C., Ma, Y., & Zhou, G. (2019). Efficient genetic transformation and CRISPR/Cas9-mediated genome editing in *Lemna aequinoctialis*. *Plant Biotechnology Journal*, *17*(11). https://doi.org/10.1111/pbi.13128

Moon, H. K., & Stomp, A. M. (1997). Effects of medium components and light on callus induction, growth, and frond regeneration in *Lemna gibba* (Duckweed). *In Vitro Cellular and Developmental Biology - Plant*, *33*(1). https://doi.org/10.1007/s11627-997-0035-5

Peterson, A., Kishchenko, O., Zhou, Y., Vasylenko, M., Giritch, A., Sun, J., Borisjuk, N., & Kuchuk, M. (2021). Robust Agrobacterium-Mediated Transient Expression in Two Duckweed Species (Lemnaceae) Directed by Non-replicating, Replicating, and Cell-to-Cell Spreading Vectors. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.761073

Rival, S., Wisniewski, J. P., Langlais, A., Kaplan, H., Freyssinet, G., Vancanneyt, G., Vunsh, R., Perl, A., & Edelman, M. (2008). *Spirodela* (duckweed) as an alternative production system for pharmaceuticals: A case study, aprotinin. *Transgenic Research*, *17*(4). https://doi.org/10.1007/s11248-007-9123-x

Rolfe, S. A., & Tobin, E. M. (1991). Deletion analysis of a phytochrome-regulated monocot rbcS promoter in a transient assay system. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(7). https://doi.org/10.1073/pnas.88.7.2683

Stomp, A., & Rajbhandari, N. (2003). Genetically engineered duckweed. *US Patent App. 10/677,441*.

Tan, X., Chen, S., Fang, Y., Liu, P., Hu, Z., Jin, Y., Yi, Z., He, K., Li, X., Zhao, L., Wang, H., & Zhao, H. (2022). Rapid and Highly Efficient Genetic Transformation and Application of Interleukin-17B Expressed in Duckweed as Mucosal Vaccine Adjuvant. *Biomolecules*, *12*(12). https://doi.org/10.3390/biom12121881

Vunsh, R., Li, J., Hanania, U., Edelman, M., Flaishman, M., Perl, A., Wisniewski, J. P., & Freyssinet, G. (2007). High expression of transgene protein in *Spirodela*. *Plant Cell Reports*, *26*(9). https://doi.org/10.1007/s00299-007-0361-4

Wang, K. T., Hong, M. C., Wu, Y. S., & Wu, T. M. (2021). Agrobacterium-mediated genetic transformation of taiwanese isolates of *Lemna aequinoctialis*. *Plants*, *10*(8). https://doi.org/10.3390/plants10081576

Wang, W., Haberer, G., Gundlach, H., Gläßer, C., Nussbaumer, T., Luo, M. C., Lomsadze, A., Borodovsky, M., Kerstetter, R. A., Shanklin, J., Byrant, D. W., Mockler, T. C., Appenroth, K. J., Grimwood, J., Jenkins, J., Chow, J., Choi, C., Adam, C., Cao, X. H., … Messing, J. (2014). *The Spirodela polyrhiza* genome reveals insights into its neotenous reduction fast growth and aquatic lifestyle. *Nature Communications*, *5*. https://doi.org/10.1038/ncomms4311

Yamamoto, Y. T., Rajbhandari, N., Lin, X., Bergmann, B. A., Nishimura, Y., & Stomp, A. M. (2001). Genetic transformation of duckweed *Lemna gibba* and *Lemna minor*. *In Vitro Cellular and Developmental Biology - Plant*, *37*(3). https://doi.org/10.1007/s11627-001-0062-6

Yang, G. L., Fang, Y., Xu, Y. L., Tan, L., Li, Q., Liu, Y., Lai, F., Jin, Y. L., Du, A. P., He, K. Z., Ma, X. R., & Zhao, H. (2018). Frond transformation system mediated by *Agrobacterium tumefaciens* for *Lemna minor*. *Plant Molecular Biology*, *98*(4–5). https://doi.org/10.1007/s11103-018-0778-x

Yang, J., Hu, S., Li, G., Khan, S., Kumar, S., Yao, L., Duan, P., & Hou, H. (2020). *Transformation Development in Duckweeds*. https://doi.org/10.1007/978-3-030-11045-1_15

Student spotlight: Maria Spektor

Mevo'ot Iron boarding school -Ecological Greenhouse Kibbutz Ein Shemer, Israel

(email: mariaspektor535@gmail.com)

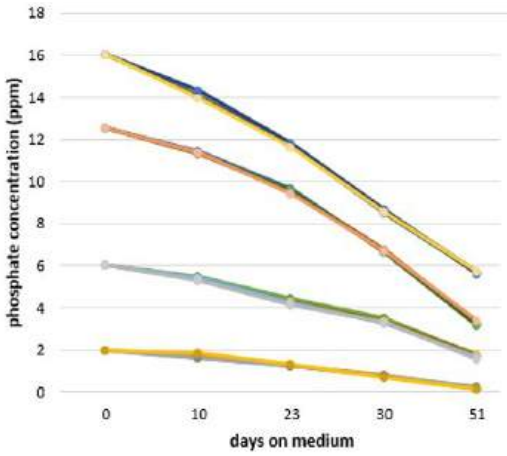
I would like to share my research-story with you. I am a 12th grade student at the Mevo'ot Iron boarding school where I recently completed my thesis on duckweed cultivation. I came to Israel on a Youth Immigration program in 2023 when I was 16. Since then, I have been studying hard at the boarding school attached to kibbutz Ein Shemer. Aside from my regular matriculation studies, I have also been learning Hebrew, and I have done an Advanced Studies project on duckweed cultivation with my volunteer guide Mike Sassoon (a Chemical Engineer by profession). The project took place at the Ecological Greenhouse of the kibbutz, which has a long tradition of hosting biology projects by high school students. The impetus for my project came from the realization that the kibbutz has a dairy herd of around 450 milking cows which generate a lot of milking parlour wastewater. This is the wastewater generated when the parlour is cleaned, twice a day, after milking. Mike suggested we use duckweed to purify the wastewater which contains, amongst others, large amounts of ammonia and phosphate. I read up on the literature and discovered that Lemnaceae grow quickly and can take up nitrogen and phosphorus at a high rate.

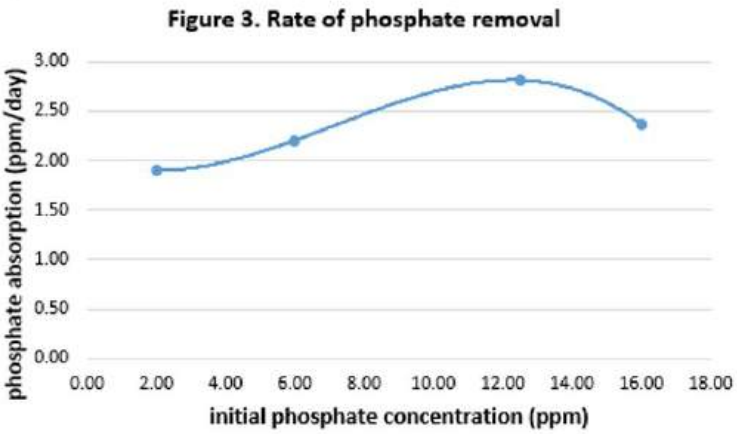
Our idea was to convert the ammonia, present in the wastewater, into nitrate using nitrifying bacteria (separate project with another student), and then use the resulting nitrate/phosphate-rich wastewater as medium for duckweed. This would remove the risk of ammonia toxicity that may occur at higher pH values. The duckweed biomass is subsequently fed to fish (tilapia, grass carp), while the remaining wastewater goes on to irrigate a vertical plant growing system for salads (yet another student project). So, the overall aim was to generate edible plants and fish by using wastewater.

Mike Sassoon comes from the industrial R&D world, so he is constantly looking at ways to commercialize research, meaning innovation. "Going vertical" is one of the innovative strategies that we used for duckweed cultivation. Figure 1 shows an innovative prototype system whereby Lemnaceae grow on a fabric that is in a vertical position slowly rotating through a tank with wastewater). However, before we got into vertical cultivation,

Figure 1: Prototype of an innovative vertical Lemnaceae cultivation system. Left, schematic overview of the system. Right, system in operation at Ein Shemer.

we wanted to establish basic facts, so my role was to look at parlour wastewater as a suitable growth medium for Lemnaceae.
I started by studying Lemnaceae growth as a function of phosphate concentration. To do this, we developed a "dummy" parlour water. This firstly involved laboratory analysis of real wastewater from our kibbutz dairy, which was found to contain 55 ppm ammonia, 12.5 ppm phosphate and 3 ppm nitrate. Using purchased chemicals, I reproduced the ammonia and nitrate concentrations and made-up solutions with different strengths of phosphate. This was replicated 4 times each and by measuring the rate of decrease of the phosphate concentration (Figure 2) I was able to determine the rate of phosphate removal (Figure 3). This informed us about optimal phosphate concentrations to aim for.





Left: Figure 2. Concentration of phosphate in synthetic medium with *Lemna minor* plants. Different lines refer to different starting concentrations of phosphate.
Right: Figure 3. Rate of phosphate removal as a function of initial concentration

Next, we wanted to see how the initial duckweed population affects growth. We realized that counting large numbers of duckweed colonies by hand was going to be impractical, so we looked at AI methods, available freely on the Internet. However, we discovered that these weren't so reliable. So, we looked around for another approach. Till now, standard practice in duckweed investigations at the school is simply to count the population of fronds and colonies. We felt that a simple population count didn't go far enough, in the sense that it didn't throw much light on the underlying reasons for duckweed population behaviour. So, we looked at the literature and discovered the concept of "population dynamics". This gives a scientific understanding of how communities grow. Figure 3 illustrates qualitatively the basic models of "exponential" and "logistic" growth. Exponential growth occurs when there are no limits to growth (plenty of nutrients, plenty of light etc). In practice the population will grow exponentially until something puts the brakes on, such as for example limited nutrient supply, overcrowding, disease, predators etc.), and growth becomes logistic.

The relevant equations for each type of behaviour are;

Exponential population growth behaviour; ($P=Poe^{rt}$):

P is population at a given time – known by counting.

Po is the starter population – known.

e is Euler's number, the base of natural logarithms -known

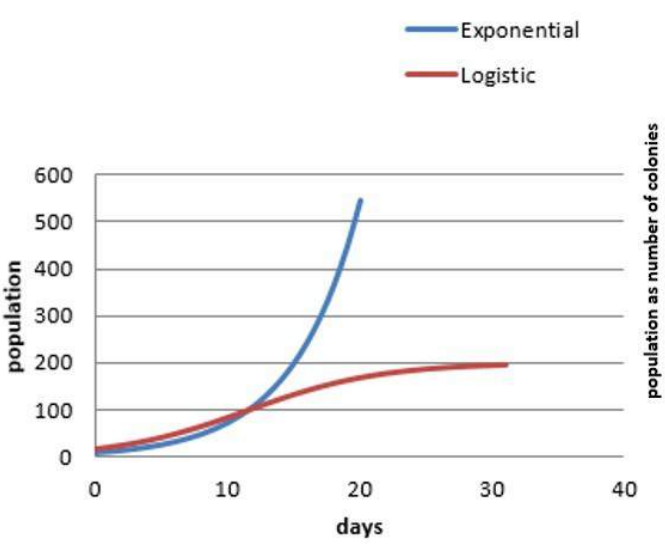
t is the elapsed time (days) – known.

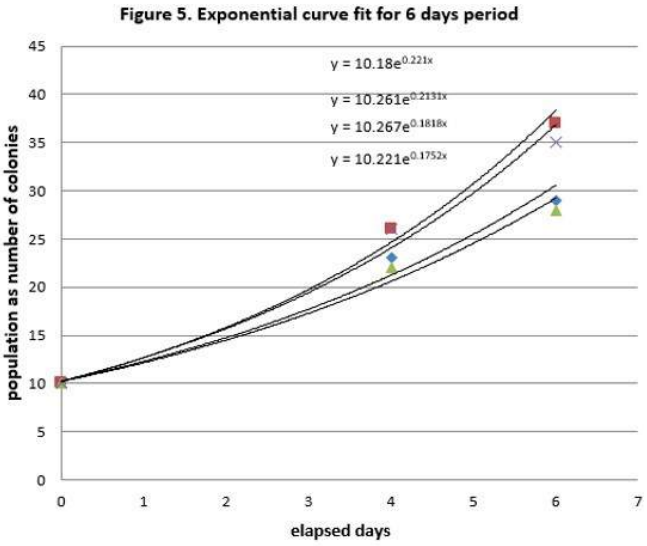
r is RGR – which is the unknown parameter to be determined.

Logistic population growth behaviour; ($P=k/1+Poe^{rt}$):

k is carrying capacity – unknown parameter, and the objective is to find it.

So, the aim was to determine both RGR and the "carrying capacity". We did a couple of "try it and see" tests and sure enough it looked feasible. So, we did the following, a controlled laboratory experiment was conducted using nutrient-limited conditions of 9 ppm phosphate. Containers (15*25 cm) were inoculated with varying numbers of a local strain of *Lemna minor* colonies and fronds to examine how the initial population size affects the growth rate. *Lemna minor* colonies were counted manually throughout the experiment. We saw that the data initially (first 6 days) followed an exponential growth pattern, with a calculated intrinsic growth rate of r ≈ 0.2 day^{-1} (Figure 4). When the experiment was extended for a total of 32 days, we saw that the growth curve started to deviate significantly from the exponential model (Figure 5). The calculated RGR decreased to approximately 0.02 day^{-1}, suggesting that the population had entered a phase influenced by resource limitation. This behaviour is consistent with the logistic growth model, in which population growth slows as environmental resources become limited. Fitting the experimental data with an S-shaped curve (logistic trend) using a third-degree polynomial approximation suggests that for an initial population of 10 colonies, the population approaches an asymptotic maximum of approximately colonies units. We concluded that using Population Dynamics is a simple, reliable and practical tool for researching effects of concentrations of various nutrients, temperature, light, turbidity etc on growth.

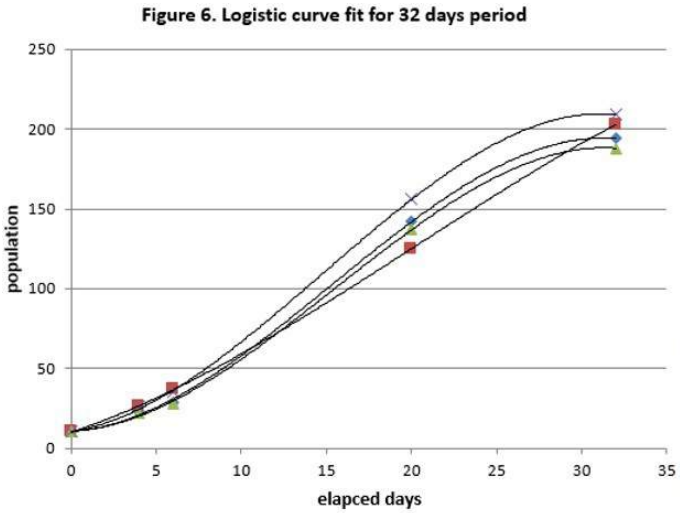


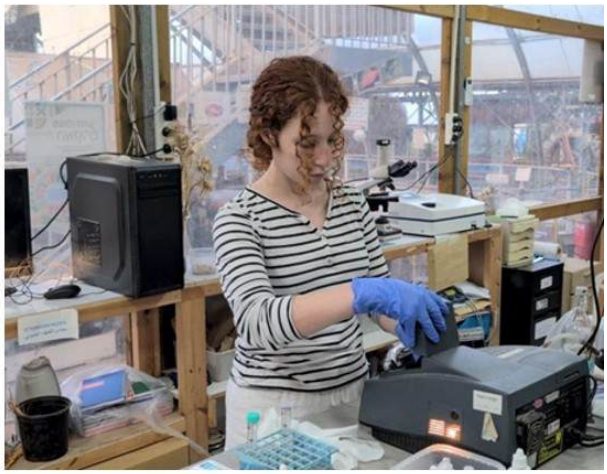


Left: Figure 4. Theoretical examples of exponential and logistic growth of populations.
Right: Figure 5. Exponential growth of a *Lemna minor* population in short-term trial. Different lines refer to replicates.

Next steps: the possibilities are endless but for the immediate future, it would be interesting to explore the growth tests using real parlour wash water (Figure 6). Also, going back to our vertical cultivation idea, we have measured quite a significant drop in light level as one travels from the top to the bottom of the fabric (see figure 1), so it will be interesting to study the effect of these different

light levels on growth using the Population Dynamics technique. For now, I am grateful for the opportunity to present my research to the international duckweed community. I didn't come to this project from a scientific background, but my duckweed-work surely paved the way to science and not only helped me to develop curiosity for the world of research but also allowed me to gain many useful skills.





Left: Figure 6. Logistical growth of a *Lemna minor* population in medium-term trial. Different lines refer to replicates.

Right: Figure 7. The author analysing phosphate concentrations in the laboratory.

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

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

Loaiza, I; Jansen, MAK (2026) International Journal of Phytoremediation
DOI10.1080/15226514.2026.2619609.

Multitiered duckweed bioreactors have been developed but have limitations due to the weight of the water column. It was hypothesized that fog-o-ponic growth systems can enable space efficient duckweed culturing by facilitating stacked cultivation systems with multiple thin layers of duckweed, in the absence of a heavy water column. In this study, the growth was assessed of *Lemna minor* suspended on a fabric textile under a nutrient-rich medium provided as a fog. The best growth of *L. minor* was a relative growth rate (RGR) of 0.24 d^{-1} with a maximum quantum efficiency (Fv/Fm) and light-adapted quantum yield (Y(II)) of around 0.8 and 0.5, respectively, which are values comparable to those achieved on traditional liquid medium under otherwise similar conditions. These results reveal that *L. minor* not only survives under fog-o-ponics culture conditions, but it thrives both in short and longer trials. Consistent with good growth, removal of nutrients by *L. minor* was considerably (e.g.,500 mg total nitrogen (TN) m^{-2} day^{-1}) under fog-o-ponics conditions. It is concluded the innovative way of duckweed culturing comprises a promising, multi-stacked, high capacity, phytoremediation system.

Strain, procedures, and tools for reproducible genetic transformation and genome editing of the emerging plant model *Spirodela polyrhiza*

Barragán-Borrero, V; Lopes, AD; Batista, EDR; Höfer, M; Elias, R; Chakraborty, A; Ponce-Mañe, A Descombes, C; Diezma-Navas, L; Petraki, L; Huber, M; Xu, SQ; Marí-Ordóñez, A (2026) New Phytologist 250: 735-756.

Duckweeds (Lemnaceae) have excellent potential for fundamental and applied research due to ease of cultivation, small size, and continuous fast clonal growth. However, their usage as model organisms and platforms for biotechnological applications is often limited by the lack of universal genetic manipulation methods necessary for transgene expression, gene editing, and other methods to modify gene expression. To identify suitable strains for genetic manipulation of the giant duckweed, *Spirodela polyrhiza*, we screened several genotypes for callus induction and regeneration and established genetic transformation. We identified SP162 to be amenable to Agrobacterium-mediated transformation via tissue culture. The procedure is robust and reproducible across laboratories, allowing stable expression of different reporter genes and selectable markers, enabling CRISPR/Cas9-mediated genome editing. In addition, due to a weak small RNA-based silencing response, *S. polyrhiza* sustains prolonged periods of transgene activity in transient expression assays. To promote duckweed research and encourage the adoption of *S. polyrhiza*, we have made SP162 (ID#: 5676) and its genome publicly available and provide here detailed procedures for its cultivation and transformation. Furthermore, we created a web server to explore its genome, retrieve gene sequences, and implement orthologous gene search and a gRNA design function for diverse CRISPR/Cas-based applications (https://agxu.uni-mainz.de/SP162/).

Aquaculture/ Agriculture

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) Agriculture Ecosystem & 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 CO2 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.

Phosphorus transport under different irrigation modes and urea application regimes in rice-duckweed systems

Chen, XY; Han, HH; Zheng, HL; Liu, XG; Li, JN (2026) Agricultural water Management 328: 110306.

A clear understanding of phosphorus transport in rice-duckweed systems is critical to enhancing phosphorus utilization efficiency (PUE) and mitigating phosphorus non-point source pollution (PNPSP). Here, we conducted a two-year field experiment with two irrigation modes (alternate wetting and drying, AWD; continuous flooding, CF), two nitrogen application rates (193 kg ha-1, N2; 135 kg ha^{-1}, N1), and two nitrogen split application ratios (tiller fertilizer: panicle fertilizer = 70%: 30%, F2; basal fertilizer: tiller fertilizer: panicle fertilizer = 50%: 30%: 20%, F3). Results indicated that phosphorus fertilizer accounted for approximately 97% of total phosphorus input in rice-duckweed systems, with other input (irrigation, wet deposition, and seedling transplanting) being negligible. Rice accumulated 30.4-41.5 kg ha^{-1} of phosphorus, while the average PUE was only 12.0%-13.9%. Soil retention accounted for 46.0% of phosphorus output, and soil total phosphorus (TP) content generally decreased with increasing soil depth. TP concentration in surface water peaked immediately after phosphorus fertilizer application, whereas that in groundwater peaked 20-25 days later, with concentrations dropping significantly with increasing depth. Phosphorus drainage and leaching accounted for less than 1% of phosphorus output, with leaching representing only approximately 10% of drainage; yet they still induced severe PNPSP by high phosphorus-to-nitrogen ratios. AWD regulated duckweed's phosphorus uptake and release to alleviate the phosphorus supply-demand mismatch for rice, thereby boosting PUE, and increasing the nitrogen application rate most notably enhanced phosphorus utilization. The AWDN2F3 treatment performed optimally in improving PUE and mitigating PNPSP in rice-duckweed systems.

Organic fertilization with cow dung modulates growth, bioactive compounds, and antioxidant capacity in duckweed (*Wolffia globosa*)

Yadav, NK; Patel, AB; Rajesh, SD; Parida, PK; Baidya, S (2026) Frontiers in Plant Science 17: 1751657.

Wolffia globosa (*wolffia*) is a fast-growing aquatic plant rich in nutrients and bioactive compounds, whose biomass and functional quality are strongly influenced by fertilization. However, the effects of organic fertilizers on bioactive composition and antioxidant potential in *wolffia* remain poorly understood. This study fills this gap by evaluating fresh cow dung as an organic fertilizer and elucidating its novel role in enhancing biomass production, biochemical composition, bioactive compounds, and antioxidant capacity of *wolffia*. A randomized complete block design was employed with six treatments and three replications each. Treatments included an inorganic fertilizer control and five levels of cow dung applied at 10, 20, 30, 40, and 50 g L^{-1}. Biomass yield was recorded, while biochemical composition (crude protein, crude lipid), bioactive compounds (total phenolics, flavonoids, carotenoids, tannins, ascorbic acid), and antioxidant activity (DPPH, ABTS, FRAP assays) were analyzed using standard protocols. Biomass was highest at 30 and 20 g L^{-1} cow dung, reaching 152.38 ± 12.25 g and 144.88 ± 1.28 g, respectively. Crude protein content was also highest at 40 g L^{-1} (30.40%) and in the control (29.31%). Crude lipid content peaked at 40 g L^{-1} (5.09%) and 50 g L^{-1} (4.91%). The highest total phenolic content (350.04 mg GAE g^{-1}), total flavonoid content (159.35 mg QE g^{-1}), and ascorbic acid (99.05 mg 100 g^{-1}) were recorded at 50 g L^{-1} cow dung. In contrast, total carotenoid content (2286.90 µg g^{-1}) and chlorophyll-b (11.82 µg g^{-1}) were significantly higher in the control. Total tannin content did not differ significantly among the control, 10, and 50 g L^{-1} treatments, whereas the 20 and 30 g L^{-1} treatments exhibited reduced levels. Antioxidant activity was highest at 50 g L^{-1} for both DPPH (57.42%) and ABTS (65.65%) assays, while FRAP activity peaked at 10 g L^{-1} (180.64 µmol Fe g^{-1}). The results demonstrate that cow dung enhances the bioactive compounds and antioxidant potential of *wolffia*. While medium doses (20-30 g L^{-1}) maximized biomass production, higher cow dung levels, particularly 50 g L^{-1}, promoted greater accumulation of phenolics, flavonoids, and overall antioxidant capacity.

DF comment: No information was given concerning origin and species identity of *Wolffia globosa*

Biochemistry

Biochemical characterization of xyloglucan galactosyltransferases MUR3 and XLT2 from *Spirodela polyrhiza*

Corulli, CJ; Graf, AS; Chapla, D; Zhang, L; Ziegler, SJ; Byrnes, J; Bomble, YJ; Moremen, KW; Urbanowicz, BR; Prabhakar, PK (2026) Plant Journal 125: e70754.

Glycosyltransferases (GTs) are the primary enzymes responsible for the biosynthesis of the complex polysaccharides in plant cell walls. Given the important role of GTs in plants, it is necessary to undertake their functional characterization to better understand plant cell wall synthesis pathways to develop improved feedstocks for efficient conversion into fuels and products to support the emerging bioeconomy. The GT47 family in plants represents a unique target for characterization due to the substantial diversity of donor and acceptor substrates observed within a single family. Here, we have carried out the biochemical characterization of MUR3 and XLT2 orthologs from the aquatic monocot *Spirodela polyrhiza*. Our findings support existing genetic and phylogenetic data classifying these enzymes as regio-specific galactosyltransferases involved in xyloglucan (XyG) sidechain biosynthesis. In addition, we have identified novel characteristics for both enzymes, such as in vitro arabinopyranosyltransferase activity and distinctiveness in xyloglucan reducing end specificity.

Biotechnology

Impact of thermal processing on amino acid composition, physicochemical properties, and bioactive compounds of watermeal (*Wolffia globosa*) extract

Suwannarong, S; Boonarsa, P; Ratseewo, J; Siriamornpun, S (2026) Applied Food Research 6: 101875.

Watermeal (*Wolffia globosa*) is a nutrient-dense aquatic plant receiving increasing attention as a sustainable plant-based protein source due to its protein content and bioactive compounds. This study investigated the effects of two thermal processes, namely boiling and autoclaving, on the amino acid composition, physicochemical properties, bioactive compound and antioxidant activities of a watermeal extract (WME). Boiling markedly increased essential amino acids (45.4 to 79.9 mg/100 mL), particularly valine, methionine, and phenylalanine. Sweet-related amino acids remained predominant, although bitterness-associated amino acids also increased slightly after heating. Both processes lowered the pH and lightness but enhanced total soluble solids and protein content, with boiled samples showing the highest protein content (176 mg/100 mL), followed by autoclaved and raw samples. The total phenolic content nearly tripled after boiling, increasing from 236 to 635 µg GAE/mL (2.7-fold), while total flavonoid content increased by 2.5-fold. DPPH radical scavenging activity increased by 7.5-fold in boiled samples (104 to 775 µg AA/mL) and by 7.1-fold in autoclaved samples, accompanied by a corresponding rise in reducing power, as indicated by FRAP values (4600 to 5700 µg $FeSO_4$/mL). HPLC analysis confirmed an increased release of gentisic acid, catechin, and apigenin after boiling. These results suggest that thermally processed WME, particularly when processed by boiling, can serve as a promising functional ingredient for plant-based beverages, protein-fortified foods, and nutraceutical formulations, while providing a basis for the further development of sustainable protein ingredients through process optimization and product formulation studies.

Lemna minor as support biomass for enhancing the biomethane yield of brewery's spent grain pulp when used in co-digestion

Di Mario, J; Nocella, S; Gambelli, AM; Del Buono, D; Gigliotti, G (2026) Agriculture 16: 545.

Pursuing the so-defined biorefinery approach, residual biomass, such as agro-industrial wastes, should first be exploited for the extraction and production of high-value-added products and then processed for energy valorisation through anaerobic digestion (AD). However, the treatments applied to achieve the first goal could impact biogas yield. This problem can be solved by co-digesting the treated biomass with others. In this study, Brewery' Spent Grain (by itself, a good biogas producer) was treated with an ionic liquid (IL) composed of triethylamine and sulfuric acid [TEA][HSO4] for lignin removal. The residual biomass (pulp, BSGp) was then used for biogas production. The tests revealed a marked reduction in the total quantity of biomethane (per unit of volatile solid-VS). In detail, 6.82 x 10^{-4} Nm^3CH_4/g VS of biomethane was produced with BSGp, against 1.31 x 10^{-3} Nm^3CH_4/g VS with BSG. The lack of organic nitrogen after the IL-based treatment prevented biogas production, resulting in a shorter production period. To compensate for the nitrogen deficiency and restore the optimal C/N ratio, BSGp was mixed with *Lemna minor* (LM), an aquatic weed with a high nitrogen content. By itself, LM cannot be considered a good biogas producer as proven in this study. However, the co-digestion of LM with BSGp extended the production period and kept the daily production close to that registered in test made with the sole BSGp, thus achieving a total biomethane production equal to 1.83 x 10^{-3} Nm^3CH_4/g VS, even higher than the one registered with untreated BSG.

DF comment: The only information about the identity of the duckweed species Lemna minor (LM) is the following: "LM was cultivated at the Department of Agricultural, Food and Environmental Sciences, University of Perugia".

Duckweed as a sustainable source: extraction and applications of bioactive nutrients for industrial applications (Review)

Wickramasinghe, M; Hiththatiyage, R; Liyanage, R; Wijesundara, S; Bhattarai, RR (2026) Sustainable Food Technology DOI10.1039/d5fb00755k

Duckweed, a fast-growing aquatic plant belonging to the Lemnaceae family, has been most commonly studied and utilised as a food source due to its excellent palatability and nutrient profile. Other genera, such as *Lemna, Spirodela*, and *Landoltia*, are less commonly consumed by humans due to issues like poor palatability. However, they remain of interest for animal feed and bioresource applications. This review synthesises peer-reviewed literature identified through structured searches of major scientific databases, focusing on studies reporting the nutritional composition, extraction technologies, analytical methods, and food-related applications of duckweed species. Therefore, duckweed is recognised as a sustainable, nutrient-rich food source, offering a viable solution to global challenges in food security, environmental sustainability, and nutrition. Across its diverse species, duckweed exhibits considerable variation in macro- and micronutrient composition, including 25-40% protein by dry weight, significant amounts of starch, polyunsaturated fatty acids (PUFAs), essential amino acids, vitamins, and minerals, contributing to its balanced and functional profile as an ingredient for food formulations. Various extraction methods have been employed to isolate bioactive compounds from the duckweed matrix. Advanced techniques, such as enzymatic hydrolysis, microwave-assisted extraction, and ultrasonication, have been applied explicitly to duckweed to facilitate the efficient recovery of high-quality starch, fats, proteins, and bioactive compounds while preserving their functional properties and nutritional value. Additionally, novel analytical methods, including chromatography, mass spectrometry, and proteomic profiling, enhance the understanding of duckweed's species-specific nutrient composition. This review emphasises the nutritional diversity of duckweed and explores innovative technologies for extracting and purifying its active ingredients. Furthermore, it discusses the various industrial applications of duckweed, including functional foods and nutraceutical preparations, and addresses safety and public acceptance challenges. By highlighting the potential of duckweed for developing new food products, this review underscores its role in promoting global food security, alleviating malnutrition, and contributing to sustainable food systems. This area remains a research gap. Given its rapid growth, high nutrient content, and positive ecological impact, duckweed emerges as a critical resource capable of meeting the needs of a growing population.

Synergistic effects of ammonia and duckweed bio-oil water emulsion in an RCCI engine: Experimental and ANN-based validation

Ramalingam, K; Elumalai, PV; Senthur, NS; Chan, CK; Mahender, M; Ritthong, W; Saleel, CA (2026) Environmental Progress & Sustainable Energy DOI10.1002/ep.70364.

This research explores reactivity-controlled compression ignition (RCCI) engine combustion with duckweed bio-oil (DBO) water emulsions and ammonia combinations to create new sustainable and low-carbon fuels. Six test fuels, including diesel base, pure duckweed bio-oil, duckweed bio-oil with 10% water emulsions, and ammonia energy compositions of 20% (A20), 40% (A40), and 60% (A60) were examined and tested under steady-state engine conditions. The emulsions were mixed using an on-the-fly mixing system to provide homogenization, and ammonia was injected separately with a dedicated port injection system to provide controlled reactivity stratification. The experimental tests indicated that the best combination for engine emissions and performance was with 40% ammonia content, which decreased emissions of hydrocarbons by 18%, carbon monoxide emissions by 9%, carbon dioxide emissions by 6%, and nitrogen oxides emissions by 11% and 19% for smoke emissions with 18% lower emissions than diesel. The improvements were realized through the combined effect caused by duckweed bio-oil's oxygen content, micro-explosions during secondary atomization from water emulsions, and ammonia's carbon-free and hydrogen-rich combustion properties. Notably, engine BTE and BSEC for this combination were both within 2% of diesel, and this combination is fit for dropping and working properly like normal diesel. The experimental findings were accurately predicted by an Artificial Neural Network that showed very accurate values with R^2 = 0.977-0.994 and RMSE and MPAE <0.04 and 4%, respectively. This research gives emphasis to new duckweed emulsions with ammonia fuels that can provide new solutions for efficiency and other emissions problems in low-temperature combustion engines for heavy vehicles.

Application of green *Lemna gibba* phytochemical extract as a corrosion inhibitor for mild steel in H_2SO_4

Gourchane, F; EL Hawary, M; Kerroum, Y; Bellaouchou, A (2026) Next Materals 11: 101659.

The effectiveness of *Lemna gibba*'s ethanolic extract as a natural corrosion inhibitor for carbon steel in a sulfuric acid environment is demonstrated for the first time in this study. Traditionally used in phytoremediation, this aquatic plant reveals new potential as a long-lasting anticorrosive agent. Electrochemical tests, including weight loss, potentiodynamic polarization (PDP), and electrochemical impedance spectroscopy (EIS), revealed inhibitory efficiencies of 82.85 %, 84.74 % and 79.99 % respectively at an ideal concentration of 800 ppm. The extract functions as an inhibitor of mixed types, and adsorption of the bioactive compounds present in the extract follows the Langmuir isotherm, making a protective monolayer on the steel surface, confirmed by SEM/EDX analysis. The inhibition mechanism is endothermic, involving both physisorption and chemisorption. Spectroscopic analyses (UV-Visible, FTIR, and GC-MS) revealed the presence of oxygen-rich functional groups - hydroxyls, carbonyls, and esters - able to form coordination bonds with ferrous ions (Fe^{2+}). The adsorption of active molecules to the metal surface is facilitated by this chemical interaction, which enhances the development of the anticorrosion layer. *Lemna gibba* could therefore be considered a promising, environmentally friendly, effective, and economically viable alternative to conventional corrosion inhibitors, without the need for external chemical additives.

Balancing biomass and starch accumulation in duckweed: Unraveling the regulation mechanism of nutrient concentration via transcriptomics

Han, DJ; Chen, ZQ; Zhang, P; Cheng, L; Hong, Y; Gu, ZB (2026) Industrial Crops and Products 239: 122547.

Duckweed has rapid growth, high starch content, and is easily regulated, making it a novel starch resource. Nutrient restriction accelerates starch accumulation in duckweed. However, the impact of changes in the nutrient concentration regulatory mechanism underlying starch accumulation remains unclear. This study optimized the nutrient concentration and analyzed key parameters of *Spirodela polyrhiza* ZH0196 starch accumulation, including starch content, photosynthetic pigments, and soluble sugars, to clarify the role of nutrient concentration. It also conducted an investigation and identification of key enzyme activities and key metabolic pathways involved in starch accumulation. Results showed that *Spirodela polyrhiza* ZH0196 growth and starch accumulation responded differently to initial nutrient levels. The 0 % nutrient group had the highest AGPase enzyme activity (12.42 ± 1.58 U/mg protein) among all groups (1.8-8.5 U/mg protein), but its final starch output was lower than that of the 40 % group. As *S. polyrhiza* ZH0196 grew and nutrient concentration declined, photosynthetic pigments and biomass decreased, while starch-synthetic enzyme activity increased, leading to starch accumulation. These processes are closely associated with photosynthetic metabolism. Analysis of differentially expressed genes revealed a downregulation in biological processes related to ATP and NADPH generation, while genes involved in starch-sucrose metabolism and glycolysis were upregulated. Overall, these findings demonstrate that *S. polyrhiza* ZH0196 exhibits notable advantages under lower (40 %) nutrient concentrations by reducing biomass production and enhancing starch accumulation. This study provides a theoretical basis for regulating starch biosynthesis in duckweed and offers a potential technical strategy for sustainable duckweed cultivation as bioenergy.

Sustainable and flexible Zn-Air batteries enabled by bifunctional electrocatalysts derived from *Lemna minor* (Duckweed) biochar

Audeves, Y; Coello-Mauleón, C; García-Estrada, JA; Alvarez-Contreras, L; Olivas, A; España-Sánchez, BL; Arjona, N (2026) Chemnanomat 12: e202500551.

Lemna minor is an aquatic plant with a high growth rate, which can cause problems in freshwater bodies. In this work, *L. minor* was valorized for the development of bifunctional electrocatalysts for rechargeable and flexible zinc-air batteries (FZABs), by synthesizing cobalt-doped and cobalt-manganese co-doped electrocatalysts (L-Co and L-CoMn). Raman spectroscopy revealed structural disorder, particularly in L-Co, which was further confirmed by TEM and attributed to a high density of surface defects. Moreover, TEM and STEM imaging indicated the formation of both spinel nanoparticles and atomically dispersed metal sites, which together with surface defects, contributed to the electrocatalytic activity. Electrochemical tests showed

that L-Co exhibited superior activity in the oxygen reduction reaction, while L-CoMn demonstrated enhanced activity for the oxygen evolution reaction, achieving a low overpotential of 1.56 V at 10 mA cm^{-2}. When used as bifunctional electrocatalysts in FZABs, the L-Co presented better performance, higher cycling stability (>100 cycles), and improved capability to operate at elevated current densities, while achieving an areal specific capacity of 14.7 mA.h cm^{-2}. These results demonstrate the potential of *L. minor* valorization for applications in electrochemical and sustainable energy technologies.

Ecology

Preliminary evaluation of *Wolffia globosa* as an alternative for biological CO_2 capture: Biomass productivity, CO_2 fixation rate, and biochemical composition

Sucunthowong, K; Plengsakul, J; Powtongsook, S; Nootong, K (2026) Journal of Environmental and Chemical Engineering 14: 121967.

This study evaluates the effects of elevated CO_2 concentration (5-15% v/v) and simulated cement flue gas (15.50% CO_2, 220 ppm NO, and 1.3 ppm SO_2) on biomass production, CO_2 fixation rate, and biochemical composition of *Wolffia globosa* cultivated in well-mixed, continuously aerated bioreactors. The highest biomass concentration (6160 mg L^{-1}) and CO_2 fixation rate (825 mg COQ L^{-1} d^{-1}) are achieved at 15% CO2. Despite a substantial decline under simulated flue gas conditions, both biomass concentration (3714 mg L^{-1}) and CO_2 fixation rate (465 mg COQ L^{-1} d^{-1}) remain approximately 45% higher than those observed under ambient air. The highest protein content (21.08%) on a dry-weight basis (DW) is obtained under ambient air, whereas lower protein contents (10.66-14.10% DW) are recorded under elevated CO_2 concentrations. At 10% CO_2, the highest carotenoid content is observed with key carotenoids including zeaxanthin, violaxanthin, neoxanthin, and beta-carotene. Overall, the high CO_2 fixation rates, particularly at 10% and 15% CO_2, and the ability to maintain relatively high CO_2 fixation rates under simulated flue gas highlight the potential of *W. globosa* as a promising alternative to microalgae for biological CO_2 capture.

Greenhouse gas and ammonia emissions from duckweed cultivation systems using diluted liquid manure

Stadtlander, T; Gomez, DM; Müller, R; Baki, C; Brueggemann, N; Leiber, F; Krause, HM; Agostini, L (2026) Scientific Reports 16: 9887.

Protein from duckweed (Araceae, subfamily *Lemnoideae*) grown on diluted animal slurries for nutrient upcycling could potentially replace plant-derived feed proteins which would be more efficiently used as human food. However, little information is available on greenhouse gas (GHG) emissions from slurry-grown duckweed, and previous studies have not reported methane emissions from a similar system. Here, we report on GHG (methane, carbon dioxide and nitrous oxide) and ammonia emissions from duckweed grown on diluted cattle slurry measured in daylight and darkness, compared with emissions from diluted slurry without duckweed. We observed (i) initially high but rapidly declining methane emissions, independent of lighting or treatment, (ii) a net carbon dioxide fixation by duckweed, independent of lighting, (iii) high nitrous oxide emissions, independent of lighting, and (iv) a > 80% reduction of ammonia emissions by duckweed, independent of lighting. Our data shows potential of duckweed protein as a sustainable protein with 3.54 to 6.54 CO^2 eq kg^{-1} protein, compared to faba bean (3.61 kg CO_2 eq kg^{-1} protein) or barley protein (5.35 CO_2 eq kg^{-1} protein). But despite the potential of slurry-grown duckweed as sustainable protein source, swapping ammonia volatilization for nitrous oxide emissions represents a limitation of the current system and mitigation strategies are needed.

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

Introducing a novel course-based undergraduate research experience using duckweed as a model system

Daniels, J; McCallum, M; Neal, N; Nkwocha, E; Machado, SR; Carter, H; Lam, E; Peng, C; O'Brien, A; Wei, N; Frederickson, M; Tan, J (2026) Integrative Organismal Biology 8: obaf049.

Course-based undergraduate research experiences (CUREs) provide a scalable model for engaging students in authentic scientific inquiry, bridging core biological concepts with real-world environmental applications. We introduce a new CURE lab tailored for introductory biology students at the undergraduate level, utilizing duckweed as a model organism to investigate ecological interactions and environmental management. Our paper presents a curriculum that engages students in hands-on research with a focus on duckweed's role in ecosystem dynamics, pollutant remediation, and its potential as a bioresource, along with scientific results from student projects that serve as tangible examples of the curriculum's outcomes. Through experimentation, students explore how duckweed can be applied to address real-world environmental challenges, utilizing advanced laboratory techniques and data analysis tools. Successfully implemented with 192 students across three semesters at our institutions, this CURE lab has produced reliable duckweed growth data with high reproducibility. This curriculum addresses the gap between traditional laboratory exercises and authentic research experiences through introducing opportunities to conduct reproducible experiments, analyze real data, and communicate scientific findings in meaningful contexts.

Feed & Food

Uncovering the protein conversion potential of alfalfa (*Medicago sativa* L.) and duckweed (*Lemna minor* L.) through enzymatic hydrolysis and digestibility assessment

Pauliukaityte, I; Zygyte, M; Salaseviciene, A; Almonaityte, K (2026) Foods 15: 885.

The growing demand for sustainable protein alternatives has increased interest in underutilized plant biomasses with high nutritional potential. This study investigated the conversion efficiency of alfalfa (*Medicago sativa* L.) and duckweed (*Lemna minor* L.) proteins through multienzyme hydrolysis, with the aim of evaluating how carbohydrate-protein matrix interactions influence enzymatic accessibility and apparent protein digestibility. Three biotechnological hydrolysis schemes were applied, involving combinations of alpha-amylase, amyloglucosidase, protease, pepsin, pancreatin, and bile salts, including an in vitro gastrointestinal digestion simulation. The first hydrolysis scheme demonstrated that starch-rich matrices formed a viscous medium that reduced protease mobility and limited protein cleavage. Improved substrate accessibility was achieved when plant material was pre-treated with amylolytic and proteolytic enzymes, which resulted in a noticeably higher release of free amino acids. Amino acid profiling revealed that this enzymatic sequence was the most effective for disrupting carbohydrate-associated protein fractions in both species. In vitro digestion assays indicated higher apparent protein conversion for duckweed compared to alfalfa under standardized laboratory conditions. Overall, the results confirm that appropriate multienzyme strategies can enhance amino acid liberation from complex plant matrices and highlight duckweed biomass as a promising candidate for sustainable protein valorization.

DF comment: The authors wrote: “Duckweed (contained 22.0% crude protein, 8.1% crude fat, 45.4% total fiber, and 8.2% starch), belonging to the Lemnaceae family, was cultivated and harvested in the Netherlands.” This does not tell the reader, which species has been investigated as in the Netherlands several Lemna species are common.

Dietary effects of duckweed on performance, carcass characteristics, hematology, immunity, sensory traits, and fatty acid profile of meat in broilers

Saei, S; Seidavi, A; Nosrati, M (2026) Scientific Reports 16: 10405.

We have evaluated the effects of duckweed (*Lemna minor*) diets on production performance, carcass characteristics, biochemical parameters and blood antioxidant status, immune system, sensory and taste traits and breast fatty acid profile in broiler chickens during three periods (starter, grower, and finisher) and the entire rearing period, using 150 broiler chickens of the Ross 308 strain. This included 3 levels of chickweed at 0, 3, and 4% with 5 replications and 10 birds in each replication, in a completely randomized design. The results showed that the duckweed did not have any negative effect on production performance, and the weight of the broilers in the waterweed groups was similar to that of the control group ($P > 0.05$). The results of the immune system trait analysis showed that for all traits except Fabricius weight, there was no significant difference between the treatment groups. However, some significant differences were observed between the waterweed groups and control group: the smell of the meat was affected after duckweed feeding, increasing with increasing duckweed percentage ($P < 0.05$). Overall, we have demonstrated that duckweed can help improve blood and antioxidant parameters while maintaining performance, although it affected the smell of the meat. However, we conclude that it has the potential to be used in functional diets for broiler chickens.

DF comment: These are the information given by the authors about the identity of the plant samples: "The powdered entire plant of *Lemna minor* species from Darvash Giah Khazar medicinal herbs complex company (Ltf) (Rasht- Guilan- Iran) was used as the test ingredient in the present study, which is abbreviated as LM in this report. LM was prepared and ground according to the protocol of Ifie et al. in a temporary pond located in the town of Kishestan, Somehsara, Guilan, Iran." Consequently, it could by any of the *Lemna* species what the authors investigated.

Water lentil rich in nutrients and bioactive components - A powerful group of vegetables for humanity's future plant-based diet

Jahreis, G; Sree, KS; Dawczynski, C; Appenroth, KJ (2026) Trends in Food Science & Technology 170: 105600.

Water lentils have the potential to be a future sustainable food source on the planet as well as for space travel. In this review, various species of the Lemnaceae family were analysed for their nutritive and antinutritive properties, and their future relevance for nutrition and health of a growing world population. Scope and approach: Several water lentil species are characterised by high content of proteins, fibre and health promoting phytochemicals. Their protein quality significantly exceeds that of cereals. Moreover, the protein digestibility-corrected amino acid score varies between 0.67 and 0.98. In addition, they have valuable techno functional properties. Protein from Lemna minor is used in the production of egg substitutes and plant-based meat alternatives. The lipids have a high omega-3 fatty acid content. Species of Wolffia have a remarkably favourable n-6/n-3 ratio of less than 1. A significant feature of water lentils is their ability to contribute to the supply of vitamin B12 through symbiotic bacteria. In addition to direct consumption and the use of individual components as food ingredients, certain Wolffia species also offer a platform for biopharmaceutical production of recombinant proteins, synthesis of bioactive metabolites and development of functional foods. Key findings and conclusions: Overall, water lentils are rich in nutrients. Noteworthy, as a constituent of plant based diet, water lentils have the potential to close gaps in the supply of vitamin B12 and omega-3 fatty acids. Additionally, antinutritive compounds such as phytate, oxalate, or nitrate do not have a substantial impact on the nutritional benefits of water lentils.

Wolffia globosa as an emerging plant-based protein source for functional and nutraceuticals

Venkatachalam, K; Phongthai, S; Puttha, R; Wongsa, J; Charoenphun, N (2026) Foods 15: 543.

Wolffia globosa, an edible aquatic plant of the Lemnaceae family, has gained increasing attention as a potential alternative protein and functional food ingredient due to its rapid biomass production, favorable amino acid profile, and micronutrient content. This review critically evaluates the current evidence on the nutritional composition, protein quality, reported bioactive properties, safety considerations, and regulatory status of *W. globosa*, focusing on its suitability for food applications. Literature data indicate that *W. globosa* biomass can contain substantial protein levels on a dry-weight basis, with reported protein quality metrics approaching those of some conventional plant proteins under specific processing conditions. In addition, studies have explored the high antioxidant, antihypertensive, and metabolism-related bioactivities of *W. globosa*, primarily based on in vitro and animal studies. However, human clinical evidence remains limited, and

reported functional effects should be interpreted with caution. Regulatory assessments, including novel food authorization in certain jurisdictions, support its use as a food ingredient under defined conditions but do not substantiate health claims. Overall, *W. globosa* represents a promising plant-based food resource; nevertheless, further standardized compositional analyses, bioavailability studies, and well-designed human trials are required to substantiate its functional and nutritional properties.

Standardized Ileal digestibility of protein and amino acids in Black soldier fly larvae and duckweed in broiler chickens

Kaewtapee, C; Thongthung, H; Petchpoung, K; Morikawa, M; Chungopast, S (2026) Animals 16: 461.

Black soldier fly larvae (BSFL) and duckweed are potential alternative feed ingredients for sustainable poultry production. This study aimed to determine the standardized ileal digestibility (SID) of amino acids (AAs) in BSFL, *Lemna*, and *Spirodela* compared with soybean meal and rapeseed meal. Six cages of eighteen broiler chickens were allocated to each treatment. The crude protein (CP) content was highest in soybean meal (511 g/kg dry matter; DM), intermediate in BSFL (391 g/kg), and rapeseed meal (335 g/kg DM) and lowest in *Lemna* (185 g/kg DM) and *Spirodela* (145 g/kg DM). Ether extract was highest in BSFL (95 g/kg DM), whereas crude fiber was highest in *Lemna* (109 g/kg DM) and *Spirodela* (171 g/kg DM). The SID of CP was higher ($p < 0.05$) in BSFL (89.0%) than in soybean meal (82.3%), rapeseed meal (71.3%), *Lemna* (70.2%), and *Spirodela* (44.9%). The SID of all essential amino acids (AAs) was higher ($p < 0.05$) in *Lemna* than in *Spirodela*, but it did not differ from that in rapeseed meal. In conclusion, the high fat content in BSFL can enhance AA digestibility, whereas the use of duckweed may be limited by its fiber fractions, which negatively impact the SID of CP and AAs in broiler chickens.

DF comment: "*Lemna* (*Lemna aequinoctialis*) and *Spirodela* (*Spirodela polyrhiza*) were provided by Advanced Greenfarm Co., Ltd. (Nakhon Pathom, Thailand)." Whereas the identification of *S. polyrhiza* even on morphological basis is not problem, *L. minor* requires molecular analysis of the species identity or the results are without relevance for digestion research.

Nutritional enhancement and glycemic regulation of breadsticks enriched with *Wolffia globosa* powder

Wai, MP; Ngamukote, S; Adisakwattana, S; Joymak, W; Suantawee, T (2026) Applied Food Reearch 6: 101711.

Wolffia globosa (Roxb.) Hartog & Plas, or duckweed, is a nutrient-dense aquatic plant from the Lemnaceae family, notably high in protein and antioxidant properties. This study is the first to investigate the incorporation of vacuum heat-dried *W. globosa* powder (WP) into breadsticks to enhance their nutritional and functional qualities. WP exhibited strong antioxidant capacity and effectively inhibited alpha-amylase and alpha-glucosidase, indicating its potential to modulate starch digestion. Incorporating WP into breadsticks produced darker green products with increased hardness. Breadsticks containing 10 % WP showed the greatest enhancement in antioxidant activity and resistant starch content, along with the largest reduction in predicted glycemic index. Sensory evaluation indicated that moderate WP incorporation (5-10 %) achieved an optimal balance between functional improvement and consumer acceptability, whereas 15 % substitution adversely affected sensory properties. Overall, these findings demonstrate the potential of *W. globosa* as a novel functional ingredient for the development of antioxidant-rich, reduced-glycemic bakery products.

DF comment: This information is not perfect but gives at least some idea about the identity of the investigated material: "*W. globosa* used in this study was a custom-selected proprietary strain developed by Advanced GreenFarm Co., Ltd. and originally isolated from Nakhon Pathom Province, Thailand"

Impact of green extraction methods for algae and aquatic plants on amino acid composition and taste detection using electronic tongue analysis

Chuon, L; Prinyawiwatkul, W; Sae-Eaw, A; Wongthahan, P (2026) Foods 15: 305.

The growing demand for sustainable protein sources has increased interest in algae and aquatic plants as alternatives to animal-derived proteins. These resources are rich in protein, amino acids, and umami compounds but require suitable extraction methods to maximize yield and quality. This study compared three green extraction techniques-maceration (MAE, 80°C, 2 h), ultrasound-assisted extraction (UAE, 750 W, 20 kHz, 50% amplitude, 35°C, pH 12, 1 h), and enzyme-assisted extraction (EAE, 5% beta-glucanase/flavourzyme, 55°C, pH 6.5, 1 h) on five raw materials: wakame (commercial seaweed), hair seaweed, sea lettuce, water silk algae, and *Wolffia*. The result revealed that both raw materials and extraction methods significantly ($p < 0.05$) affected protein yield, amino acid, physicochemical properties, and taste detection with e-tongue. *Wolffia* extracted by MAE yielded the highest protein overall, followed by UAE and EAE methods, when compared with commercial seaweed. The relationship between amino acid profiles and taste detection was investigated by principal component analysis (PCA) and hierarchical cluster analysis (HCA); the samples with higher glutamic and aspartic acids were linked with umami taste, while histidine contributed to bitter taste. Overall, the findings highlighted that extraction efficiency was influenced more by the extraction method-material compatibility than the raw material alone.

DF comment: The authors gave *W. arrhiza* as plant species without identification but most probably this species does not exist in Thailand.

Production of protein hydrolysates from *Wolffia* (*Wolffia globosa*) using bromelain and its application to oyster sauce imitation products

Singkhum, U; Lapsongphon, N (2026) International Journal of Food Science 2026: 7646872.

Wolffia is a superfood. It is a fantastic source of alternative protein and is one of the aquatic food plants of the future. The determination of the optimum conditions for extracting protein hydrolysate from fresh *Wolffia* using bromelain revealed that a bromelain concentration of 0.75% (w/w), a digestion time of 6 h, and a digestion temperature of 55°C were the optimal conditions, as the protein hydrolysate produced under these conditions contained 4.42% protein. The obtained protein hydrolysate was developed into a *Wolffia* sauce product using a mixture design. The three factors studied included *Wolffia* protein hydrolysate content (55%-60%), salt (0%-5%), and low-sodium soy sauce (0%-10%). The results indicated that WSF3, which was produced using 53.3% *Wolffia* protein hydrolysate, 3.3% salt, and 8.3% low-sodium soy sauce, was given the highest ratings for texture and overall acceptability. All attributes rated this recipe at "like moderately-like very much" levels. The resulting *Wolffia* sauce had a protein content of 2.29%, sodium content of 1252 mg or 2.81%, pH of 5.35, and viscosity of 8409 cPs, while commercial oyster sauce has a protein content of 0.52%, sodium content of 1853 mg or 4.73%, pH of 4.34, and viscosity of 9710 cPs, on average. This study confirmed that the *Wolffia* sauce had a low sodium content and a high protein content, similar to oyster sauce. The research indicated that the *Wolffia* sauce made with WSF3 to mimic oyster sauce could last at least 3 months at 35°C, and its viscosity, pH, and microbiological properties met the Thai Industrial Standard for Oyster Sauce (TIS 1317-2538), Thailand.

Duckweed as a sustainable aquafeed: Effects on growth, muscle composition, antioxidant and immune markers in grass carp

Song, YJ; Hu, ZL; Yang, XW; An, YX; Lu, YL (2026) Animals 16: 53.

Duckweed (*Spirodela polyrhiza*), a fast-growing aquatic plant rich in protein and bioactive compounds, offers a sustainable alternative to conventional aquafeed protein sources. This study evaluated the effects of incorporating 25-75% duckweed meal into a commercial feed on grass carp (*Ctenopharyngodon idella*) over a 6-week trial. Fish meal, wheat starch, and vegetable oil was added in amounts to obtain isonitrogenous and isoenergetic diets. Additionally, another grass carps were used for extended feeding until they reached approximately 1000 g, using the feed with the optimal duckweed inclusion rate (25%). Fish fed a diet consisting of 75% commercial feed and 25% duckweed meal (F75D25) exhibited significantly higher weight gain. Muscle analysis revealed increased protein content (up 15%, $p < 0.05$) and improved amino acid and fatty acid profiles. Liver, muscle, and blood assays showed elevated antioxidant enzyme activities (SOD up 20%, LYS up 18%; $p < 0.05$) and immune markers (CRP, GOT; $p < 0.05$), indicating enhanced health status. Transcriptomic and metagenomic analyses confirmed the upregulation of immune-related genes (e.g., SOD1, IL-6; fold change

> 2, p < 0.01) and beneficial shifts in gut microbiota (e.g., increased Firmicutes). These findings highlight duckweed's potential as a nutrient-rich, health-promoting ingredient for sustainable aquaculture diets.

Growth & Development

SIPEREA: A scalable imaging platform for measuring two-dimensional growth of duckweed

Jung, SK; Nandi, S; McDonald, KA (2026) Applied Sciences 16: 66.

Biomass production in organisms is closely linked to their growth rate, necessitating rapid, in situ, nondestructive, and accurate growth measurement. Existing imaging platforms are often limited by high cost, lack of scalability, wired connections, or insufficient automation, restricting their applicability for high-throughput growth monitoring. Here, we present SIPEREA, a scalable imaging platform built on cost-effective ESP32-CAM modules. SIPEREA comprises three graphical user interface (GUI) based applications: (1) an embedded program for the ESP32-CAM responsible for imaging, (2) an image acquisition program for automatic wireless image transmission from multiple ESP32-CAMs, and (3) an image analysis program that automatically segments organisms in the images using a deep neural network (DNN) and calculates their area. The implementation of asynchronous, sequential wireless image acquisition enables the efficient management of multiple ESP32-CAM modules. To demonstrate the usefulness of this platform, we analyzed images captured over a two-week period using four ESP32-CAM units during Lemna sp. (duckweed) cultivation to compute doubling time.

History

Exploring duckweed diversity at the dawn of its cultivation era: The invaluable legacy of the Landolt Collection

Morello, L; Lee, YR; Braglia, L (2026) Plants 15: 345.

The aquatic plant family Lemnaceae, commonly called duckweed or water lentil, has attracted increasing interest in the scientific literature over the past two decades. It holds extraordinary potential as a new crop due to its multiple applications: as an alternative protein source for feed and food production, as a starch producer for renewable biofuel, and for its capacity to provide valuable ecosystem services. Its high biomass productivity, ability to thrive under a wide range of environmental conditions, lack of requirement for arable land, and aptitude for nutrient recycling from wastewater align with the criteria for future sustainable crops. The Lemnaceae is a small plant family comprising a still uncertain number of species and hybrids with largely unexplored genetic diversity, owing to its taxonomic complexity. We focus on critical aspects that must be addressed to establish duckweed as a viable crop: the availability and accessibility of genomic resources to understand the genetic basis of key agronomic traits; the development of protocols for flower induction and crossing; and the establishment of effective methods for genetic transformation and plant regeneration, all aimed at enabling selection and breeding strategies. We highlight the importance of duckweed germplasm collections, including accessions from a wide geographic and ecological range, as essential resources for addressing duckweed diversity and supporting both fundamental research and agronomic applications.

Interaction with other organisms

Diversity and plant growth-promoting potential of duckweed-associated bacteria on *Wolffia globosa* biomass production and nutritional quality

Kettongruang, S; Morikawa, M; Boonmak, C (2026) Envionmental Microbiology Reports 18: e70312.

Wolffia (Lemnoideae) is recognised as a nutritional superfood with increasing interest in commercial cultivation. Its growth and biomass quality are influenced by abiotic factors and duckweed-associated bacteria (DAB) that support nutrient cycling, stress tolerance and metabolism. This study assessed DAB diversity and their effects on *Wolffia* growth to select effective plant growth-promoting bacteria (PGPB) and elucidated beneficial plant-microbe interactions. A total of 108 isolates, representing 66 species from 41 genera across four phyla, were obtained from duckweeds collected in six provinces of Thailand. The culturable DAB community showed high taxonomic diversity, dominated by the phylum Pseudomonadota, particularly Alphaproteobacteria. The isolates, along with the known PGPB of *Spirodela polyrhiza*, were evaluated for growth promotion in axenic *Wolffia globosa* using co-cultivation. Six PGPB enhanced growth by 54.67%-77.75% without reported pathogenicity. Several PGPB originally isolated from *S. polyrhiza* also showed growth-promoting effects on *Wolffia*. Large-scale co-cultivation demonstrated that *Pseudomonas toyotomiensis* W5-11 most effectively increased dry weight and chlorophyll content (3.18- and 2.75-fold, respectively). All selected isolates produced indole-3-acetic acid (IAA), siderophores and solubilised phosphate. Correlation analysis revealed that IAA production was associated with protein accumulation. These findings suggested that PGPB represent promising biofertilisers that enhance *Wolffia* productivity and nutritional quality through various mechanisms.

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

Streptomyces lemnae sp. nov., a novel actinomycete isolated from *Lemna aequinoctialis*

Boonchu, P; Butdee, W; Phatthalung, CN; Boonmak, C; Suriyachadkun, C; Duangmal, K (2026) Journal of Antibiotics DOI10.1038/s41429-026-00905-3

A novel *Streptomyces* species, designated strain DW26H14(T), was isolated from duckweed (*Lemna aequinoctialis*) and characterized using a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain DW26H14(T) belonged to the genus *Streptomyces* and showed the highest similarity to *Streptomyces tremellae* Js-1(T) (98.8%) and *Streptomyces fuscigenes* JBL-20(T) (98.1%). The average nucleotide identity via BLAST (ANIb) and digital DNA-DNA hybridization (dDDH) values between strain DW26H14(T) and these closely related type strains ranged from 84.15-84.60% and 29.8-31.5% respectively, which were below the established thresholds for prokaryotic species delineation. Strain DW26H14(T) has a genome size of 8,003,460 bp with DNA G + C content of 72.18%. Chemotaxonomic analysis revealed that strain DW26H14(T) contained glucose, mannose, rhamnose, and ribose in its whole-cell hydrolysates. The major cellular fatty acids (>10%) were C-16:0 and summed feature 8 (C-18:1 omega 7c/C-18:1 omega 6c). The polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylinositolmannosides, an unidentified aminolipid, and five unidentified phospholipids. The major menaquinones were MK-9(H-4) and MK-9(H-6). Based on the results of a polyphasic taxonomic analysis, strain DW26H14(T) represents a novel species within the genus Streptomyces, for which the name *Streptomyces lemnae* sp. nov. is proposed. The type strain is DW26H14(T) (=TBRC 17042(T) = NBRC 116115(T)).

DF comment: The only information about the identity given by the authors is the following: “Lemna aequinoctialis samples were collected from a ditch in Tha Yang district, Phetchaburi province, Thailand (12°56'52.7“N,99°55'55.3“E).”

A nature-based duckweed-bacteria-fungi consortium enables comprehensive sulfamethoxazole degradation and detoxification in synthetic wastewater

Song, YJ; Hu, ZL; Yang, XW; Lu, YL (2026) Bioresource Technology 44: 134213.

Antibiotic residues in wastewater pose ecological and health risks, demanding efficient and sustainable removal strategies. A nature-based tripartite system was developed combining *Spirodela polyrhiza*, a sulfamethoxazole (SMX)-degrading bacterium (*Pseudomonas* sp. N6), and the white-rot fungus *Trametes*

versicolor N105 for SMX removal. In sterile batch reactors (1 mg/L SMX), the consortium reduced SMX below the quantification limit (0.05 mg/L) within 12 days, outperforming duckweed alone and duckweed with a single microbial partner. Mass balance analysis showed that biodegradation (0.90 mg/L) dominated SMX dissipation, with fungal oxidative cleavage complementing bacterial hydroxylation and duckweed-mediated deamination. Physiological assays indicated that microbial partners, particularly the fungal component, alleviated SMX-induced oxidative stress and enhanced pigment synthesis, thereby improving duckweed growth. These results demonstrate that integrating duckweed, bacteria, and fungi yields enhanced SMX removal and mitigates phytotoxicity, highlighting a promising nature-based strategy for antibiotic-contaminated wastewater.

Microbiota modulate metformin phytoremediation and stress responses in *Lemna minor*

Gomes, MP; Malinoski, L; Maranho, LT; Carneiro, DNM; Richardi, VS; Martinez, MG (2026) Journal of Hazardous Materials 505: 141427.

The phytoremediation of pharmaceuticals by aquatic plants is influenced by both plant physiology and microbial interactions. This study investigated how microbial symbiosis modulates the uptake, transformation, and physiological responses of *Lemna minor* to metformin. Plants were cultivated under axenic and non-axenic conditions and exposed to 10, 50, and 100 µg/L metformin for 7 days. Both systems removed > 99 % of metformin from water, but exhibited distinct accumulation patterns, stress biomarkers, and metabolic profiles. Axenic plants accumulated 2.1-fold more metformin and 1.7-fold more guanylurea, a key metformin metabolite, at 100 µg/L, along with increased oxidative stress (up arrow MDA) and elevated cytochrome P450 activity. Non-axenic systems exhibited extracellular guanylurea concentrations up to 0.9 µg/L, indicating a reliance on intrinsic detoxification pathways. Guanylurea was detected in both plant types, but appeared in water only under non-axenic conditions, suggesting microbial-mediated excretion. Principal component analysis revealed that guanylurea accumulation was correlated with elevated P450 activity, lipid peroxidation, and hormonal shifts, especially in axenic plants. These results confirmed that *L. minor* can biotransform metformin independently of microbes, albeit with a greater physiological burden. Microbial presence mitigates stress and enhances extracellular degradation. Overall, the data demonstrate complementary roles of plants and microbiota, with microbiota reducing internal contaminant load and protecting plant homeostasis.

Observing weak adaptation of duckweeds to their local microbiome depends on local pond water

Rose, AM; O'Brien, AM (2026) American Journal of Botany 113: DOI10.1002/ajb2.70165.

Populations can locally adapt to the biotic and abiotic factors of environments. However, detecting adaptation to biotic factors can depend on the abiotic conditions in which the adaptation is tested, and vice versa. The microbiome is one important aspect of the biotic environment: Interactions between microbiomes and their hosts are critical for host fitness and trait expression. If hosts adapt to local microbiomes, they may therefore depend on interactions with local microbes to express trait values adapted to the local abiotic environment. Using *Lemna minor* (duckweed) as a model host, we examined differences in host fitness when grown in local and nonlocal microbiomes and in local and nonlocal water. We experimentally recombined duckweeds, microbes, and water from four ponds around Durham, New Hampshire (United States) in well-plate microcosms in a growth chamber. The source of duckweeds, microbes, and water all affected microbial growth, duckweed growth, and duckweed traits. However, weak, marginally significant local adaptation resulted in higher frond area only when duckweeds were paired with their local water and local microbes. Microbial growth was also marginally reduced when duckweeds were paired with microbes and water from their local site. While microbiome impacts on duckweed growth and traits varied across abiotic contexts, local microbiomes provided only limited growth benefits. Harnessing the effects of plant microbiomes is an exciting area of applied research. Despite our findings, bioprospecting in local microbiomes could still be fruitful: It may be ecologically safer, and other plants may locally adapt to microbiomes.

Species-level profiling of *Landoltia punctata* (duckweed) microbiome under nutrient stress using full-length 16S rRNA sequencing

Bunyoo, C; Phonmakham, J; Morikawa, M; Thamchaipenet, A (2026) PEERJ 14: e20648.

Duckweed is a rapidly-growing aquatic plant utilized as food/feed and for wastewater remediation. It coexists with complex microbial communities that play crucial roles in its growth and capability for phytoremediation. In a previous study, microbiomes associated with four duckweed species (*Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis*, and *Wolffia globosa*) grown under natural and nutrient-deficient conditions, were investigated using V3V4 16S rRNA sequencing. However, species-level classification was not achieved due to the partial 16S rRNA sequences obtained, restricting the selection of potential microbial species for further application. In this study, *L. punctata* samples from the previous work were investigated further by employing full-length 16S rRNA sequencing. A total of 31 predominant microbial species were identified. Under stress, the proportion of *Proteobacteria* increased significantly, along with potentially beneficial bacteria such as *Roseateles depolymerans, Pelomonas saccharophila, Acidovorax temperans, Ensifer adhaerens* and *Rhizobium straminoryzae*. Functional metagenomic predictions suggest that associated microbes adapt to stressors and may confer benefits to duckweed, including pathways related to host adhesion, biofilm formation, microbial growth modulation, and co-factors and vitamin biosynthesis. Furthermore, the study demonstrates both the advantages and limitations of full-length 16S rRNA amplicon sequencing. The findings provide more insight into *L. punctata* microbiomes at species-level, facilitating establishment of stable, beneficial microbial communities for duckweed applications. Ongoing investigations aim to isolate key microbial species from *L. punctata* and validate their roles through co-cultivation, along with establishing potential synthetic microbial communities based on the metagenomic findings.

Molecular Biology and Genomics

Systematic optimization enables high-efficiency stable and transient transformation of *Spirodela polyrhiza* (Greater Duckweed)

Islam, T; Ligaba-Osena, A; Josephs, EA (2026) Plant Cell Tissue and Organ Culture 164: 43.

The in vitro transformation of plants, or the delivery of foreign genetic material that is incorporated into their genomes, represents a powerful tool both for elucidating genotype-phenotype relationships and for generating plant cultivars which have desirable traits. However, outside of a few model species, the processes involved in transformation are often inefficient, taking months to perform for many plant species, with several bottlenecks at the different stages of calli induction, genetic transfection, and plant regeneration. While duckweeds - aquatic monocots that are the smallest and fastest-growing flowering plants - have distinguished themselves with several emerging biotechnological applications, they too are the subject of conflicting reports regarding their transformation potential. Here, we synthesized and optimized the protocols for in vitro transformation of duckweed *Spirodela polyrhiza* (Greater Duckweed) from start-to-finish: achieving > 90% - 100% efficiencies for each of calli induction; transient and stable genetic transformation; visual marker-free selection of transformants; and regeneration of genetically modified plants with stable transgene expression for over 100 generations - and which in *S. polyrhiza* can be achieved over the course of weeks instead of months. These approaches overcome many bottlenecks and help to pave the way for functional genomics studies and synthetic biology applications in this biotechnologically important species.

Genome sequence assembly of the 5S rDNA loci informs haplotype specificity and evolution in the greater duckweed *Spirodela polyrhiza*

Stepanenko, A; Schubert, V; Chen, G; Kishchenko, O; Michael, TP; Lam, E; Hrmova, M; Schubert, I; Borisjuk, N (2026) Communications Biology DOI10.1038/s42003-026-09598-8.

Despite the rapid expansion of information on eukaryotic genomes, data on ribosomal DNA (rDNA) loci encoding ribosomal RNAs, crucial for the biogenesis of ribosomes, are absent in almost all cases due to difficulties in assembling the long regions of tandemly repeated DNA units. Taking advantage of the uniquely low rDNA copy number in the aquatic plant *Spirodela polyrhiza*, we resolved the species' complete 5S rDNA

architecture at a nucleotide level. A combination of in situ hybridization, extra-long DNA reads, and conventional DNA sequencing allowed the assembly of near-complete loci sequences of 40,878bp, specific for one haplotype of chromosome ChrSp6, and of 110,911bp specific for a haplotype of ChrSp13. The completely resolved 5S rDNA locus of ChrSp6 contains 40 copies of tandemly repeated gene units with an intergenic spacer (NTS) of 400bp for one haplotype, and more than 60 highly homogenized gene copies for the second haplotype. The ChrSp13 locus contains 5S rDNA clusters with NTSs of 1,056 or 1,069bp arranged in two sub-clusters. The G/C-rich 5S rDNA arrays in both loci are embedded in A/T-enriched chromosome regions. This work advances our understanding of the basic principles of rDNA organization and evolution of rRNA genes in plants by revealing the molecular architecture and evolutionary dynamics of 5S rDNA loci.

A novel frond-based genetic transformation method in *Spirodela polyrhiza*: Reliability reconfirmed through functional analysis of S*pNramps* co-overexpression under cadmium stress

Chen, Y; Xi, J; Sun, ZL; Yang, JJ; Li, GJ; Zhao, XY; Zhang, LY; Hu, HW; Ye, CN; Hou, HW (2026) Aquatic Botany 204: 103993.

Spirodela polyrhiza has great advantages for energy production, sewage treatment, toxicity experiments, and biomonitoring. However, low transformation efficiency and long-cycle transformation time remain an urgent problem. In this research, we established an optimized frond-mediated genetic transformation protocol achieving 37.35 % efficiency within 51 days-a 3.2-fold improvement over existing methods (11.48 %, 164 days). Frond-based transformation data indicated that vacuum treatment could be applied to increase the frond transformation efficiency in *S. polyrhiza*. To validate this system's reliability, we employed it to investigate the cooperative functions of three natural resistance-associated macrophage protein (Nramp) transporters (SpNramps) in *S. polyrhiza* under cadmium (Cd) stress. Heterologous co-expression of SpNramps in yeast resulted in reduced Cd accumulation and heightened Cd sensitivity. However, SpNramps overexpression increased the tolerance to Cd by promoting its growth and enhancing Mn absorption at 150 μM Cd. Collectively, this study presented the first report on the frond-based transformation system in *S. polyrhiza*, which enables rapid functional validation of multi-gene regulatory networks in aquatic plants, laying the foundation for further functional studies. Our study also offered a new solution to increase Cd tolerance in plants and gained new insights into both basic and applied research by using the co-overexpression strategy.

Physiology & Stress

Thermal performance of *Wolffia globosa* under climate change: heat waves impair population growth

Cuddington, K; Kuntze, M; Andrade-Pereira, D; Gasmen, Y; Wu, JY; Ferns, A; Geng, XW (2026) AOB Plants 18: plaf068.

Climate change impacts on temperature may alter the availability of plants used for food. Some species may have asymmetric responses to temperature, with growth rates that fall rapidly at temperatures above the optimum. As a result, even if mean temperatures increase towards optimal conditions, fluctuations about this mean can substantially decrease growth. We use *Wolffia globosa*, a tropical duckweed harvested for food in Southeast Asia, to examine the impacts of predicted changes in temperatures. This aquatic plant has a fast growth rate, a high protein content, and is also a source of important nutrients. Therefore, it could play an important role in food security under climate change. For constant temperatures there is no significant difference between growth at current conditions and those predicted in the next 40 years according to the high emissions scenario (SSP5-8.5 scenario) in Thailand, Laos and Myanmar. However, when temperatures are allowed to fluctuate about the mean in a pattern similar to recent heatwave conditions in Thailand, we find significantly lower growth rates at the optimum than at current mean temperatures. This decrease is driven by an increase in frond death at higher temperatures. Nevertheless, given the fast growth rate of this species relative to other food crops, and the mitigating impact of water on the magnitude of temperature fluctuations, it seems likely that *W. globosa* may more rapidly recover from extreme heat events than other crop species, and is therefore a suitable candidate for adapting food systems to climate change impacts.

Abscisic Acid Induces Triacylglycerol Accumulation and Lipid Remodelling in Chloroplast-Containing Green Tissues of *Lemna minor*

Kim, E; Choi, BY; Je, S; Kang, J; Shin, S; Roh, Y; Kim, M; Ito, S; Oyama, T; Lee, Y; Shim, D; Yamaoka, Y (2026) Plant Cell & Environment DOI10.1111/pce.70386.

Lipid remodelling is a fundamental component of plant responses to environmental stress and development, yet its regulation in fast-growing aquatic plants remains poorly understood. Here, we investigated how abscisic acid (ABA) regulates triacylglycerol (TAG) accumulation and fatty acid (FA) composition in the duckweed *Lemna minor*. A 3-day treatment with 1 μM ABA induced a 2.9-fold increase in TAG content, accompanied by extensive remodelling of plastidial and extraplastidial membrane lipids. Reduced monogalactosyldiacylglycerol (MGDG) likely served as a FA source for TAG synthesis. Transcript analyses revealed strong induction of diacylglycerol acyltransferase (DGAT) genes, catalysing the final step of TAG formation, and repression of fatty acid desaturase (FAD) genes, resulting in a marked reduction in polyunsaturated FA levels. Confocal imaging confirmed substantial lipid droplet accumulation in both fronds and chloroplast-containing roots. Notably, this sustained ABA-induced TAG accumulation was unique to *L. minor*, with no comparable response observed in other duckweed species or in Arabidopsis under identical treatment. These findings reveal a species-specific ABA-driven lipid remodelling pathway in duckweed, linking phytohormone signalling to carbon storage in aquatic plants.

DF comment: The authors reported the source of four duckweed clones but not how they were identified.

The ionome of aquatic plants *Lemna minor* L. under abiotic stresses

Bodnar, I; Cheban, E (2026) Aquatic Botany 204: 103996.

The content of macroelements in plants is regulated by genetic factors and depends on the environment. Plant ionome is species-specific, the impact of stressors, imbalance of mineral nutrition, including excessive intake of heavy metals, changes the level of macro- and microelements, which affects the physiological state of plants, growth and development. In this work, it is shown that the impact of heavy metals (Cu^{2+}, Cd^{2+}, Zn^{2+}) and gamma radiation led to a change in the accumulation of Ca^{2+}, Mg^{2+} and K^{+} in *Lemna minor* L., which is a model plant in ecotoxicological experiments and is promising for phytoremediation of polluted water bodies. With the considered effects, a dose-dependent decrease in growth was observed in the form of a decrease in the total area of duckweed fronds; the toxicity series for this group of metals is Cu^{2+}>Cd^{2+}>Zn^{2+}. Duckweed can be classified as an accumulator of heavy metals; aquatic plants accumulated Zn^{2+} most efficiently (BCF>1000). The general change in the macronutrient profile under the influence of heavy metals and radiation was an increase in Ca^{2+} accumulation. The Mg^{2+} level increased after irradiation (42, 63 Gy), exposure to Cd^{2+} (12.6 μM), Zn^{2+} (3.15, 6.3 μM) and Cu^{2+} (3.15, 6.3 μM). The K^{+} content decreased under the influence of chemical agents (Cu^{2+}), but did not change after irradiation. The obtained data can be used in assessing the quality of natural and waste waters with the interpretation of the plant ionome as a stress biomarker.

DF comment: “The plant material was used from the culture of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences.” No identification of the species was reported.

Growth variation among Thai duckweed species under axenic conditions

Jansantia, S; Harnvanichvech, Y; Senayai, A; Sanevas, N; Oyama, T; Kraichak, E (2026) Biology 15: 159.

Duckweed has attracted considerable attention for its high protein content, rapid growth, and broad potential in biotechnological applications. Understanding key phenotypic traits is crucial for unlocking and maximizing this potential. While most studies on duckweed growth have been conducted under natural or non-sterile conditions, here we minimize environmental influences and focus on the genetic component of growth by assessing growth performance under axenic culture. In this study, we measured relative growth rate (RGR) in four duckweed species, *Landoltia punctata* (G. Mey.) Les & D. J. Crawford, *Lemna aequinoctialis* Welw., *Spirodela polyrhiza* (L.) Schleid., and *Wolffia globosa* (Roxb.) Hartog & Plas. collected from various natural locations across Thailand. A total of six to seven strains were tested for each species. The relative growth rates of studied species ranged from 0.012 day^{-1} in *S. polyrhiza* to 0.162 day^{-1} in *W. globosa*. Significant intraspecific variation was observed in *L. punctata*, *S. polyrhiza*, and *W. globosa*, with the coefficients of variation between 9.6 to 109.9 percent. Each strain showed distinct growth characteristics: Most displayed a

steady growth pattern, whereas *W. globosa* showed exponential growth at Day 35 after the start of experiment. The results provide the first systematic comparisons of baseline growth rate data for duckweed species in Thailand. These findings advance the understanding of strain-specific growth traits in duckweed and establish a standardized protocol for evaluating growth traits under axenic conditions, providing a basis for future research and applications.

Demographic senescence and lifespan extension by caloric restriction in two duckweed species (*Wolffia brasiliensis* and *Lemna aequinoctialis*)

Senayai, A; Chmilar, SL; Abeyrama, D; Burg, TM; Laird, RA; Kraichak, E (2026) Aquatic Botany 204: 103991.

Demographic senescence involves population-level declines in survival and reproduction with increasing age. Duckweeds provide excellent models for plant senescence research due to their rapid clonal propagation and short lifespans. While caloric restriction (CR) extends lifespans in heterotrophs, its effect on plants remains poorly understood. We investigated demographic senescence and CR effects by reducing light intensity in two duckweed species: *Wolffia brasiliensis* Wedd. and *Lemna aequinoctialis* Welw. Using longitudinal laboratory studies of 336 individual plants per species, we recorded daily reproduction from birth to death under controlled light treatments. Both species exhibited significant demographic senescence, with survival data fitting logistic models for *W. brasiliensis* across treatments, while *L. aequinoctialis* fit Weibull (full light) and logistic (dim light) models. Generalized estimating equations revealed significant age-related declines in daily reproduction probability across all treatments ($p < 0.001$). CR significantly extended mean lifespan in both species: from 26.11 days (full light) to 28.45 days (dim light) in *W. brasiliensis* ($p < 0.001$) and from 24.74 days (full light) to 33.67 days (dim light) in *L. aequinoctialis* ($p < 0.001$). While total offspring production remained similar between treatments, the intrinsic rate of increase measured at the individual level (r) was significantly higher under full light. These findings demonstrate demographic senescence across duckweed genera and suggest that CR can extend lifespan in photoautotrophic organisms. Temporal scaling analysis revealed complete conservation of senescence trajectories in *W. brasiliensis*, while a marginally non-significant difference was detected in *L. aequinoctialis* ($p = 0.065$), suggesting potential species-specific responses to CR that warrant further investigation.

DF comment: Whereas *W. brasiliensis* was identified by barcoding, the identification of *L. aequinoctialis* by morphological markers remains risky.

Evaluation of the tolerance of duckweed (Lemnaceae) to abiotic stress factors associated with nutrient recycling systems via multispectral imaging

Audenaert, L; De Souza, MF; De Zutter, N; Rousseau, DPL; Meers, E (2026) Plant Stress

Lemnaceae, commonly known as the duckweed family, include the smallest flowering plants known to science. Although duckweed holds significant potential for wastewater treatment and nutrient recycling, abiotic stress factors prevailing in nutrient recycling systems can lead to die-off. Given the considerable inter-species and inter-clone morphological and physiological variability, this study aimed to assess the physiological and functional tolerance of 14 duckweed clones belonging to three different species (*Lemna minor, Spirodela polyrhiza*, and *Wolffia globosa*) to four abiotic stress factors: electrical conductivity (salinity stress), ammonium concentration, phosphorus concentration, and potassium concentration. In all experiments, the physiological and functional tolerance of duckweed was monitored using a multispectral imaging platform, which enabled the assessment of presymptomatic stress responses. The results demonstrated that *L. minor* clones exhibited greater physiological tolerance to the imposed stress factors, with clone *L. minor 7022* emerging as the most tolerant. No health deterioration was observed upon exposure to high concentrations of phosphorus, ammonium, and potassium. This study uniquely demonstrates the use of multispectral imaging as a powerful and non-destructive tool for screening duckweed clones, offering a novel approach to optimize clone selection for its potential application in wastewater treatment and nutrient recovery systems.

DF comment: All clones were documented with well-known clone-ID. However, this does not mean that the species are reliably identified.

Phytomedicine

Gut microbiota modulation of *Wolffia globosa* (Roxb.) Hartog & Plas and synbiotic effect with probiotic strain *Lactiplantibacillus plantarum* HM04-80 in an in vitro colonic fermentation from obese adults

Kaewsaen, R; Honwichit, O; Mok, K; Nakphaichit, M; Chaiworapuek, W; Charoensiddhi, S (2026) Journal of Agriculture and Food Research 27: 102857.

Duckweed, *Wolffia globosa* (Roxb.) Hartog & Plas, has gained attention as a highly nutritious food source due to its rich protein and dietary fiber content. This study aimed to investigate the chemical composition and digestibility of dried duckweed powder (DDP) and duckweed powder extract (DPE), as well as to evaluate their synbiotic potential with the probiotic *Lactiplantibacillus plantarum* HM04-80 (PHM) for modulating gut microbiota in obese individuals. Nutritional analysis revealed that DDP primarily comprised carbohydrates (38.41%) and proteins (23.93%), whereas DPE contained higher levels of protein (34.47%) and ash (33.15%). Both DDP and DPE underwent partial digestion, with degrees of hydrolysis based on reducing sugar release at 4.88% and 27.02%, and on amino acid release at 29.73% and 29.50%, respectively. Following 24 h of in vitro colonic fermentation using fecal samples from obese adults, DDP and its combination with PHM exhibited a bifidogenic effect, while DPE was associated with increased relative abundance of Parabacteroides. Moreover, DDP in combination with PHM synergistically suppressed the growth of *Escherichia, Fusobacterium, Sutterella, Megamonas*, and *Dorea*, which are positively associated with obesity. Both DDP and DPE, as well as their combinations with PHM, significantly enhanced total short-chain fatty acids (SCFAs) production, particularly acetic, propionic, and butyric acids. Correlation analysis revealed strong positive associations between SCFAs levels and the abundances of *Bifidobacterium, Suterella*, and *Parabacteroides*. These findings highlight the potential of duckweed and its synergistic combination with *L. plantarum* to improve gut health in obese individuals through modulation of the gut microbiota and enhanced SCFAs production.

Phytoremediation

Long-term bioaugmentation of a carrier enhanced duckweed-based wastewater treatment system via the addition of activated sludge and denitrifying phosphate-accumulating bacterium

Zhao, YG; Ma, RD; Li, XX; Li, JQ; Liang, TN; Li, W; Chang, JJ; Duan, CQ (2026) Ecological Engineering 226: 107919.

Duckweed exhibits distinct advantages over other aquatic plants in phytoremediation. Bioaugmentation is a promising approach for enhancing the treatment performance of various wastewater treatment systems, including duckweed-based systems (DS). However, there is a lack of suitable inoculants for field-scale DS bioaugmentation, and the long-term viability and mechanisms of these inoculants are still poorly understood. To address this, a single strain (*Pseudomonas* sp. DWP1) and a mixed culture (activated sludge) were separately added into two carrier-enhanced DS to systematically evaluate their application potential for DS bioaugmentation by comparing with another control DS over a year. The results indicated that adding strain DWP1 and activated sludge increased the dissolved oxygen concentration and oxidation-reduction potential of pond water. Meanwhile, they promoted duckweed growth (by 64.95% and 41.39%, respectively), enhanced nutrient recovery (by 68.97% and 44.83% for nitrogen, and 71.43% and 42.86% for phosphorus, respectively) and pollutant removal in the DS, and reduced CH_4 emission (by 68.88% and 33.16%, respectively) from the DS. Compared with activated sludge, strain DWP1 had a more significant impact on microbial diversity, community composition, and the relative abundance of dominant assemblages probably due to its high adaptability and successful survival in the duckweed system, which results in the better bioaugmentation performance of strain DWP1 in terms of duckweed biomass production, water purification, and carbon emission reduction in the DS. Therefore, strain DWP1 has greater application potential than activated sludge for the bioaugmentation of the DS.

DF comment: Experiments were carried out using Lemna japonica 0223

From purification to energy: Biogas and biomethane production from *Schoenoplectus americanus* and a mixture of duckweed from phytoremediation of domestic wastewater

Quevedo, MR; González, PS; Bertaina, G; Paisio, CE (2026) Journal of Environmental Management 404: 129451.

Anaerobic digestion (AD) is a promising strategy for converting organic biomass into biogas and methane. In this context, plant biomass generated after domestic wastewater phytoremediation represents an opportunity for integrated waste management and energy recovery. This study evaluated the biochemical composition and the biochemical methane potential (BMP) of biomass from *Schoenoplectus americanus* and a mixture of duckweeds (*Lemna gibba, L. minuta* and *Wolffia columbiana*) collected after use in phytoremediation of domestic wastewater. The BMP were determined through batch anaerobic digestion assays. *S. americanus* exhibited a higher carbohydrate content (71% dry weight) compared to the duckweed mixture, which showed a higher protein content (58% dry weight). Anaerobic digestion was conducted until stable methane production was achieved for each substrate. BMP values were similar for both substrates (146 ± 1.2 and 153.4 ± 3.3 mL CH_4 g^{-1} VS for *S. americanus* and duckweed mixture, respectively). However, the biodegradability was higher for *S. americanus* (55%) than for the duckweed mixture (40%). These results indicate that, under the applied experimental and biomass preparation conditions, both biomasses have potential as substrates for biogas and methane production, with trade-offs between biodegradability and operational advantages such as rapid growth and continuous biomass availability in duckweed-based systems.

DF comment: The identification of these three species on the basis of morphological markers is not very convincing.

Elucidating the phytoremediation potential of aquatic macrophytes for Cd, Cr, and Pb under varying pH and salinity

Mussarat, M; Ahmad, W; Muhammad, D; Adnan, M; Saeed, B; Sabah, NU; Mushtaq, Z; Tahir, MA; Ahmad, M; Romman, M (2026) International Journal of Phytoremediation DOI10.1080/15226514.2026.2642353

Water contamination by heavy metals threatens human and environmental health. This study evaluated *Pistia stratiotes* L. and *Lemna minor* L. for remediation of cadmium (Cd), chromium (Cr VI), and lead (Pb) applied at the rate of 0, 5, 10, and 20 mg L^{-1} under varying pH (6-8) and electrical conductivity (EC; 1,450 and 2,150 µS cm^{-1}) in Hoagland solution. Higher moisture content was observed at neutral pH in *L. minor* L (98.1%) and *P. stratiotes* L. (96.8%) which were decreased with increasing EC and acidic pH. *P. stratiotes* sustained comparatively higher moisture between pH 6-8, proving its adaptability to pH stress. Heavy metal stress significantly decreased growth of both aquatic macrophytes. Higher growth (75% and 54.5%), tolerance index (41.9% and 62.1%), and plant growth rate (0.16 and 0.07) were recorded in *L. minor* and *P. stratiotes* respectively, at neutral pH. However, acidic pH and increasing heavy metal content adversely affected mentioned parameters. The potential of macrophytes for heavy metal absorption/uptake significantly varied between the species depending upon pH and EC. The *P. stratiotes* absorbed higher Cr (1,614 mg kg^{-1}), Pb (1,469 mg kg^{-1}), and Cd (487 mg kg^{-1}) at pH 6-7, while *L. minor* showed higher accumulation (1,644 mg Cr kg^{-1}, 617 mg Cd kg^{-1} and 593 mg Pb kg^{-1}) under salinity stress. Both species showed hyper-accumulation having a bio concentration factor (BCF) > 1,000) of Cd, Cr, and Pb under acidic pH and elevated EC. *P. stratiotes* exhibited greater overall adaptability across pH conditions while *L. minor* performed better under saline environments. Therefore, *P. stratiotes* is recommended for remediation of metal-contaminated waters across variable pH regimes, whereas *L. minor* is better suited for saline and alkaline environments.

Lemna minor as a phytoremediator of cobalt nanoparticles: Insights into toxicity and remediation

Mallin, AC; Marques, RZ; Kitamura, RSA; Prodocimo, MM; de Moraes, LC; Figueredo, CC; Juneau, P; Gomes, MP (2026) Water Biology and Security 5: 100442.

The extensive use of cobalt nanoparticles (CoNPs) in industrial and biomedical applications has raised environmental concerns, necessitating effective mitigation strategies. This study examines the phytotoxicity and phytoremediation potential of CoNPs using *Lemna minor* L., a recognized Co-hyperaccumulator. CoNPs

exhibited significant sublethal toxicity at concentrations ≥1000 µg Co^{2+}/L, leading to oxidative stress and impaired growth, photosynthesis, and respiration. Despite these challenges, *L. minor* effectively removed over 99 % of CoNPs from the medium, even at high concentrations (up to 20.000 µg Co^{2+}/L), with gravimetric cobalt concentrations reaching 1771 µg Co^{2+}/g dry weight in plant tissues. Physiological responses to CoNPs were similar to those induced by equivalent concentrations of $CoCl_2$, suggesting that CoNPs dissolve into Co^{2+} ions upon interaction with plants. However, ultrastructural analysis revealed distinct intracellular cobalt localization with CoNPs causing more severe chloroplast damage than ionic Co^{2+}. These findings suggest two toxicity mechanisms: ionic effects from dissolved Co^{2+} and direct physical damage from intact nanoparticles. The resilience and hyperaccumulation capacity of *L. minor* makes it a promising candidate for the phytoremediation of cobalt contaminated waters. The study underscores the importance of further research to elucidate CoNP environmental behavior and optimize phytoremediation approaches.

DF comment: "L. minor was obtained from the Laboratory of Plant Stress Physiology at the Federal University of Paraná, Curitiba, Brazil" – but this does not tell how reliable this identification of *Lemna minor* is.

The comparison of fresh and dry duckweed (*Lemna minor* L.) on metal (Cr^{6+}, Cd^{2+}, and Zn^{2+}) removal from wastewater

Islam, R; Smith, N; Jang, B; Guo, L (2026) Plants 15: 848.

Heavy metals contaminating the environment is a global concern. Duckweed (*Lemna minor*) is a promising plant for the phytoremediation and biosorption of metal-contaminated water. Although studies have shown that duckweed can remove multiple metals, there is limited research comparing the efficiency of fresh and dried biomass for wastewater treatment. To evaluate the performance of both forms, fresh and dried duckweed were exposed to metal solutions containing varying concentrations of Cr^{6+}, Cd^{2+}, and Zn^{2+} (5 mg/L Cr^{6+} + 1 mg/L Cd^{2+} 10 mg/L Zn^{2+}; 10 mg/L Cr^{6+} + 5 mg/L Cd^{2+} + 50 mg/L Zn^{2+}; or 50 mg/L Cr^{6+} + 25 mg/L Cd^{2+} + 250 mg/L Zn^{2+}) for a duration of 168 h. Metal uptake in fresh duckweed followed zero-order kinetics for Cr^{6+}, Cd^{2+}, and Zn^{2+} sequestration or Michaelis-Menten kinetics for Cd^{2+} and Zn^{2+} uptake, rather than a first-order model. In contrast, dried duckweed reached equilibrium more rapidly, within 4-48 h, exhibiting pseudo-second-order kinetic and fitting the Langmuir isotherm model. Zn^{2+} reached equilibrium the fastest (4 h), Cd^{2+} required 4-24 h, and Cr^{6+} required up to 48 h to reach equilibrium. In general, fresh duckweed uptakes more metals over the 168 h period, depending on the metal type and concentration. However, dried duckweed demonstrated a rapid remediation capability. The findings highlight the complementary potential of applying both fresh and dried duckweed for wastewater treatment.

DF comment: "Fresh duckweed, purchased from Play It Koi (Bothell,WA, USA)" – but this does not tell how reliable this identification as *Lemna minor* is.

Whole-genome and microbial diversity analyses reveal mechanism of GY8 for enhancing cadmium removal rate of duckweed

Zhang, M; Liu, YT; Li, XQ; Chen, ZQ; Lan, Y; Tan, AJ; Yang, GL (2026) International Biodeterioration & Biodegradation 210: 106313.

The enhancement of heavy metal remediation efficiency by endophytes in hyperaccumulators has been widely demonstrated. However, research on the influence of endophytes on cadmium (Cd) remediation efficiency of hyperaccumulating duckweed remains limited. In this study, a Cd-resistant endophyte, *Agrobacterium fabrum* GY8 (GY8), was identified, which increased Cd removal rate of duckweed to 93%. And, colonization of GY8 enhanced duckweed growth rates (up to 117.78%) and alleviated Cd-induced oxidative damage by activating key antioxidant enzymatic pathways. Further investigation revealed that GY8 facilitated phytoremediation through reducing Cd accumulation within duckweed organelles and transforming bioavailable NaCl-extractable Cd into stable HCl-extractable complexes. Whole-genome sequencing identified genes of GY8 associated with Cd resistance and plant growth promotion, including those involved in glutathione metabolism, sulfur cycling, ABC transporter systems, and phenylpropanoid biosynthesis pathways. Microbial diversity analyses confirmed the successful colonization and predominance of GY8 within duckweed. The results provide innovative insights into the mechanisms of endophytes enhancing the Cd removal rate of duckweed, offering theoretical basis for the development of plant-microbe remediation strategies for heavy metal contamination.

DF comment: "The duckweed (*L. minor* 0014) was obtained from the Duckweed Germplasm Bank of the College of Life Sciences of Guizhou University." Most probably, the species identity was investigated as the clone ID points to the stock collection of CAS Chengdu. Nevertheless, the method for species identification should be reported.

Complementary removal of municipal pollutants via biochar-amended wetlands planted with *Lemna minor* and *Arundo donax*

Ahmed, AM; Kareem, SL (2026) International Journal of Phytoremediation
DOI10.1080/15226514.2026.2627325

This study evaluates the treatment performance of pilot-scale vertical subsurface flow constructed wetlands (VSSF CWs) for decentralized municipal wastewater management in Iraq's arid and semi-arid regions. Two vegetated systems-VFpL (*Lemna minor*) and VFpA (*Arundo donax*)-were assessed against an unplanted control (VF) in Al-Midhatiya, Babylon, Iraq. Each unit (0.82 m diameter x 0.80 m depth) operated in batch mode between November 2024 and March 2025, with a hydraulic retention time (HRT) of 5 days, extended to 8 days for nutrient removal. The influent rate averaged 0.0527 ± 0.012 m(3)/day, simulating real-world decentralized flow conditions. All wetland beds were filled with gravel amended by locally sourced date palm frond biochar, selected for its high porosity, mineral content, and functional group diversity. FTIR, SEM, and XRD analyses confirmed the presence of surface-active groups (e.g., carboxyl, hydroxyl), crystalline phases such as quartz and calcite, and microstructural changes associated with microbial colonization. Spectral shifts and morphological smoothing in the planted units reflected strong microbial-root interactions and biofilm formation. Treatment performance was superior in vegetated systems: VFpL achieved 86.2% (COD), 93.3% (BOD), 71.8% (TN), 71% (TP), and 86.2% (TSS) removal, while VFpA recorded 84.3%, 91%, 68.4%, 73.4%, and 89.2%, respectively. TDS removal was moderate (26.7% VFpL, 29.3% VFpA). The unplanted VF exhibited significantly lower efficiency. Statistical analysis via one-way ANOVA ($p < 0.001$) confirmed the enhanced performance of biochar-plant-integrated VSSF CWs as a sustainable approach for wastewater treatment in water-scarce environments.

Biochemical and physiological insights into *Lemna minor* as a remediator of multi metal-microplastic contaminated waters

Farid, M; Irshad, S; Ullah, Q; Haider, W; Asam, ZU; Zubair, M; Alasmari, A; Alomrani, SO; Munir, M; Ali, S (2026) B;MC Plant Biology 26: 447.

Aquatic ecosystems face escalating threats from heavy metals (HMs) and microplastics (MPs), which bioaccumulate and disrupt plant physiology, necessitating effective phytoremediation strategies. This study evaluated the impacts of synthetic wastewater (WW at 25%, 50%, 100%) containing Cd, Cr, Cu, Pb, and polyethylene MPs (0.5 g/L, 1 g/L) on *Lemna minor* in a 42-day hydroponic experiment. Morphological traits declined significantly, with fresh weight reduced by 92.33% and dry weight by 88.04% at 100% WW + 1 g MPs compared to controls. Photosynthetic pigments decreased up to 91.27% in total chlorophyll, while antioxidant enzymes surged (e.g., CAT by 533.18%, SOD by 423.81%). Markers of reactive oxygen species (ROS), such as H_2O_2 and MDA, increased by 108.06% and 154.52%, respectively, alongside HM accumulation exceeding the highest concentration for Cu. These findings elucidate synergistic stress mechanisms, highlighting *L. minor* potential for hyperaccumulating HM-MP, which informs sustainable wastewater treatment and ecosystem restoration practices.

DF comment: "a sub-sample of the collected plants were submitted to the Herbarium Department of Botany, University of Gujrat to confirm its identity and the issuance of the herbarium number (voucher ID) Ara-13/25-UOG".

Duckweed-derived magnetic adsorbent for efficient removal of methylene blue

Seng, KWK; Zheng, ALT; Jeffrey, KB; Hii, TT; Chung, ELT; Lease, J; Andou, Y (2026) Biomass Conversation and Biorefinery 16: 111.

Low-cost and easily recoverable adsorbents are urgently needed for efficient treatment of dye-contaminated wastewater. In this study, a green magnetic duckweed-derived adsorbent (MDW) was synthesized via co-precipitation of Fe_3O_4 nanoparticles (NPs) onto dried duckweed using ascorbic acid (AA) as a reducing agent. The MDW exhibited a maximum methylene blue (MB) adsorption capacity of 78.59 mg/g under optimal conditions of pH 10, 50 mg/L initial MB concentration, 10 mg adsorbent dosage, and 60 min contact time, achieving a removal efficiency of 85.27%. Kinetic analysis showed that adsorption followed the pseudo-second-order (PSO) model ($R^2 > 0.999$), while equilibrium data were best described by the Temkin isotherm model, indicating heterogeneous surface interactions. Thermodynamic analysis revealed an exothermic and spontaneous process with negative Delta G degrees values across the temperature range of 298-318 K. The MDW retained over 69% of its initial removal efficiency after five regeneration cycles, confirming good reusability. Cost analysis estimated the synthesis cost at approximately RM 29.51/kg, highlighting economic feasibility. These findings demonstrate that MDW is a sustainable, low-cost, and magnetically recoverable adsorbent with strong potential for scalable wastewater treatment applications.

DF comment: The only information about the identity of the used Lemnaceae species is the following: "Natural duckweed (*Lemna minor* L.) was sourced from a retention pond in Bintulu".

Development of a two-stage nature-based system for efficient Naphthol Blue Black dye removal

Muduli, M; Bambhaniya, RV; Mishra, N; Ray, S (2026) Bioresource Technology Reports 33: 102566.

The textile industry is generating a large volume of dye-laden wastewater, among which, Naphthol Blue Black (NBB) is one of the most toxic and recalcitrant dyes. In this study, a two-stage nature-based system (NBS), comprising a Cynodon dactylon-based horizontal flow constructed wetland (HFCW), followed by a *Spirodela polyrhiza* duckweed pond, was studied to achieve sustainable NBB dye removal from textile wastewater. The system was assessed using influent dye concentrations ranging from 25 to 200 mg/L, and treatment performance was evaluated in terms of dye, chemical oxygen demand (COD), and nutrient removal (NH_4^+-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P). The maximum removal efficiencies for dye and COD were 91.34% and 49.22%, respectively, at 25 mg/L influent concentration, decreasing to 53.59% and 25.13% at 200 mg/L, indicating reduced system tolerance at higher pollutant loading. Nutrient removal was substantial with NH_4^+-N and PO_4^{3-}-P reductions ranging from 65 to 94% and 65-74%, respectively. ANOVA results ($p < 0.05$) confirmed a statistically significant difference between the control and CW systems. The planted HFCW contributed predominantly to pollutant removal, while the duckweed pond provided a minor polishing effect. Overall, the proposed two-stage NBS offers a low-cost, sustainable, and decentralized solution for NBB-laden textile wastewaters.

Comprehensive study of domestic wastewater phytoremediation using duckweed in mesocosms: Physiological, biochemical, hormonal and metabolic aspects

Quevedo, MR; González, PS; Llanes, A; Magallanes-Noguera, C; Paisio, CE (2026) Bioresource Technoogy Reports 33: 102551.

Domestic wastewater (DW) presents considerable environmental and public health challenges if not adequately treated, owing to its high levels of organic matter and pathogens. Phytoremediation offers a sustainable alternative for their treatment, due to the natural capacity of plants to remove contaminants, often in synergy with microbial communities. In this study, phytoremediation of DW by a duckweed mixture in mesocosm scale and natural environmental conditions was evaluated for a period of 30 days in the summer and 7 days in both summer and winter was implemented. The results revealed removal efficiencies exceeding 70% for chemical oxygen demand and phosphorus, 52% for nitrogen and nearly complete pathogen removal. Furthermore, the plants maintained a stable osmotic potential; increases were detected in the relative permeability of cell membranes and in the concentration of chlorophyll, indicating an increase in the rate of photosynthesis. However, the reduction in carbohydrate concentration could have been due to decreased synthesis, energy expenditure, or other metabolic reactions. The stability observed in the concentrations of abscisic acid, jasmonic acid, and salicylic acid indicates that the plants were either not subjected to significant stress or had effectively activated adaptive hormonal responses to mitigate its impact. Gas chromatography-

mass spectrometry analysis identified elevated levels of erucamide, which could enhance the removal of nutrients, pollutants, and microorganisms. Overall, this study highlights the potential of duckweed for effective DW treatment, as well as its ability to maintain homeostasis and physiological balance. This highlights its application in wetlands on a larger scale to achieve sustainable wastewater treatment.

DF comment: The authors wrote "duckweed mixture composed of *L. gibba, L. minuta* and *W. columbiana*" without giving the way of identification.

Evaluating nutrient removal efficiency of aquatic plants in pond aquaculture discharges

Eljasik, P; Edzarek, AN; Sobczak, M; Lisiecki, S; Roy, K; Smietana, P; Panicz, R (2026) Aquaculture 615: 743645.

Semi-intensive common carp (*Cyprinus carpio*) pond aquaculture in Central and Eastern Europe provides valuable ecosystem services, such as water retention and biodiversity hotspots, but may also contribute to nutrient pollution and eutrophication. This study assessed the phytoremediation potential, bioconcentration efficiency, and biomass yield of two aquatic plants-watercress (*Nasturtium officinale*) and duckweed (*Lemna minor*)- cultivated using carp farm effluents. Watercress yielded 7.99-11.92 kg m^{-2}, while duckweed yielded 2.54 kg m^{-2}. Watercress bioremediated 5.93-8.63% of total nitrogen (TN) and up to 23.43% of total phosphorus (TP), whereas duckweed removed 19.15% of TN and 57.89% of TP. Despite negligible carbon (C) retention, duckweed and watercress systems effectively reduced N (-9.9%) and P (-25.6%) concentrations of the carp pond effluents, with duckweed performing best in terms of nutrient uptake relative to the influx of nutrients. All phytoremediated water met the European Union Water Framework Directive limits for nitrogen concentration; duckweed-treated water also approached compliance with the limits for phosphorus concentration, safe for riverine discharge. Furthermore, both macrophytes remained within food-grade limits for heavy metals, providing safe and marketable biomass. These findings suggest that integrating watercress and duckweed production into carp aquaculture can diversify farm income while mitigating eutrophication through scalable, nature-based bioremediation solutions.

DF comment: No identification of *L. minor* was reported.

Duckweed-based systems in the water-energy-food nexus: controlled environment agriculture (CEA) for industrial water reuse and nutrient recovery

Jurga, A; Jugowicz, A; Lipinska, MB; Rodziewicz, T; Kazmierczak, B (2026) Water Resource and Industry 35: 100343.

Duckweed (Lemnaceae) is gaining increasing attention as a multifunctional bioresource for wastewater treatment, nutrient recovery, food, feed, and bioenergy production, as well as for closed-loop life support systems. Its rapid growth, high nutrient uptake capacity, and aquatic growth habit make it particularly suitable for integration into controlled environment agriculture (CEA) systems within the water-energy-food nexus. This review synthesizes recent advances in indoor duckweed cultivation, focusing on laboratory- and pilot-scale systems with working volumes exceeding 1 L. The aim is to identify key biological and engineering factors governing system performance and to assess the feasibility of scaling duckweed-based CEA for industrial and municipal water reuse applications. Comparative analysis of reported studies shows that short-term laboratory experiments frequently overestimate long-term productivity, with relative growth rates declining as cultivation duration and system complexity increase. System performance is strongly influenced by hydraulic design, culture depth, mat density, harvesting strategy, nutrient dosing, microbiological control, and energy input, particularly for lighting and climate regulation. Pilot-scale studies demonstrate that stable operation and realistic productivity require integrated management of biological processes and engineering subsystems rather than optimization of single parameters. Major knowledge gaps remain in long-term nutrient management, microbial stability, standardized performance metrics, and techno-economic assessment. Overall, duckweed-based CEA represents a promising but still emerging technology for sustainable water reuse and biomass valorization. Progress toward practical deployment will depend on pilot-scale validation, improved process integration, and robust evaluation of economic and safety constraints, with additional insights emerging from space life-support research.

Phytotoxicity

Rapid assessment of pesticide toxicity in aquatic ecosystems using deep learning-based automatic duckweed counting method

Cheng, D; Kurnia, KA; Hsiao, CD (2026) Aquatic Toxicology 294: 107791.

Pesticides are widely used in agriculture to control weeds, insects, and diseases that threaten crop yields. However, their extensive use raises concerns about environmental impacts, particularly in aquatic ecosystems, which are vulnerable to contamination through runoff and leaching. To assess the toxicity of pesticides to aquatic plants, we applied an optimized automated duckweed (*Wolffia globosa*) frond-counting tool based on the StarDist technique. Using this method, we tested twenty-eight commonly used pesticides, including herbicides, fungicides, and insecticides effects on duckweed growth. The herbicide paraquat showed the strongest growth inhibition (IC_{50} < 10 ppb), followed by diuron (IC_{50} = 384.2 ppb). Simazine and pendimethalin exhibited moderate toxicity, while glyphosate, triclopyr, and glufosinate showed lower toxicity. Surprisingly, metamifop did not inhibit duckweed growth up to the highest tested concentration (10^6 ppb). Isoprothiolane was the only fungicide tested that exhibited significant toxic effects on duckweed (IC_{50} approximate to 924.3 ppb). All others, including azoxystrobin, hexaconazole, difenoconazole, picoxystrobin, tebuconazole, and cyproconazole, only inhibited plant growth at unnaturally high concentrations. Interestingly, cyazofamid promoted duckweed growth under the test conditions. Among 12 insecticides tested, 8 exhibited relatively low toxicity to duckweed (IC_{50} > 10^5 ppb). Cypermethrin, carbofuran, fenpropathrin, and nitenpyram showed very low toxicity, with IC^{50} values exceeding 10^6 ppb. Our results both enhance understanding of agrochemical toxicity and demonstrate the utility of automated, high-throughput quantification of *W. globosa* growth, providing a rapid and effective approach for pesticide toxicity assessment in aquatic environments.

Commentary: Rare earth elements affect the growth and fitness of free-floating plant *Lemna minor* L

Szabó, S; Koleszár, G (2026) Frontiers in Plant Science 17: 1805616.

The study by Gjata et al. (2025) revealed the toxicological impact of rare earth elements (REEs) on the free-floating species Lemna minor where they measured the effects of REEs on growth rate, photosynthetic pigment concentrations, and oxidative stress markers of the plants. A key point of such toxicity studies is the precise measurement of the relative growth rate (RGR) of the plants which is the primary metric for determining inhibitory thresholds (Naumann et al., 2007). The authors correctly described RGR by the formula: $RGR=(\ln(N_t) - \ln(N_0))/t$, where N_0 and N_t are the initial and final frond numbers, and t is time in days. However, examination of the RGR values in their Table 1 reveals great mathematical and biological contradiction. This commentary reveals the error source, recalculates growth rates, and discusses suggestions for the study's conclusions on REE toxicity.

Impact of sulfamethoxazole, trimethoprim, diclofenac, carbamazepine, and their mixture on the metabolism of *Lemna minor*: a targeted metabonomic study

Wahman, R; Schröder, P; Duporté, G; Chiron, S; Drewes, J; Sauvêtre, A; Cruzeiro, C (2026) 22: 33.

Metabolomics is an analytical profiling technique that measures and compares large numbers of metabolites in biological samples, providing insight into metabolic mechanisms. There are few studies concerning the effects of xenobiotics and their transformation products on aquatic plant metabolites, which can uptake and detoxify them using untargeted metabolomics. This study investigates how pharmaceuticals, including diclofenac (DCF) and carbamazepine (CBZ), as well as sulfamethoxazole (SMX) and trimethoprim (TRIM), present in aquatic environments, can influence the biosynthetic pathways of *Lemna minor*. Based on previous research on the effects of DCF, SMX, and TRIM on *Lemna* pathways, specifically phenylalanine, tyrosine, and tryptophan biosynthesis, folate biosynthesis, and the phenylpropanoid pathway, including flavonoid and anthocyanin metabolism. *Lemna* was incubated with DCF, CBZ, SMX, and TRIM alone and in a mixture (MIX) at 5 ppb (5 µg/L) for 5 days, at concentrations near environmental levels. The methanolic extract was analysed

using a Q Exactive Focus Orbitrap to investigate changes in the aforementioned biosynthetic pathways, as reported in previous studies. *Lemna* can modulate its pathways to produce more phenolic compounds as a defence mechanism against various drugs. This modulation can be considered an indicator for each drug. The presence of pharmaceuticals in the aquatic environment can affect the biosynthetic pathways of *Lemna*. Therefore, *L. minor* can be used as a model to study the stress-response of different pharmaceuticals on plant metabolites and their pathways.

DF comment: The authors did not report any identification of the species *Lemna minor*

Manganese thresholds govern antagonism-synergy switching in tetracycline phytotoxicity in *Lemna minor*

Tu, JF; Tong, Y; Xi, YL (2026) Ecotoxicology and Environmental Safety 312: 119930.

Tetracycline antibiotics frequently co-occur with manganese (Mn) in freshwater environments, yet how Mn background gradients reshape mixture phytotoxicity remains insufficiently resolved. The objective of this study was to systematically investigate how varying Mn background levels modulate the phytotoxicity and interaction patterns of tetracycline antibiotics. Using *Lemna minor* as a model species, we employed full-factorial Mn x tetracycline concentration matrices (tetracycline, TC; oxytetracycline, OTC; and chlortetracycline, CTC) to quantify Mn-dependent modulation of tetracycline toxicity and identified operational Mn regimes supported by breakpoint robustness analysis. Growth-rate responses delineated a low-Mn window (0.10-0.40 mg L^{-1}), a transition interval (0.40-1.60 mg L^{-1}), and a high-Mn domain (≥ 1.60 mg L^{-1}). Under low Mn conditions, Mn generally promoted antagonistic interactions, with inhibition weaker than expected, a pattern consistently observed across growth, pigment, and antioxidant endpoints. In contrast, under high Mn conditions, synergistic interactions emerged at specific Mn x antibiotic combinations, characterized by stronger-than-expected inhibition, forming spatially heterogeneous interaction "islands" on the response surfaces. These regime-dependent patterns were concordant between integrated multi-endpoint Delta Bliss interaction landscapes, which quantify deviations from Bliss independence, and potency-surface validation using delta ZIP, a zero-interaction potency (ZIP)- based deviation metric, based on chlorophyll a. Overall, the results support a threshold-based, operational reporting framework linking Mn regimes to mixture interaction landscapes and indicate that mixture outcomes cannot be inferred from antibiotic dose alone when background metal levels vary, underscoring the need to explicitly incorporate metal gradients in mixture risk assessment.

DF comment: The only information for the identity given by the authors is the following: “Fronds of *L. minor* were collected from a freshwater pond in Huainan, Anhui Province, China (32◦37'N, 116◦59'E).” This does not give how the plants were identified.

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

Sackey, LNA; Quansah, YM; Aidoo, I; Mensah, EF (2026) Environmental Toxicology DOI10.1002/tox.70045

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

the river samples showed confirmed toxicity, indicating the impact of illegal mining on aquatic ecosystems. This highlights the need for monitoring and strong regulations to protect aquatic ecosystems.

DF comment: No information is given concerning source or identification of the test plant *Lemna minor*

A short-term preliminary experimental study on the effect of microplastics on *Chlorella vulgaris* and duckweeds

Sasikumar, AP; Vinod, V; Babu, HP (2026) Biologia 81: 61.

Surface or ground waters are heavily contaminated with numerous toxic substances. One of the emerging contaminants is the Microplastics (MP). MPs are polymers smaller than 5 mm in diameter, which are harmful. Plants are affected in terms of growth inhibition and photosynthetic pigment reduction. *Chlorella* and *Lemna minor* are freshwater algae and floating plants, respectively, which are considered a good source of protein for humans and fish due to their high protein content and environmentally friendly production properties. The study aimed to investigate the effect of the plastic microspheres on *Lemna minor* and *Chlorella*. The plastic microspheres were exposed to *Chlorella* and duckweed for 21 and 14 days, respectively. The effects of different doses of microspheres on the growth, photosynthetic pigments, algal cell density, root length, number, and root cell viability were assessed. The growth rate, content of photosynthetic pigments, algal cell density, root length, and root growth have been reduced with an increase in the dose of MP concentration. Evan's blue staining provided evidence for the incorporation of MPs deep into the layers of roots. It can be concluded that the microspheres can negatively impact floating organisms of freshwater ecosystems.

DF comment: "The *Lemna minor* were collected from a pond in Thrissur (10.22.77°N; 76.19.71°E)." Unfortunately, the method of species identification was not given. There are serious doubts that *L. minor* does exist in Thrissur. The most common Lemna species in this area is *Lemna aequinoctialis*.

Multifaceted toxicity assessment of di-octyl phthalate on morphology, antioxidant biomarkers, and chlorophyll a fluorescence kinetics of *Spirodela polyrhiza*

Soni, V; Chaudhary, HD; Bhatt, U (2026) Environmental Sustainability DOI10.1007/s42398-025-00407-2.

Di-octyl phthalate (DOP), a widely used plasticizer, is an emerging aquatic pollutant with limited information regarding its phytotoxic effects on floating macrophytes. This study systematically investigates the morphological, biochemical, and photosynthetic responses of *Spirodela polyrhiza* exposed to increasing DOP concentrations (0-9 ml/L) over 5 days. Progressive frond discoloration and chlorosis became apparent at ≥ 5 ml/L, indicating visible morphological degradation. Biochemical analyses revealed a marked increase in malondialdehyde (MDA) content and altered superoxide dismutase (SOD) activity, indicative of oxidative stress and cellular damage. A significant dose-dependent reduction in total chlorophyll content further underscored photosynthetic impairment. Chlorophyll a fluorescence (OJIP) transients showed substantial disruption in PSII functionality, evidenced by a decline in maximum fluorescence (Fm), variable fluorescence (Fv/Fo), and quantum yields PHI(Po), PHI(Eo), alongside enhanced energy dissipation PHI(Do). Performance indices PI(abs) and PI(csm) sharply declined at 7 and 9 ml/L, with PI(csm) approaching zero at the highest concentration, denoting near-total loss of photosynthetic capacity. Per cross-section parameters, including RC/CSm (active reaction centers), ETo/CSm (electron transport), and ABS/CSm (light absorption), were also significantly diminished at higher concentrations. Multivariate analyses (PCA and heatmap) confirmed clear separation of high-dose treatments, highlighting cumulative damage to the photosynthetic apparatus. Lethal dose calculations (LD_{50}: 2.897 ml/L; LD_{90}: 9.304 ml/L) emphasize the narrow margin between sub-lethal and lethal exposures. Collectively, these findings demonstrate that DOP severely compromises photosynthetic efficiency and redox homeostasis in *S. polyrhiza* within just 5 days, underscoring its ecological threat to aquatic primary producers.

Ecotoxicological effects of synthetic food dyes on *Daphnia magna* and *Lemna minor*

Aidoo, IA; Sackey, LNA; Quansah, YM; Mensah, EF; Bosomtwi, A (2026) Journal of Applied Toxicology DOI10.1002/jat.70064.

Synthetic food dyes are widely used in Ghana, yet their ecological effects on freshwater ecosystems remain understudied. This study investigated the acute toxicity of five commonly used synthetic food dyes (tartrazine, allura red, sunset yellow, carmoisine, and green S) on two bioindicators, *Daphnia magna* and *Lemna minor*, following OECD 202 (2006) and OECD TG 221 protocols. The physicochemical analysis suggested that synthetic dyes, even in dilute form, can alter water chemistry. Although the electrical conductivity (EC), salinity, and total dissolved solids (TDS) values for sunset yellow were relatively low (8.12 mS/cm, 7.50 psu, and 2.98 g/L, respectively), tartrazine, carmoisine, and green S recorded high levels of EC, TDS, and salinity. Levels of Cd and Hg in all the dyes were below the detection limit (<0.001 mg/L). Carmoisine, however, recorded 0.003 mg/L for Cd. Levels of Cr, Pb, Fe, and Mn were above the limits for food dyes (≤0.002, ≤0.043, and ≤0.006 mg/L), raising environmental concern. The EC_{50} showed that allura red is the most toxic, and tartrazine is the least toxic. IC_{50} showed that green S is the most toxic and sunset yellow is the least toxic. All the dyes showed potential toxicity, and when released into the environment without treatment, they will have adverse effects on the aquatic environment. Bioaccumulation potential revealed that Pb, Mn, As, and Hg were accumulated in daphnia, whereas Cr, Cd, and Fe were not due to their low solubility, ionic competition, and strict cleansing mechanisms by daphnia. These findings emphasize the ecological risks of unregulated dye pollution in Ghanaian waters and highlight the importance of region-specific ecotoxicological data to guide environmental policies, regulations, and sustainable wastewater management practices.

DF comment: No information about origin or identity of *L. minor* was given.

Ibuprofen surface waters contamination: phytotoxic effects on the aquatic macrophyte *Lemna minor*

Kornacka, H; Sitarska, M; Wolf-Baca, M (2026) International Journal of Enironmental Science and Technology 23: 229.

Ibuprofen is one of the most widely used analgesic and anti-inflammatory pharmaceuticals due to its availability and wide range of medical properties. High utilization is associated with the presence of the drug in aquatic ecosystems, raising concerns about potential environmental effects. This research assesses the influence of ibuprofen on the common aquatic macrophyte *Lemna minor*. The study was conducted for seven concentrations of ibuprofen: 5, 10, 20, 50, 100, 200 and 400 mg/L. The duration of the experiment was 21 days for the lower concentrations (5-20 mg/L) and 15 days for the higher concentrations (50-200 mg/L). To determine the toxicity of IBU, changes in weight gain, chlorophyll a and b content, and alterations in plant appearance were analyzed. Exposure to very high concentrations (≥ 100 mg/L) resulted in plant necrosis. Lower concentrations caused a significant reduction in fresh weight of 20% (5 mg/L), 36% (10 mg/L) and 16% (20 mg/L) after two weeks of exposure. Analysis of the results of the chlorophyll content of the plants showed a notable decrease in all IBU-treated groups as exposition time increased. Microscopic images showed signs of alterations suggesting the possibility of chlorosis and necrosis even under the low level of IBU. However, some results suggest that *L. minor* may show an adaptation to small amounts of IBU. These observations provide a basis for further research on the tolerance mechanisms and on the potential of *L. minor* for the phytoremediation of waters contaminated with pharmaceuticals.

DF comment: The authors wrote: "*Lemna minor* was collected in September/October (2024) from the natural environment, specifically the water reservoir located in the Wrocław city park", no identification of the species was described.

The effect of single and combined stress of microplastics and heavy metals on growth, biochemical components and antioxidant activity of *Lemna gibba* (Duckweed)

Senturk, T; Oskay, M (2026) Journal of Agricutural Sciences – Tarim Bilimleri Dergisi 32: 408-422.

The increasing presence of microplastics (MPs) and heavy metals (HMs) in aquatic ecosystems poses a growing concern due to their potential ecotoxicological effects. While the individual toxicity of MPs and HMs has been widely investigated, limited attention has been given to their combined effects on aquatic macrophytes. In this study, we aimed to evaluate the single and combined impacts of two types of MPs [polypropylene (PP) and acrylonitrile butadiene styrene (ABS)] at concentrations of 25, 50, and 100 mg L^{-1}, along with three typical HMs (Zn^{2+}, Cu^{2+}, and Ni^{2+}), on the growth, biochemical components, and antioxidant activity of the model macrophyte *Lemna gibba* under laboratory conditions over a 7-day exposure period. The results revealed that both contaminants alone negatively impacted growth and biochemical performance, but the combined application caused a more pronounced decrease, suggesting a synergistic inhibitory effect on plant metabolism. The simultaneous application of ABS-MP with nickel, copper, and zinc resulted in more pronounced adverse effects on *L. gibba* growth parameters, photosynthetic pigments, and carbohydrate content compared to single-pollutant exposures. Co-application of copper and nickel induced pronounced oxidative stress in plant tissues, as evidenced by increased malondialdehyde levels. Furthermore, significant reductions were observed in total protein, total phenolic, and total flavonoid content across all treatments. Conversely, total antioxidant activity showed variable results dependent on the specific contaminant and concentration applied. These findings provide preliminary evidence that co-occurring MPs and HMs may exert additive or synergistic stress effects on aquatic macrophytes. In particular, the comparative evaluation of ABS and PP microplastics, along with Cu, Zn, and Ni treatments, highlights polymer and metal-specific toxicity patterns and integrated antioxidant response profiles that have not been previously reported for *L. gibba*.

DF comment: The authors wrote: "whole *L. gibba* plants were collected in April (2024) from the Yagcilar area of Manisa province, Türkiye but no identification was reported.

Serotonin application decreases fluoxetine-induced stress in *Lemna minor* and *Spirodela polyrhiza*

Wierzbicka, M; Michalczyk, DJ; Piotrowicz-Cieslak, AI (2026) International Journal of Molecular Sciences 27: 2.

The aim of this study was to evaluate the impact of fluoxetine, a widely used selective serotonin reuptake inhibitor, on two aquatic plants: *Lemna minor* and *Spirodela polyrhiza*. Additionally, the effect of exogenous serotonin on the level of fluoxetine-induced stress in duckweed will be studied. Increasing presence of antidepressants in surface waters poses ecological risks, and the duckweed species are ideal model organisms for ecotoxicological studies due to their rapid growth and ability to accumulate pollutants. For 14 days, plants were exposed to fluoxetine (0.001-150 mg L^{-1}), followed by a recovery phase in a drug-free medium or a medium supplemented with exogenous serotonin. We analysed morphological/physiological parameters (frond length and area, fresh and dry mass, hydration, stomatal size), the activity of antioxidant enzymes (catalase, ascorbate peroxidase, superoxide dismutase), cell viability, and the level of heat-shock proteins. The plants' ability to remove fluoxetine from the medium was also assessed. High fluoxetine concentrations (50-150 mg L^{-1}) significantly reduced fresh mass (by 63-98% in *L. minor* and 56-97% in *S. polyrhiza*), frond area (by 21-48% in *L. minor* and 11-25% in *S. polyrhiza*), and cell viability (by 36-94% in *L. minor* and 49-94% in *S. polyrhiza*), and induced oxidative stress. Despite this, both species showed high regeneration potential after the stressor's removal. Serotonin supplementation did not affect morphology but increased antioxidant enzyme activity, improved cell viability, and elevated heat-shock proteins levels. Crucially, serotonin significantly increased the efficiency of fluoxetine removal. The data can provide a basis for predicting fluoxetine removal efficiency in plants with different levels of endogenous serotonin. *Lemna minor* and *S. polyrhiza* exhibit substantial tolerance to fluoxetine, and antioxidative enzymes are sensitive markers of this stress.

DF comment: "The experimental material consisted of axenic plants of common duckweed (*Lemna minor* L.), and greater duckweed (*Spirodela polyrhiza* L.) obtained from the Department of Plant Physiology, Genetics, and Biotechnology at the University of Warmia and Mazury in Olsztyn." No information about the identification of *L. minor*

Nanoplastics in duckweed: Single-cell responses and recovery

Yuan, WK; Xu, EG; Zhu, D; Zhang, WH; Liu, WZ; Monikh, FA; Lin, L; Li, LZ; Grossart, HP; Yang, YY (2025) ACS Nano 19: 42869-42880.

Micro- and nanoplastics have emerged as critical contaminants in aquatic ecosystems due to their small size, persistent nature, and potential for bioaccumulation. Nanoplastics are particularly concerning because they can be widespread in aquatic environments and ingested by aquatic organisms, posing potential risks to ecological health and environmental sustainability. However, the response and recovery of aquatic plants to nanoplastics, as well as the cell-specific molecular mechanisms underlying these processes, remain unclear. By integrating single-cell transcriptomics, enzymatic assays, and europium-doped nanoplastic tracing, we comprehensively investigated the response of duckweed to polystyrene nanoplastics at environmentally relevant and high doses over exposure and recovery phases. Nanoplastics exposure reduced plant reproduction and root length by inducing oxidative damage, with partial recovery after removal. Single-nucleus RNA sequencing revealed cell-type-specific responses of duckweed to nanoplastics, particularly in mesophyll, mestome sheath, epidermis, and parenchyma cells. Interestingly, recovery triggered a greater number of differentially expressed genes mechanistically linked to carbon metabolism, membrane transport, and stress-responsive pathways. Nanotracer quantification demonstrated root/frond absorption and 36.8-51.4% postrecovery excretion. These multiscale lines of evidence decipher the molecular strategies of duckweed to nanoplastics at single-cell resolution, providing mechanistic insights into the interactions between aquatic plants and nanoplastics contamination.

Taxonomy & Geobotany

Comparative population genetic structure of *Spirodela polyrhiza* and the *Lemna minor* species complex (*Lemna minor* and *Lemna japonica*) across the Northern United States

Hobble, TT; Kerstetter, JE; Turcotte, MM (2026) International Journal of Plant Sciences DOI10.1086/739851

Duckweeds (Lemnaceae) have become an increasingly used model system for ecological and evolutionary studies as a result of their rapid clonal propagation and diverse applications. However, their facultative asexual reproduction and the limited morphological differences among species have complicated efforts to characterize natural duckweed population structures across large geographical scales. Understanding how intraspecific diversity varies spatially is critical to identifying unique genotypes and accurate use as a model system. We characterized the genetic composition of two duckweed species, *Lemna minor* and *Spirodela polyrhiza*, across three states in the northern United States. Using microsatellite markers, we genotyped 1262 duckweed individuals, sampled from 12 freshwater ponds, for nine species-specific microsatellite loci. Our collection revealed the widespread presence of the cryptic hybrid *Lemna japonica*. We found that, on average, *L. minor* and *L. japonica* populations had greater genotypic and allelic richness than *S. polyrhiza*, with *L. japonica* showing significantly more genotypes per population than *S. polyrhiza*. While *S. polyrhiza* populations all contained fewer than five genotypes, *L. minor* (two to 10 genotypes) and *L. japonica* (one to 18 genotypes) showed greater genotypic richness. Both *L. minor* and *L. japonica* populations had significantly greater Shannon genetic diversity when compared to *S. polyrhiza*. All species showed differentiation in population structure among states. Only two genotypes were found across multiple populations: one *S. polyrhiza* genotype, present in all Michigan ponds, and one *L. minor* genotype, found in both the Michigan-south triad and the Wisconsin mixed-species pond. Our findings suggest that *Lemna* species tend to be more diverse than their *Spirodela* counterparts, although variation in genotypic and allelic richness among species may be less than previously suggested. The widespread presence of *L. japonica* across our populations echoes the emphasis on accurately classifying and reporting duckweed species for future use as a model system.

Phylogenetic relationships and climate-driven range shifts of Lemnaceae in South Africa

Ndou, UH; Tshabalala, T; Mankga, LT; Bezeng, BS (2026) Frontiers in Ecology and Evolution 13: 1715912.

Duckweeds (Lemnaceae) are among the smallest flowering plants with ecologically significant components of freshwater ecosystems, yet their extreme morphological reduction complicates species identification, biogeographic classification, and invasion assessments. This study integrates multi-locus molecular phylogenetics and species distribution modeling (SDM) to clarify the evolutionary relationships, native versus

non-native status, and climate-driven range dynamics of duckweed species in South Africa. Phylogenetic reconstruction based on five plastid markers (matK, rbcL, rpl16, trnK-3 ', and trnK-5 ') resolved two well-supported subfamilies (Lemnoideae and Wolffioideae) and confirmed the monophyly of the genera *Lemna, Landoltia, Spirodela, Wolffia*, and *Wolffiella*. Of the 38 taxa analysed, nine were identified as native and 29 as non-native, providing new evidence that *Lemna minor* and *Lemna gibba*, previously regarded as invasive in South Africa, are native species. Ecological niche models developed using MaxEnt and bioclimatic variables projected current and future habitat suitability under four Shared Socioeconomic Pathways (SSP1-2.6, SSP2-4.5, SSP3-7.0, SSP5-8.5) across three global climate models (MIROC6, EC-Earth3-Veg, and UKESM1-0-LL). Current suitability was found to be the highest in the coastal provinces (KwaZulu-Natal, Eastern Cape, and Western Cape). Future projections revealed model-dependent outcomes, with MIROC6 and EC-Earth3-Veg predicting net expansion of climatically suitable habitat, particularly in inland provinces such as Mpumalanga, Limpopo, North-West, and Gauteng while UKESM1-0-LL consistently predicts contraction. The congruence between phylogenetic identity and projected range shifts highlights regions and freshwater systems vulnerable to future duckweed invasions. By integrating evolutionary history with climate projections, this study provides a robust framework for refining invasive species management, conserving native freshwater flora, and guiding adaptive conservation planning under climate change in South Africa.

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