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DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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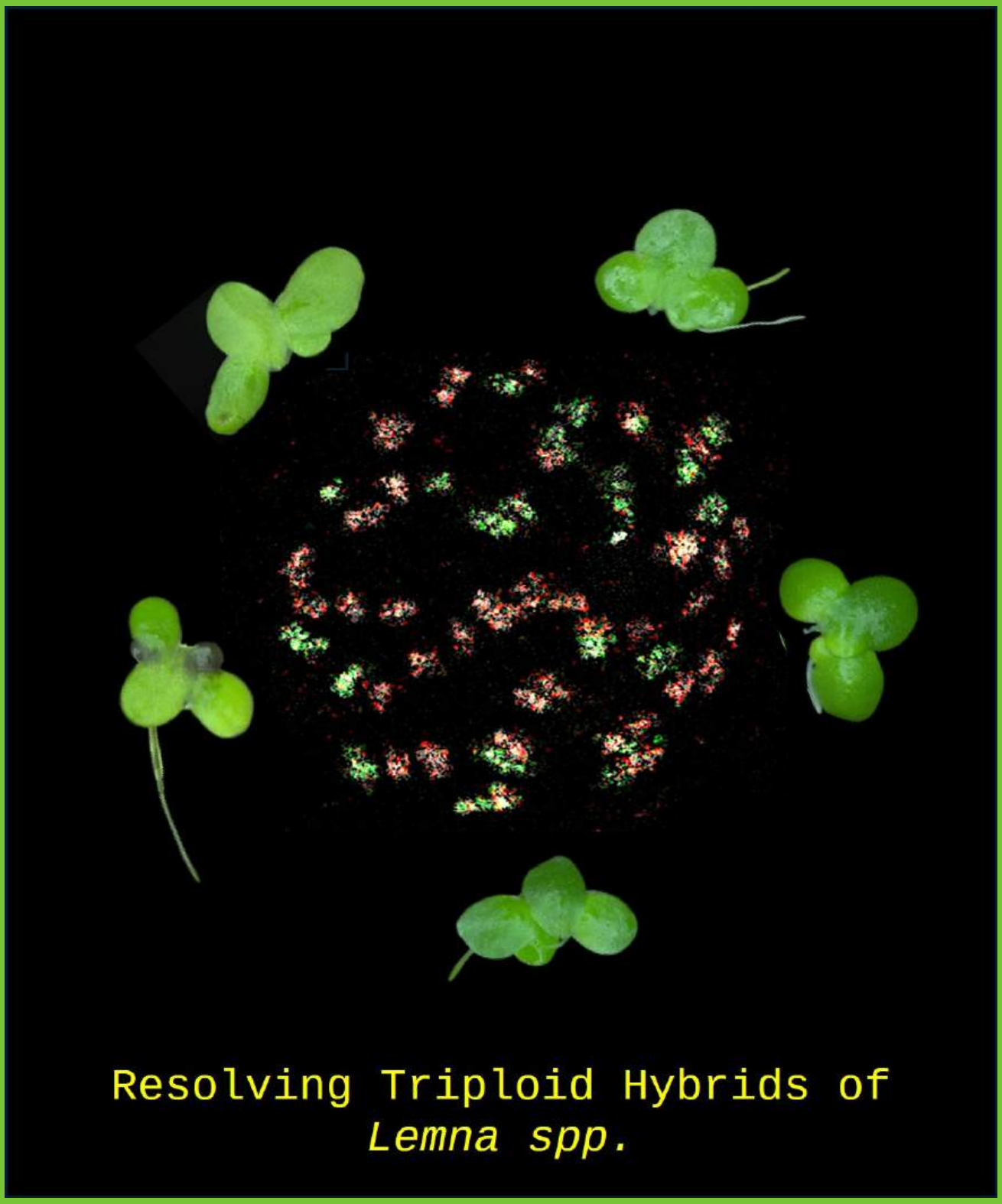

Cover page

Resolving Triploid Hybrids of *Lemna* spp.: Some duckweed species, and hybrids between them, are morphologically challenging to distinguish. This is illustrated by a collage of five distinct genotypes of *Lemna*, taken from data described in *Ernst et al. (2025)* in a selected paper of the Highlight section of this DF issue. Starting from 9 o'clock, clockwise: *Lemna turionifera 9434*, TT*; Lemna minor 9252*, MM*; Lemna japonica 9421*, MT*; Lemna japonica 7182*, MTT*; Lemna japonica 8627*, MMT. In the center is shown a Genomic In Situ Hybridization result with chromosome spread of the clone Lj8627 which demonstrated it is a triploid hybrid of *L. minor* and *L. turionifera* with two sets of the *L. minor* and one set of the *L. turionifera* chromosomes. The *L. minor* chromosomes are stained red while those of the *L. turionifera* genome are stained green. (Cover design: Eric Lam, Rutgers University, with contributions by Evan Ernst, Phuong Hoang, and Veit Schubert).

In this issue

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

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

Letter from the Editor:

April 30th, 2025

Dear Duckweed Community,

Greetings from the ISCDRA!!!

The onset of spring highlights a wonderful spread of colourful blossoms in nature. In this issue of Duckweed Forum, we have a bouquet of articles addressing some of the basic questions in the field of Lemnaceae as well as those showcasing the vast range of practical applications of duckweeds.

It has been proven time and again that the taxonomic status of Family Lemnaceae for duckweeds has to be retained. Concerning a recent journal publication which debated for the integration of duckweeds as a subfamily of Araceae, one of our articles in this issue by Tippery et al. systematically and scientifically explains why the recent study still does not disprove the family status of Lemnaceae. Studies like this are and should be based on data generated from a large number of clones belonging to each species. Understaning this very early on, duckweed researchers like Late Prof. Elias Landolt had maintained for decades a painstakingly large collection of duckweed clones. Currently several researchers house small to large duckweed clone collections and the ISCDRA has been instrumental in streamlining the nomenclature of these clones which is significant for strengthening the research efforts. An update to the list of duckweed clone collections across the globe has been presented here for use by the duckweed community. And, a commentary on the highlight article from the previous issue by Hidehiro Ishizawa and Hongwei Hou explores the compatibility of duckweeds and time series analysis for understanding the intricate relationships in the ecosystem.

In the series of articles focusing on the applications of duckweeds, a detailed article on the contribution of duckweeds as wholesome greens and their role in addressing the global health issue of phytonutrient deficiency has been brought forward by one of our ISCDRA members, Dr. Tsipi Shoham. Another article on the potential of duckweeds for phytoextraction of minerals as shown in Liberia is presented by Rafael Sarji Ngumbu. And, bringing back our memories from the previous duckweed meeting, Chiemchaisri et al. described the duckweed based wastewater treatment set up that was demonstrated to several of us who attended the 7th ICDRA in Thailand last year.

In preparation for the 8th ICDRA, the organizers have now announced the dates of the conference from 28th September to 2nd October, 2026 giving us enough time to earmark these dates for attending the next duckweed meeting in Naples, Italy. Thanks to the organizers for also setting up the conference website with preliminary information and we strongly recommend you to follow the announcement and fill in the 'stay informed' page already now to facilitate the organizers to disseminate the upcoming conference details from time to time.

It is heartening to see the increasing trend in the number and quality of duckweed publications as can been read through in the database section collated by Klaus Appenroth. The coverpage designed by Eric Lam presents one of the selected highlight articles in the database section on 'Resolving Triploid Hybrids of *Lemna* spp.'.

Enjoy reading and researching duckweeds!!!

Best regards,

Sincerely,
K. Sowjanya Sree
Chair, ISCDRA

New phylogenetic data have not disproven years of precedent – Lemnaceae should not be integrated into the Araceae family

[1]Nicholas P. Tippery, [2]Manuela Bog, [3]K. Sowjanya Sree, [4]Donald H. Les, [5]Klaus J. Appenroth*

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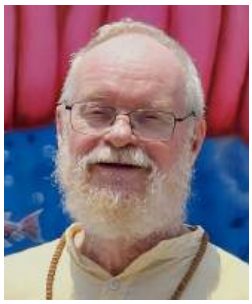

A recent article (Sururi et al., 2025) presents a new evaluation of DNA sequence data from the chloroplast *psbA-trnH* spacer region and asserts that the new evidence supports the taxonomic status of duckweeds as a subfamily of Araceae (i.e., Lemnoideae). Classifying duckweeds within Araceae has become popular among some influential taxonomists (e.g., French et al. 1995) and the Angiosperm Phylogeny Group forwarded this suggestion (APG 1998). However, other biologists, particularly those whose research has specialized in studying duckweeds, assert that duckweeds should continue to be regarded as a taxonomic family Lemnaceae. Although the taxonomic term Lemnoideae has a double meaning as subfamily within the family Lemnaceae (comprising the genera *Spirodela*, *Landoltia* and *Lemna*) and as subfamily of the family Araceae s.l., Tippery et al. (2021) showed that many more research papers are using the term Lemnaceae rather than Lemnoideae, and this was more than 10 years after the APG suggested to accept duckweeds as Lemnoideae. We therefore suggest to revise the APG (1998) suggestion, because it confuses the taxonomic situation.

The family was defined by Ivan I. Martinov (1771 – 1833) already more than 200 years ago (Martinov, 1820) resulting in the complete botanical name "Lemnaceae Martinov". Other authorities defined the family of Lemnaceae also around the same time, like Dumortier (1827) and S.F. Gray (1821) (review in Landolt, 1986), but definitively later than Martinov. Besides upholding the precedent of scientific literature over 200 years, the family rank for duckweeds is also compatible with molecular phylogenetic data, so long as the 'aroid' clade consists of the three families Araceae, Lemnaceae, and Orontiaceae (Tippery et al., 2021).

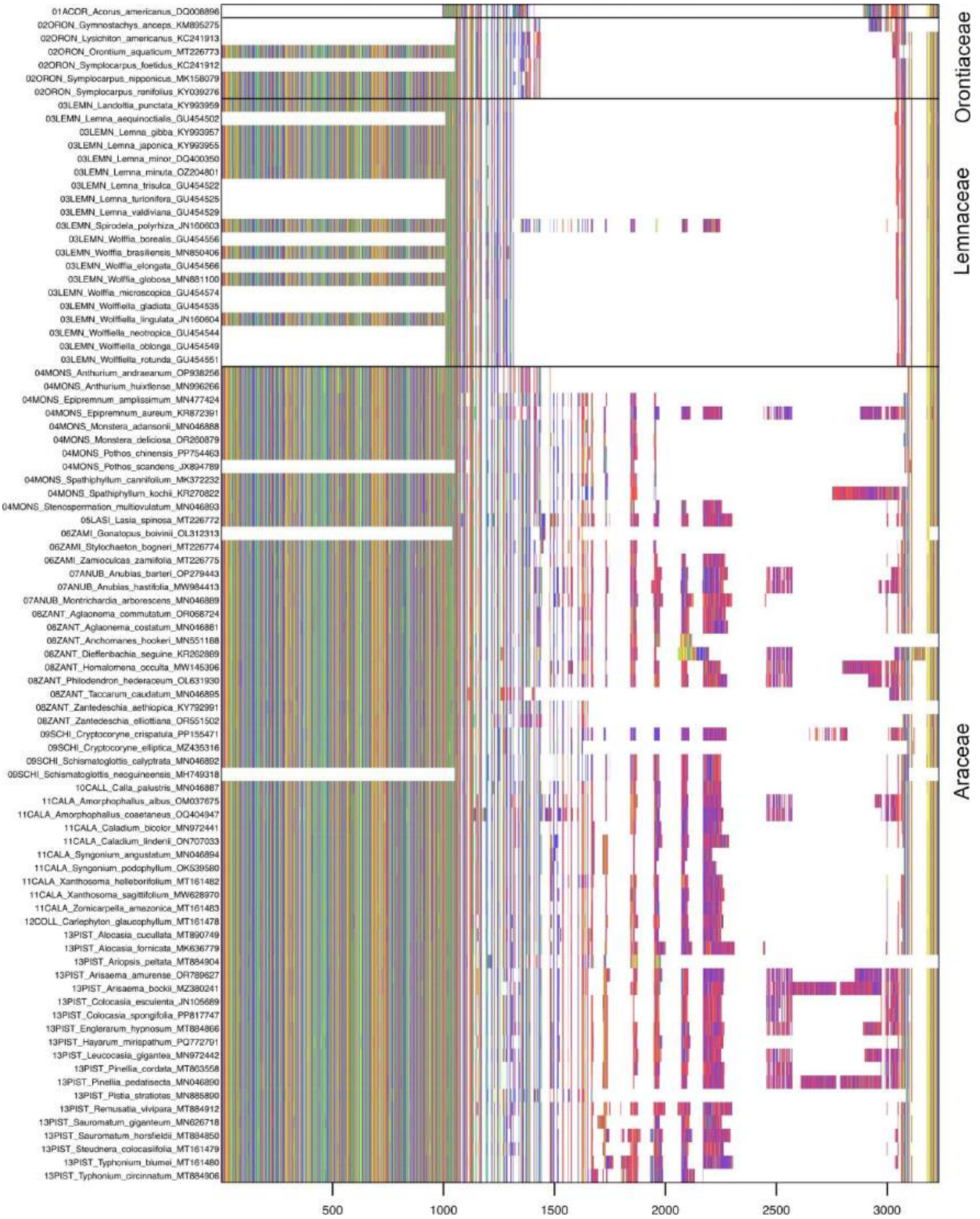

Fig. 1. Alignment of *psbA* gene (left side of alignment), trnH gene (right side of alignment), and the *psbA-trnH* intergenic spacer. Colors represent different nucleotides. Sequences are organized by taxonomic group, indicated before the species name, either family (Acoraceae, Lemnaceae, Orontiaceae) or Araceae subfamily or tribe. GenBank accession numbers are given after the species names. White space in the alignment reflects missing data or gaps in the alignment.

Sentences like "molecular taxonomy studies revealed the integration of Lemnaceae into the Araceae family" (Sururi et al., 2025) belie the rules of taxonomy. Two concepts are possible according to the taxonomic rules. That Lemnaceae not necessarily need to be integrated in the Araceae, also considering the molecular data, was shown in Tippery et al. (2021).

Using previously published sequences, many of which were generated during a barcoding study of duckweeds (Wang et al., 2010), Sururi et al. (2025) presented data that are comprehensive in scope, yet their conclusions should be regarded with caution. They included 20 duckweed species (of four genera) and 21 species of Araceae s.l. from six subfamilies. Firstly, in principle there should not be a single chloroplast region that somehow upends years of precedent from studying other chloroplast regions. The chloroplast genome is a single molecule that basically has evolved as one unit, and every gene region should essentially support the same phylogenetic hypothesis. Sequences of chloroplast DNA in duckweed species (*trnL–trnF* intergenic spacer) were already published by Rothwell et al. (2004). Current data for duckweeds include not only several isolated chloroplast gene sequences (*rbcL*, *trnK/matK*, *rpl16, trnL–trnF*) (Les et al., 2002; Cabrera et al., 2008; review in Bog et al., 2019), but also many whole chloroplast genomes (Wang et al., 2011; Ding et al., 2017) and there are ample data for Araceae s.s. (Ross et al., 2016; Choi et al., 2017; Henriquez et al., 2014, 2020, 2021; Kim et al., 2019). The phylogenies presented by Sururi et al. (2025) fail to recover monophyletic subfamilies (under their classification): not only Lemnoideae, but also Orontioideae, Pothoideae, and Zamioculcadoideae, clades that have been corroborated repeatedly with molecular evidence.

Secondly, the *psbA-trnH* spacer region is exceptionally variable among aroids and duckweeds (Wang et al., 2010), and such variability can be problematic for phylogenetic reconstruction. Whereas the *psbA* and *trnH* genes themselves are highly conserved and reliably aligned, the spacer between them is so different among species in the target group that the DNA sequences cannot be aligned with confidence. For example, the length of the spacer varies from about 190–1030 bp across aroid species. Phylogenetic reconstructions using such variable data might support erroneous relationships, depending on the way in which the sequences are aligned.

At least one sequence identified as *psbA-trnH* for *Spirodela intermedia* (GenBank accession number GU454484) was a close match by BLAST search (Altschul et al., 1990) to a portion of the mitochondrial genome of *Spirodela polyrhiza* (GenBank accession number JQ804980). It should be noted, however, that the mitochondrial sequence in question was not annotated. At any rate, the potential similarity to a mitochondrial region could explain the divergent phylogenetic position for *S. intermedia* (Sururi et al., 2025).

To investigate the source data used by Sururi et al. (2025), we downloaded and aligned available *psbA-trnH* sequences for aroids, including the sequences used in that study as well as data from plastid genomes and otherwise published *psbA-trnH* sequence data. Our independent sequence alignment showed the extreme variation present in the spacer portion of the sequences (Fig. 1). The alignment also shows length variation that is consistent within Lemnaceae and within Orontiaceae and sets them apart from Araceae s.s.

It is always welcome to see new molecular data for duckweeds, and we support scientific debates about phylogenetic relationships and taxonomy. However, the recent study of *psbA-trnH* data in duckweeds and aroids (Sururi et al., 2025) is generally flawed and presents a poorly developed phylogenetic hypothesis that is derived from a narrow subset of available data. Therefore, we recommend keeping the plant families Lemnaceae and Orontiaceae separated from the family Araceae as suggested before (Tippery et al., 2021) – in agreement with taxonomic rules.

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Update: Registration and Collections of duckweed clones / strains

Table: Update to the Details of Duckweed Stock Collections across the globe.

S.No.	Director of the stock collection (Email ID)	Affiliation	Clone ID in private system	Available clones with Landolt ID system	Available clones with private ID system
1	Klaus J. Appenroth, Klaus.Appenroth@uni-jena.de	Matthias Schleiden Institute - Plant Physiology, University of Jena, Jena, Germany	KJAxxx	394 live, 36 herbarium specimens	66 Other IDs: 42
2	Manuela Bog, manuela.bog@uni-greifswald.de	Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany	BOGxxx	500	350
3	Nikolai Borisjuk, nborisjuk@yahoo.com	Jiangsu Key Lab Ecoagr Biotechnol Hongze Lake, Huaian, China	NBxxxx	11	26
4	Hongwei Hou, houhw@ihb.ac.cn	Chinese Acad Sci, Inst Hydrobiology, Wuhan, Hubei, China	WHxxx, GDxxx, BJxxx, SHxxx	28	50
5	Marcel Jansen, M.Jansen@ucc.ie	University College Cork, Earth and Environmental Sciences, Cork, Ireland	MJxxx	2	7
7	Laura Morello, lauraemmamariamorello@cnr.it	Landolt Stock Collection, Milano, Italy	LMxxx	550	80
8	Robert Laird, robert.laird@uleth.ca	Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada	RLxxx	2	58 (with RDSC and CPCC)
9	Eric Lam, ericl89@hotmail.com	Rutgers Duckweed Stock Cooperative (RDSC), Rutgers	ELxxx SGxxx	912	98 (91 ELxxx; 7 SGxxx)

		University, NY, USA www.ruduckweed.org			
10	Yubin Ma, mayubin@ouc.edu.cn	Ocean University of China, Qingdao, China	MYBxxxx	103	40
11	Viktor Olah, olahviktor@unideb.hu	Institute of Biology and Ecology University of Debrecen, Debrecen, Hungary	UDxxxx	3	90
12	Ingo Schubert, schubert@ipk-gatersleben.de	Leibniz Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany	Private IDs from others	133	29
13	K. Sowjanya Sree, ksowsree@gmail.com; kssree9@bhu.ac.in	School of Biotechnology, Banaras Hindu University, Varanasi, India	KSSxxx JPKxx	45 live, 36 herbarium specimens	45
14	Shuqing Xu, shuqing.xu@uni-mainz.de	University of Mainz, Mainz, Germany	XUxxx	246	107
15	Jiaming Zhang, zhangjiaming@itbb.org.cn	Institute of Tropical Bioscience and Biotechnology, Hainan, Haiku, China	ZJxxxx	50	700
16	Hai Zhao, zhaohai@cib.ac.cn (c/o Yang Fang, fangyang@cib.ac.cn)	Chengdu Institute of Biology, Chinese Acad Sciences, Chengdu, China	ZHxxxx	640	174
17	Leone Ermes Romano, leoneermes.romano@unina.it	Dept. of Agricultural Sciences, University of Naples Federico II, Italy	LERxxx	51	33
18	Tsipi Shoham, tsipi@greenonyx.biz	Greenonyx, Israel	GOxxxx	13	15

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Commentary: Duckweed meets time-series analysis – A unique synergism to unveil critical factors in complex ecosystems

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Duckweed is often cultivated in complex environments where numerous biotic and abiotic factors fluctuate and interact simultaneously. Understanding this complexity is essential for optimizing cultivation systems; however, traditional approaches that investigate individual factors by controlled experiments may not fully account for the phenomenon occurring in natural environments. Furthermore, changes in many factors (e.g., water properties and coexisting microbes) can be both the cause and the consequence of the change in duckweed cultivation performance, making it difficult to identify true causal relationships influencing the cultivation performance.

In ecology, researchers have employed time series analysis to address such ecosystem complexity. While correlation-based analysis cannot distinguish between factors that merely change synchronously and those with true causality, several analytical frameworks utilizing time-series data are able to identify genuine causal relationships and their directions. One such framework is empirical dynamic modeling (EDM) (Sugihara et al., 2012; Chang et al., 2017), which has been successfully applied across various fields. For example, Ushio et al. (2018) used EDM to reveal interspecies interactions within natural fish communities solely from time series data of fish abundance. Similarly, Deyle et al. (2016) predicted factors that induce influenza outbreaks based on time-series epidemiological and meteorological datasets.

We posit that duckweed and time series analysis represent a highly compatible combination, creating a unique synergy not easily attainable with other plant systems. This compatibility stems from duckweed's clonal propagation, which enables continuous sampling of plant material over time. Unlike typical plants, where destructive sampling terminates cultivation and prevents collection of time-series data, duckweed's clonal growth allows for repeated destructive sampling while maintaining the cultivation system largely intact. This property makes it feasible to apply time series analysis involving in-depth biological datasets that include plant gene expression, metabolic state, and microbiome composition.

In our recent paper (Ishizawa et al. 2024), we employed EDM on a lab-scale cultivation system of *Lemna japonica* #5512 grown in municipal wastewater, specifically focusing on the roles of the duckweed microbiome (Fig. 1). During the 63-day cultivation period, we obtained daily time-series data including duckweed growth rate, wastewater treatment performance, environmental conditions, and microbial community structure. The analysis of the dataset revealed several important causality factors. For instance, abundance of the bacterial family Comamonadaceae was found to have positive causal influence on inorganic nitrogen removal, possibly through its associated denitrification activities. In addition, predicted abundance of bacterial xenobiotic degradation genes were suggested to contribute to the removal of organic pollutants in wastewater,

supporting previous findings based on controlled experiments (Ogata et al., 2013; Kristanti et al., 2014). While this study is still preliminary in terms of cultivation scale and duration, our analysis demonstrates the ability of the time-series analysis to reveal candidate causal relationships in duckweed cultivation environments.

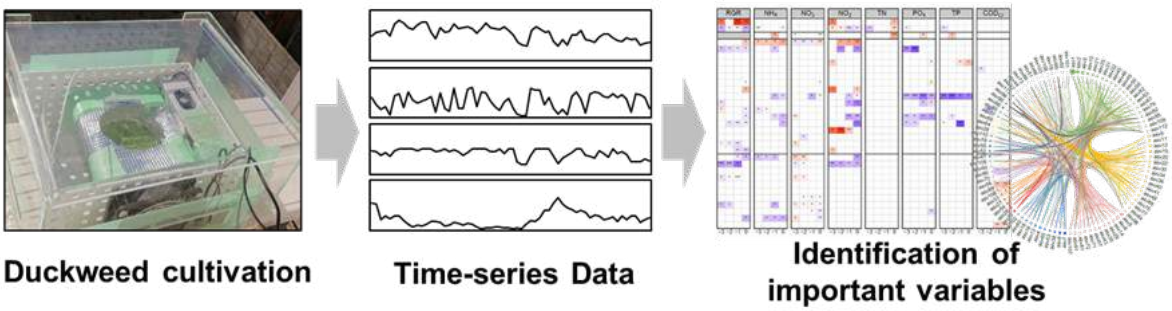

Fig. 1 Schematic of time-series analysis for duckweed cultivation systems

Duckweed demonstrates remarkable productivity under suitable environments, which could reach 149.6 t ha^{-1} yr^{-1} in dry biomass and exceeds all the mainstream crops (Sun et al. 2023b). Key environmental factors (light, temperature, and nutrient composition) significantly impact the individual metabolic processes and population growth dynamics, and their influence is ultimately reflected in the yield of biomass and metabolic products (Sun et al. 2022; Yang et al. 2022; Sun et al. 2023a; Sun et al. 2023b). In view of the rapid clonal growth and the flexible metabolic plasticity of duckweed, it would be interesting to characterize the physiological dynamics both at the level of individual fronds and entire populations through time-series analysis. We hope extended studies like these will help pave the way for more effective and sustainable cultivation strategies.

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8th ICDRA-2026 at Naples, Italy: Announcement

Dear Duckweed Community,

We are pleased to present two exciting updates about the 8th ICDRA – International Conference on Duckweed Research and Applications.

1. Conference dates: 28 September – 2 October 2026
2. Official website launched: https://www.8thicdra.info/

On the website you will already find some details about the venue, the Department of Agriculture, University of Naples Federico II, housed in the historic Royal Palace of Portici, Naples, Italy, as well as a preliminary program outline, travel tips, and information on the natural and cultural treasures awaiting you in Naples and the Campania region.

To fine-tune the organization process, we kindly ask every potential participant to complete the short Google Form in the "Stay Informed" section of the site. Your quick response will also ensure you receive first notice of the official opening of registration and abstract submission.

We are actively seeking partners who wish to engage with and contribute to the global duckweed community. Sponsorship offers prominent branding on conference materials, exhibition space in the main hall, and tailored networking opportunities throughout the meeting. If your company or Institution is interested, please reach out to us; we would be delighted to discuss a package that meets your goals and showcases your commitment to innovation in plant science.

Thank you for your continued enthusiasm and support. We look forward to welcoming you to Naples for a week of inspiring science, collaboration and discovery.

Warm regards,

Organizing Committee

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Unravelling duckweed's potential for phytoextraction of gold and other valuable minerals from mine spoils in Liberia

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Population growth and fast economic expansion are driving a surge in demand for electricity, putting strain on power systems and requiring increased investment in infrastructure and sustainable energy generation (IEA, 2021). According to the Internation Energy Agency (IEA, 2025), global energy demand surged in 2024 to almost twice the annual average of the past decade. This has resulted in a corresponding increase in demand for valuable metals such as gold (Au), platinum (Pt), manganese (Mn), Copper (Cu), Silver (Ag) that are all essential components of wind turbines, electronic appliances, aerospace equipment, solar panels, batteries and other industrial and renewable energy technologies (Akinbile and Mbohwa, 2025).

Industrial exploitation of metal ores requires the use of chemicals for extraction, separation and refining. Uranium, copper and gold require most chemicals for processing, often due to the complex extraction and refining processes. Other metals like nickel, lead and zinc also require significant chemical inputs for processing, but the complexity and chemical intensity are far less than for uranium, copper and gold (Gkika *et al,* 2024). While extraction and processing of many precious metals presents environmental challenges, gold mining generally stands out due to the intensity and scope of its negative impact (Adeeyo *et al,* 2023). This is primarily due to (1) the extensive use of toxic chemicals like cyanide, mercury, copper sulfate, ferrous sulfate, hydrated lime, lead nitrate, thiourea, powder polymer and other chemicals which are employed in gold leaching, (2) the large volumes of waste produced (typically some 5 million m^3 of mine-contaminated water from waste rock dumps and 2-3 million m^3 of tailings wastewater from a tailing storage facility per annum), and (3) the overall high energy consumption of gold extraction (Fashola *et al*, 2016). The huge quantities of wastewater are collected in mine tailing ponds to be treated either by biodegradation, photo-degradation and other natural and/or artificial processes. Through leaching, these tailings can contaminate local water sources and soil, impacting ecosystems and human health (Vaca-Escobar *et al,* 2024). For example, several epidemiological studies (Kamunda *et al*, 2016; Orisakwe *et al*, 2017) have linked contaminants from gold mine tailings with a variety of health impacts including renal failure, bronchial cancer, cardiac dysfunction and hypertension.

The use of different chemicals at varying stages of gold processing presents a complex tailings management challenge. Tailings from the gold leaching process are stored in a large pond called tailings storage facility (TSF). The TSF is designed to allow storage of gold mine tailings and wastewater on the facility and enable controlled release of supernatant into the environment once environmental compliance is met. Typically, the TSF is surrounded by waste rocks or overburdens from ore exploitation and a layer of geo-membrane to reduce the risk of tailings spillage. However, this method of tailings management is not always immune to

failure as evident by published cases of tailing dam collapses and spill events (Van Niekerk and Vijoen, 2005; Glotov *et al*, 2018).

Figure 1: Partial view of an open pit gold mine in Ndablama, Grand Cape Mount County, Liberia

Figure 2: Ore Crushing Plant at New Liberty Gold Mine, Liberia

There is growing concern that the surge in demand for precious metals including gold may drive unsuitable and unsustainable mining practices resulting in resource scarcity, increased environmental footprints and health risks to bio-receptors (Baofo *et al*, 2024). To address this threat, it is crucial to embrace more sustainable approaches to metal recovery. This involves minimizing resource depletion, reducing environmental impacts, and ensuring responsible use of extraction and processing of chemicals. By treating tailing ponds as a resource of valuable metals, and by investing in technologies to recover metals from this end-of-life product, demand for new extractions can be significantly reduced (Kirchherr *et al*, 2023).

Figure 3: Drone imagery of the Tailings Storage Facility (TSF) of New Liberty Gold Mine

One way to recover economically valuable metals, while reducing environmental footprints, is through phytomining. Phytomining relies on the ability of selected plant species to absorb, transport and accumulate metals from low-grade ores, tailings and contaminated soils into aerial parts for economic benefits. While phytomining is not a substitute for conventional mining, it provides an opportunity to recover valuable natural resources that would have otherwise been left to waste. Moreover, this technology is eco-friendly, efficient and cost-effective, while reducing the waste burden from mineral processing (Dinh *et al*, 2022). The biggest challenge to the successful implementation of phytomining lies in finding suitable candidate plant species for accumulation of the desired metal (Akinbile and Mbohwa, 2025). Identifying the right plant species is critical as plants will be exposed to low nutrient levels and high concentrations of metals and processing chemicals.

Finding suitable plants for gold phytoextraction is challenging because gold is naturally insoluble, making it difficult for plants to absorb through their roots (Akinbile and Mbohwa, 2025). Additionally, plants do not naturally hyperaccumulate gold, and even when forced to accumulate the metal, the concentration in plant tissues is often low, hindering the economic viability of phytomining (Anderson *et al,* 2005). This explains why there have been few attempts to find suitable plant candidates for phytomining of gold. Cyanide could hold the key to this mystery! The ability of cyanide to dissolve gold from its ore can be harnessed to modify the environment, making it more conducive for plant uptake of gold. Understanding how cyanide solubilises gold can possibly show how to manipulate soil chemistry to enhance plant uptake of gold. This approach can be

used to reclaim contaminated gold mining sites and/or mine tailing ponds. The biggest obstacle to achieving this is finding a suitable hyperaccumulator of gold that is capable of withstanding varying concentrations of cyanide and other contaminants in goldmine tailings. This is where duckweed comes in!

Duckweed is widely known for its ability to absorb heavy metals (e.g. Ni, Pb, Cd, Cu) and other pollutants. Its effectiveness in phytoremediation stems from its high biomass production, rapid growth, and ability to accumulate a variety of contaminants. There is preliminary evidence suggesting that duckweed is a viable candidate for gold phytoextraction (Hegazy *et al,* 2009; Sasmaz-Kislioglu *et al.,* 2023). A study by Timofeeva *et al* (2020) assessed the potential of several aquatic plants to phytoremediate cyanide contaminated wastewater in Uzbekistan. The results showed high resistance of selected aquatic plants, including star duckweed (*Lemna trisulca*), to concentrations of up to 100 ppm cyanide. Typically, cyanide concentrations in a tailing pond are less than 50ppm as required by the International Cyanide Management code to protect biodiversity. The tolerance of duckweed to moderately high concentrations of cyanide is of substantial scientific interest, as it can enable duckweed to accumulate gold.

The current study, therefore, seeks to bridge the information gap by investigating the potential of duckweed to accumulate gold and other valuable minerals from goldmine tailings in Liberia, a West African country with enormous natural resources including gold. Gold mining presents the greatest mine-related environmental challenge in Liberia, not least because of the proliferation of artisanal and small-scale mining (ASM) activities but also due to the use of large amounts of processing chemicals at industrial gold mines. According to the Stanbic Bank Trade Club, Liberia's gold production increased by 16.4 % year-over-year, reaching an estimated 11.7 metric tons (377,000 ounces) in 2023, and this translates to the presence of very large tailings facilities in the country.

While tailing ponds should theoretically protect the local environment, breaches can occur. For example, between December 2015 and February 2023, over five tailing spill events occurred at the New Liberty Gold Mine (Grand Cape Mount County, Western Liberia) and MNG Gold Mine (Bong County, North Central Liberia) with varying degrees of impact on human health, biodiversity and the environment. The reported tailings spill events present a reminder of the need to employ appropriate tailings and mine wastewater management measures to reduce the impact of gold mining on water resources and biodiversity. Conversely, the large volumes of gold-containing wastewater present an opportunity for waste valorisation using local duckweed species.

Thus, the current project proposes duckweeds as candidates for two important tasks:

- *Phytoextraction of environmental contaminants from tailings storage facilities:* this will involve the use of duckweed to facilitate the removal of chemical contaminants resulting in the remediation of wastewater from gold processing. Cyanide recovered from the wastewater and tailings could be reintroduced in the gold leaching process, presenting a potential economic benefit to the mining companies and better environment and public health outcomes to downstream communities.

- *Phytomining of valuable minerals:* this will involve the extraction of valuable metals including gold, copper and arsenic; presenting an opportunity to valorise the extracted minerals. A typical tailings pond will contain about 10 million m^3 of wastewater with an average gold concentration of 0.96 ppm. Cyanide is linked to gold accumulation and could potentially accelerate gold absorption and uptake in plants.

The current study is conducted using gold mine tailings from two tailing storage facilities in Liberia: New Liberty Gold Mine TSF in Kinjor and the MNG Gold Mine TSF in Kokoya. The New Liberty Gold Mine is in Kinjor, Grand Cape Mount County, approximately 100 km northwest of the Liberian capital, Monrovia. The MNG Gold Mine is in Kokoya, Bong County, approximately 167 km northeast from Monrovia.

A unique aspect of the ongoing study is that success will benefit both local communities through water remediation, and mining companies through additional income. With the price of gold at a record high and continuing to surge, success in recovering gold from mine tailings, even at a low recovery rate, would present an economic venture worth considering in pursuit of gold and other valuable minerals.

Figure 4: Drone imagery of the southern embankment of the Tailing Storage Facility (TSF) at New Liberty Gold Mine, Liberia

Figure 5: Drone imagery of the northern embankment of the Tailing Storage Facility (TSF) at New Liberty Gold Mine, Liberia.

Figure 6: Drone imagery of the western embankment of the Tailing Storage Facility (TSF) at New Liberty Gold Mine, Liberia.

Figure 7: An image of a channel which conveys gold mine effluents from the TSF into the environment

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Demonstration of Duckweed Based Wastewater Treatment System set-up on University Campus in Thailand

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The field excursion for participants of the 7th ICDRA held in Bangkok was arranged to visit the demonstration unit of duckweed-based wastewater treatment system set-up at the central wastewater treatment facility of Kasetsart University in Bangkok, Thailand (Figure 1).

Figure 1: Participants of the 7th ICDRA visited the demonstration site

This demonstration set-up is part of the Japan International Cooperation Agency (JICA) supported research project on "The Development of the Duckweed Holobiont Resource Values Towards Thailand Bio-Circular-Green (BCG) Economy"[1]. Holobiont is an assemblage of host and its associated microorganisms. The study investigated the use of specialized duckweed holobiont for wastewater treatment and biomass production at the central wastewater treatment plant of Kasetsart University. The investigation focused on organic removals and nutrient (N and P) conversion into duckweed biomass from low-strength campus wastewater. Two experimental units with plug-flow type flow patterns were set up to compare the performance of a duckweed-based treatment system (Figure 2) with a control system without the presence of duckweed. Each experimental unit has a working volume of 200 L. It was fed with inflow wastewater pumped from a pump sump after the primary screen unit at a constant flow rate of 50 L/day thus yielding a hydraulic retention time of 4 days in the treatment unit. In the duckweed treatment unit, *Spirodela* sp. duckweed (Figure 3) was obtained from the local water source and acclimatized to the campus wastewater, which was initially provided at 100% of the water surface. The

transition of the microbiome before and after the acclimatization to the wastewater was investigated. This specialized duckweed holobiont was allowed to grow naturally while the duckweed biomass was harvested regularly twice a week to its original condition (100% coverage). The treatment performance of both experimental units was examined through sampling and analysis of influent and effluent qualities on the same days as duckweed biomass harvesting.

During short-term monitoring of the experimental unit operation of 3 months, it was found that the duckweed system demonstrated improvement in organic carbon and nutrient removals from the wastewater compared to the control unit. The gradual improvement of organic and nutrient removals was also found associated with an increase in duckweed biomass production. The enhancement of the treatment capability of duckweed was associated with the development of duckweed holobiont as they became acclimatized to degrade organic substances in the wastewater while promoting the nutrient uptake capacities of duckweed. Moreover, there was a clear indication of a reduction in the accumulation of settleable solids in the duckweed unit. These preliminary results demonstrate the beneficial effect of duckweed holobiont in contributing to an improvement of wastewater treatment performance and highlighting its potential for environmental applications. The duckweed wastewater treatment system can serve as a sustainable and environmentally friendly alternative for wastewater management[2]. The development of duckweed holobiont which was well acclimatized to the targeted wastewater not only improved wastewater purification performance but also increased production of duckweed biomass. The produced duckweed biomass can be utilized for other beneficial purposes, such as serving as feedstock for bioenergy production[3].

Figure 2: Demonstration unit with duckweed.


Figure 3: *Spirodela* sp. duckweed cultivated in wastewater.

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Duckweeds: Nature's power leafy greens for health and longevity

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ABSTRACT: The global burden of phytonutrient deficiency demands innovative, natural solutions capable of supporting healthspan and longevity. Among all plant foods, leafy greens stand out as fundamental to human health, with strong clinical evidence linking their consumption to the prevention of chronic diseases. Within this category, duckweed family in general, and selected species specifically, emerge as a uniquely potent, highly bioavailable, and sustainable food source. This article reviews the global nutrition gap, the essential role of leafy greens, and highlights duckweed's extraordinary nutritional profile, clinical health benefits, and future potential health benefits.

LEAFY GREEN VEGETABLES: THEIR ESSENTIAL ROLE IN HEALTHSPAN AND DISEASE PREVENTION

The Global Pandemic of Phytonutrient Deficiency: Phytonutrients—including vitamins, minerals, antioxidants, and many other bioactive plant ("phyto") micro-nutrients, are crucial for maintaining metabolic balance, immune function, and cognitive health. Despite widespread awareness, a significant portion of the global population fails to meet the recommended daily intake of key micronutrients — a gap that persists regardless of a country's income level. Often termed "hidden hunger", Phytonutrient Deficiency is now recognized by the World Health Organization (WHO) as a global pandemic, affecting billions of people worldwide - 25–50% of the global population[1,2] , and nearly 50% of children under five years old[3], even in developed countries such as the United States and across the European Union[4,5,6]. These deficiencies are directly associated with increased risks of cancer, cardiovascular disease, diabetes, cognitive decline, and early mortality[7]. Diets high in processed foods and low in fresh, whole plant foods are major contributors to this widespread issue[8]. Addressing phytonutrient deficiency is therefore a critical step in combating non-communicable diseases on a global scale.

Traditional Diets Offer a Blueprint for Healthy Longevity: Extensive demographic and nutritional research[9], including studies on the Okinawan, Mediterranean and Nordic diets[10.11] ,—demonstrates that populations consuming predominantly fresh, whole-plant-based diets experience lower rates of chronic diseases such as heart disease, cancer, and dementia, along with higher life expectancy and prolonged disease-free longevity[12,13]. These dietary patterns emphasize the importance of plant-based nutrition, along with regular physical activity, and strong social cohesion as foundational pillars of healthy aging. Rich in anti-inflammatory compounds, chlorophylls, and a diverse array of antioxidants and phytochemicals, these diets are also low in calories and marked by minimal consumption of processed foods. Recent clinical studies validate the health benefit of Okinawan-based Nordic diet demonstrated the correlation between food, gut microbiota, metabolism (e.g. improved glucose and lipid metabolism), weight management, and inflammation[14,15].

Clinical Evidence: Daily Intake of Fresh Fruits and Vegetables prevent chronic diseases and premature death: Multiple cohort and meta-analyses confirm that increasing fruit and vegetable intake—especially fresh greens —is associated with decreased risk of, cancer, cardiovascular disease, type 2 diabetes, obesity, and cognitive decline[16,17,18,19]. A landmark study of over 65,000 adults in England, conducted over 7.7 years, identified vegetable consumption as the strongest dietary predictor of reduced mortality risk[20]. Recent research also demonstrates the impact of anti-inflammatory, plant-rich diets on mental health and neurodegenerative disease prevention, mediated through gut microbiota modulation and oxidative stress reduction[21]. According to the WHO and the Food and Agriculture Organization (FAO), low intake of fruit and vegetables is among the top 10 risk factors for mortality in the world[22,23], yet only 1 in 10 adults get enough fruits or vegetables[24]. They established clear recommendations for daily consumption: at least 400g of total fruit and vegetables among

which at least 50-80 g leafy greens, and excluding potatoes, sweet potatoes, cassava and other starchy roots[25,26,27].

Greens as Our First Line of Defense: The Gut-Health Connection: Leafy greens are among the richest natural sources of phytonutrients per calorie, providing among other essential vitamins, minerals, fibers, antioxidants, and anti-inflammatory compounds. Regular consumption of these vegetables is an underutilized yet powerful strategy for preventing chronic diseases and maintaining metabolic and immune health. In the past years, breakthrough studies (Nature Chemical Biology and Nature Immunology) has expanded our understanding of why a daily intake of fresh greens is critical[28,29,30], revealing that greens are not merely a source of vitamins and minerals, but contain specific active molecules that directly program gut-associated immune cells, promoting anti-inflammatory pathways, nourish the gut microbiota, thus may regulating the gut-brain axis, and reducing risks for diseases ranging from metabolic syndrome to depression and dementia.

The human gut harbors a complex community of trillions of microorganisms, collectively called the gut microbiota, that plays a central role in maintaining health. Increasingly referred to as the "second immune system", the gut microbiome helps regulate digestion, metabolism, immune function, and even brain health[31,32]. Disruptions to the gut microbiome, a condition known as dysbiosis, have been directly linked to the development and progression of chronic diseases, including inflammatory bowel disease (IBD), obesity, type 2 diabetes, cardiovascular disease, depression, and even certain cancers[33]. Thus, the gut microbiome acts as a critical interface between diet and systemic health[34].

Fresh leafy greens uniquely nourish the gut microbiota. They provide complex fibers, polyphenols, chlorophylls, and flavonoids that serve as prebiotic substrates, promoting the growth of beneficial microbes[35,36] and enhancing the production of short-chain fatty acids like butyrate, which strengthens gut barrier integrity and modulates systemic inflammation[37]. Moreover, chlorophyll and its derivatives have been shown to contribute to detoxification processes and reduce gut inflammation[38], while polyphenols in greens act synergistically with fibers to reshape the microbiota composition favorably. Notably, the unique structural properties of fresh, minimally processed greens preserve their molecular complexity, maximizing their gut-supportive and health-promoting effects.

Nature Designed It Right — But Our Food System Breaks It: Minimally processed, fresh whole foods retain the a nonrandom, evolutionarily orchestrated, full spectrum of phytonutrients and bioactive compounds that designed to work in a complexed synergy to support human health. Industrial food processing, by contrast, strips foods of their natural protective molecules, reducing their nutritional value and disrupting food synergy[39] — the natural interplay of nutrients and bioactives that enhances absorption and effectiveness, emphasizing that the health benefits of whole foods far exceed the sum of their isolated components[40], and often depend on their food matrix in complex and highly interactive ways[41,42].

The NOVA food classification system[43], endorsed by the WHO, categorizes foods based on the extent and purpose of industrial processing, highlights the importance of unprocessed whole-food consumption, and underscores that diets high in ultra-processed foods are strongly linked to increased risks of obesity, diabetes, cardiovascular disease, and early mortality[44]. Whole foods, particularly fresh vegetables, provide complex, synergistic support for human physiology that isolated nutrients or supplements cannot replicate.

The Gap: Most Consumers Want It — But Don't Get It MASSIVE UNMET NEED –Fresh Wholesome Nutrition at the Convenience, Fun, & Affordability of Industrial Food. Mass consumers are well-educated and crave fresh, wholesome food - "55% of U.S. consumers are willing to pay 30% more than their annual grocery budget for fresh food products that are clean-labeled and contain functional ingredients"[45,46]. This shift reflects a growing awareness of the severe impact fresh phytonutrient deficiency has on health. Yet modern food systems fail to deliver these fresh foods efficiently. Fragile shelf life, contamination risks[47], and supply chain degradation result in nutrient loss before consumption, particularly for leafy greens — the very foods most essential for health.

DUCKWEEDS: THE SOLUTION TO A PRESSING HEALTH AND NUTRITION GAP

Among the most promising candidates to address the urgent need for natural wholesome dietary solutions that can enhance healthspan and longevity is the duckweed family (**Lemnaceae**), the smallest flowering plants on Earth. Duckweed comprises five genera (**Spirodela**, **Landoltia**, **Lemna**, **Wolffiella**, and **Wolffia**) and 36 recognized species, and are distributed globally across diverse aquatic environments[48].

The Ideal Leafy Greens: Fresh, wholesome, and packed with phytonutrients, Wolffia and other Duckweed species are leafy greens that offer an extraordinary nutritional profile. They are rich in flavonoids, polyphenols, carotenoids, chlorophylls, and other antioxidants known to counteract oxidative stress, a key driver of aging and chronic diseases[49,50,51]. Furthermore, duckweed provides complex fibers and polysaccharides that function as prebiotic substrates, promoting the growth of beneficial gut microbiota, strengthening gut barrier function,

and enhancing systemic anti-inflammatory responses[52]. Thus, the unique and rich phytochemical composition of duckweed can support healthy longevity through multiple biological pathways, including the reduction of chronic inflammation, support of detoxification processes, modulation of the gut microbiome, and mitigation of oxidative damage and cellular senescence.

Exceptional Nutrient Density: The Good Without The Bad: Nutrient density can be assessed using various indices and scoring systems, such as the Nutrient Rich Foods (NRF) index, which considers both the presence of beneficial nutrients and the absence of less desirable components like sodium, sugars and saturated fats[53]. Calculating a food's nutrient density involves determining the nutrient content and their % DV (Recommended Daily Value). DVs are the recommended amounts of nutrients to consume or not to exceed each day, and the % DV indicates how much a nutrient in a single serving contributes to your daily diet[54,55]. A well-established scoring system for assessing nutrient-rich foods is the NRF9.3 score[56], which is based on 9 nutrients to encourage (protein, fiber, vitamins A, C, D, calcium, iron, potassium, and magnesium) vs. 3 nutrients to limit (saturated fat, added sugar, and sodium) (figure 1, right). We have further analyzed an extended score based on 17 essential nutrients to encourage (including protein, fiber, vitamins A, C, D, E, K, B2, B6, minerals calcium, iron, potassium, magnesium, phosphorus, zinc, and essential fatty acids omega-3 and omega-6) versus the 3 nutrients to limit (figure 1, left). *Wolffia* offers a superior phytonutrient-to-calorie ratio, supporting dense nutrition without unwanted additives.

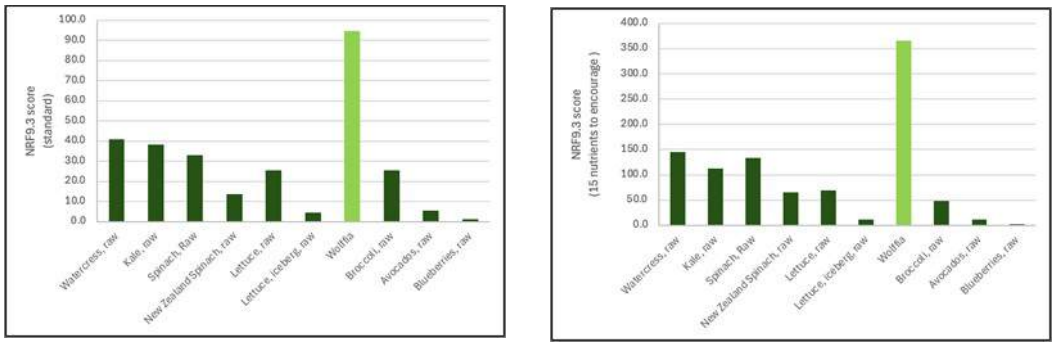

Figure 1: Using the well-established NRF9.3 scoring system (righ panel) and an extended NRF9.3 (left panel) for assessing fresh Wolffia (*Wolffia ahrriza* and *Wolffia globosa*) nutrient density as compared to other leafy green vegetables representing the category and other nutrient-rich foods

High Mineral Bioavailability - Low Oxalic Acid: A further advantage is *Wolffia* species extremely low oxalic acid content. Oxalic acid is an organic compound found in many plants, including vegetables, fruits, cocoa, nuts and seeds. In leafy greens, members of the spinach family and the brassicas are high in oxalates. Oxalic acid in plants is usually bound to minerals, forming oxalate, thus, high Oxalic acid foods have been known to exert a negative effect on calcium and iron absorption. For example, calcium availability from spinach, a high oxalate and high calcium food, is low at 5.1%[57].Calcium oxalate is also the most common component of kidney stones, which may lead to the development of chronic kidney disease. A daily intake of 180 mg oxalic acid has been suggested as a critical value for renal stone formation, thus as a maximum recommended daily amount[58].

Duckweeds have been studied for oxalic acid metabolism, concentration and calcium oxalate formation[59,60,61]. It has been found that the concentration of the chemical and physical form of oxalate differs in the various duckweeds. While *Spirodela* and *Lemna* genera can contained large amounts of oxalic acid, accumulating calcium oxalate crystals, *Wolffia* and *Wolffiella* genera contain very low levels of oxalic acid, much lower than any other food component, and do not exhibit calcium oxalate crystals[62,63,64]. As shown in figure 2, while many leafy greens, such as spinach and brassicas, are rich in

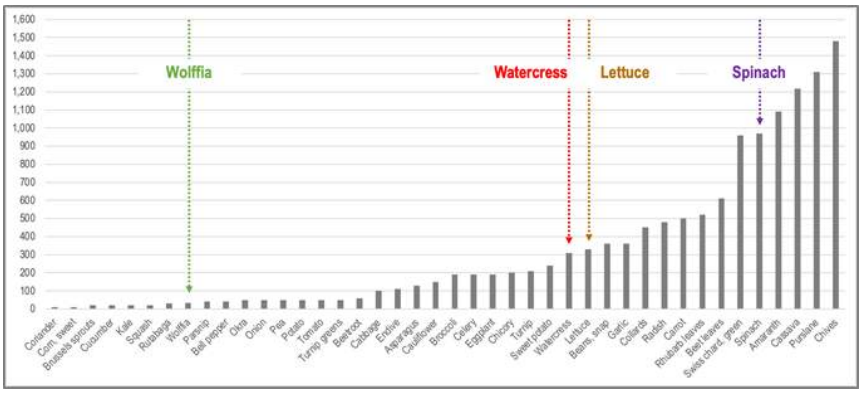

Figure 2: Raw Wolffia - among the vegetables with the lowest Oxalic acid

oxalates, ***Wolffia*** species exhibit minimal oxalate levels. This enhances the bioavailability of calcium and iron, while enabling higher safe daily intake of fresh greens.

Modern clinical research has begun to validate duckweed's health-promoting potential: Studies focusing on the nutritional impact of incorporating ***Wolffia globosa*** into human diets have demonstrated significant improvements in protein and mineral intake, reductions in visceral adiposity[65], and weight loss[66], decreased risk of fatty liver disease[67,68], and prevention of cardiometabolic diseases through modulation of the gut microbiome[69]. Additional findings suggest potential benefits for brain health, with a reduced risk of age-related brain atrophy[70]— conditions often linked to neurodegeneration, seizures, aphasia, and dementia. These health outcomes can be mediated by duckweed's rich content of vitamin K, antioxidants, fibers, nitrates, and carotenoids. Overall health and other metabolic health parameters such as blood sugar levels, cholesterol levels, and other markers of cardiovascular health, have been also associated with the consumption of diet reach in duckweed plants (*Wolffia globosa*)[71].

Safety Assessment and Validation: In addition to its impressive biochemical properties, Duckweeds, particularly *Wolffia* species, have been known as a safe traditional food in different parts of the world. In regions such as Thailand, Laos, and Vietnam, *Wolffia* is harvested from natural freshwater bodies and used as a fresh vegetable. It is typically eaten in soups, salads, or stir-fried dishes, involve minimal processing — usually just rinsing to remove debris before consumption. Its role in local diets highlights its historical significance as a safe nutritious, readily available, and culturally accepted green food. Modern cytotoxicity studies[72,73]and early clinical trials[74], have demonstrated that specific duckweed species, particularly ***Wolffia*** species and ***Lemna*** species, are safe for human consumption when properly cultivated and handeled[75,76].

Considerations for Safe and Effective Use: While promising, duckweed's use as food necessitates species-specific evaluation and consideration. Nutritional composition and safety can vary significantly depending on selected species, growth conditions, harvesting times, and post-harvest handling. In addition, although, Duckweed's simple cellular structure may facilitates highly efficient digestion and nutrient absorption, comprehensive studies on the digestibility, bio-accessibility and bioavailability of duckweed nutrients in humans remains need to be further performed[77]. Moreover, understanding how processing methods - such as drying, freezing and cooking - affect the stability, accessibility, and nutritional quality of duckweed's bioactive components is critical for optimizing its functional food potential.

CONCLUSION

Leafy greens are foundational to human health, but modern diets, food systems, and processing practices have eroded access to their benefits. Duckweed species represent an unparalleled opportunity to restore this nutritional power in a scalable, sustainable, and scientifically validated form. Combining extraordinary nutrient density, bioavailability, minimal anti-nutrients, traditional safety record, and emerging clinical validation, duckweed stands poised to become it as a next-generation wholesome food capable of closing the global phytonutrient gap and supporting global health and longevity initiatives.

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From the Database

Highlights

Duckweed genomes and epigenomes underlie triploid hybridization and clonal reproduction

Ernst, E; Abramson, B; Acosta, K; Hoang, PTN; Mateo-Elizalde, C; Schubert, V; Pasaribu, B; Albert, PS; Hartwick, N; Colt, K; Aylward, A; Ramu, U; Birchler, JA; Schubert, I; Lam, E; Michael, TP; Martienssen, RA. Current Biology (2025) DOI10.1016/j.cub.2025.03.013.

The Lemnaceae (duckweeds) are the world's smallest but fastest-growing flowering plants. Prolific clonal propagation facilitates continuous micro-cropping for plant-based protein and starch production and holds tremendous promise for sequestration of atmospheric CO_2. Here, we present chromosomal assemblies, annotations, and phylogenomic analysis of *Lemna* genomes that uncover candidate genes responsible for the unique metabolic and developmental traits of the family, such as anatomical reduction, adaxial stomata, lack of stomatal closure, and carbon sequestration via crystalline calcium oxalate. Lemnaceae have selectively lost genes required for RNA interference, including Argonaute genes required for reproductive isolation (the triploid block) and haploid gamete formation. Triploid hybrids arise commonly among *Lemna*, and we have found mutations in highly conserved meiotic crossover genes that could support polyploid meiosis. Further, mapping centromeres by chromatin immunoprecipitation suggests their epigenetic origin despite divergence of underlying tandem repeats and centromeric retrotransposons. Syntenic comparisons with *Wolffia* and *Spirodela* reveal that diversification of these genera coincided with the "Azolla event" in the mid-Eocene, during which aquatic macrophytes reduced high atmospheric CO_2 levels to those of the current ice age. Facile regeneration of transgenic fronds from tissue culture, aided by reduced epigenetic silencing, makes *Lemna* a powerful biotechnological platform, as exemplified by recent engineering of high-oil Lemna that outperforms oil-seed crops.

Duckweed: a starch-hyperaccumulating plant under cultivation with a combination of nutrient limitation and elevated CO_2

Guo, L; Fang, Y; Wang, SH; Xiao, Y; Ding, YQ; Jin, YL; Tian, XP; Du, AP; Liao, ZH; He, KZ; Chen, S; Zhao, YG; Tan, L; Yi, ZL; Che, YQ; Chen, LC; Li, JM; Zhao, LY; Zhang, P; Gu, ZB; Zhang, FY; Hong, Y; Zhang, Q; Zhao, H. Frontiers in Plant Science (2025) 16: 1531849.

The increasing global demand for starch has created an urgent need to identify more efficient and sustainable production methods. However, traditional starch sources, such as crop-based options, experience significant bottlenecks due to limitations in land use, water consumption, and the impacts of climate change. Therefore, there is a pressing need to explore and develop new sources of starch. We develop a novel duckweed cultivation technology that combines nutrients limitation and CO_2 supplementation to achieve very high starch content. In this study, we integrated whole-genome sequencing, epigenomics, transcriptomics, enzyme activity, and composition variation to elucidate the mechanisms of efficient starch accumulation in duckweed in terms of starch accumulation and carbon partitioning, regulation of the expression of genes in the starch metabolic pathway, and sucrose biosynthesis and transportation. Although *Landoltia punctata* exhibits dramatic gene family contraction, its starch content and productivity reached 72.2% (dry basis) and 10.4 g m^{-2} d^{-1}, respectively, in 10 days, equivalent to a yield of 38.0 t ha^{-1} y^{-1}, under nutrient limitation treatment with elevated CO_2 levels. We also examined the mechanism of high starch accumulation in duckweed. This phenomenon is associated with the regulation of DNA methylation and transcription factors as well as the significantly upregulated transcription levels and the increased activities of key enzymes involved in starch biosynthesis. Moreover, while nitrogen redistribution was increased, sucrose biosynthesis and transportation and lignocellulose biosynthesis were reduced. These alterations led to a reduction in lignocellulose and protein contents and ultimately an increase in the accumulation of starch in the chloroplasts. This work demonstrates the potential of duckweed as a highly efficient starch producer.

Aquaculture/ Agriculture

Encapsulated fronds technology modified for long-term preservation of *Lemna aequinoctialis*

Phuong, HTN; Phung, TQ. Plant Cell Tissue and Organ Culture (2025) 161: 18.

Duckweeds, the Lemnaceae family, are employed in a wide range of industries, including agriculture, animal feed, wastewater treatment, biofuel production, and human food. Duckweeds are also of interest for researchers. However, due to their rapid growth, development, and susceptibility to contamination by bacteria and algae during the storage process, duckweed samples require a great deal of time and effort to be maintained. In order to overcome the difficulties in preserving duckweed samples for scientific research, we developed an approach for duckweed storage by modification of the 'artificial seed' procedure. We found that encapsulation of entire fronds of *Lemna aequinoctialis* in Na-Alginate (3, 4, and 5%) and calcium chloride (150, 200, and 250 mM) were appropriate for maintaining them in a metabolically reduced state. After being stored for two months, there were no obvious variations between artificial seeds having an endosperm composition of 1/2 "N" medium, "N", and water. After two months, duckweed samples kept in seeds with "N" endosperm medium containing 150 mM $CaCl_2$ and 3% sodium alginate recovered the best. Following a year of storage, a noticeable difference could be seen between the treatments with various endosperm medium compositions. The optimal conditions were encapsulation in an aqueous medium containing 200 mM $CaCl_2$ and 3% sodium alginate. This procedure offers an effective, cost-saving long-term preservation strategy that may be adapted in future for further duckweed species.

From waste to resource: Use of *Lemna minor* L. as unconventional fertilizer for lettuce (*Lactuca sativa* L.)

Baldi, A; Verdi, L; Piacenti, L; Lenzi, A. Horticulturae (2025) 11: 20

Duckweeds, such as *Lemna minor* L., are invasive aquatic species that can proliferate on the surface of the nutrient solution in hydroponic systems, requiring removal operations from the cultivation tanks and disposal as waste. Several studies have demonstrated the potential use of duckweeds as an organic fertilizer. Recycling plant waste as a nutrient source for crops may be a circular approach to enhancing the sustainability of intensive horticultural production systems. Two pot experiments were carried out to evaluate the possibility of using the biomass of *Lemna* as a fertilizer for lettuce. The following fertilization treatments were applied: Control (no fertilization), *Lemna* biomass (60, 120, and 180 kg ha^{-1} nitrogen), urea (60 kg ha^{-1} nitrogen), and commercial organic fertilizer (60 kg ha^{-1} nitrogen). Lettuce head diameter, fresh and dry weight, the number of leaves, and the contents of minerals, nitrates, chlorophyll and carotenoids were determined. In addition, nitrogen use efficiency was calculated. Fertilization with *Lemna* resulted in a significant increase in yield compared to control (+50% considering the average of the three *Lemna* doses) and both inorganic (+65%) and organic (+71%) fertilization treatments. No differences in yield and quality were observed between the three doses of *Lemna*, but the lowest one was the treatment with the best performance in terms of N productivity. These results suggest that *Lemna* biomass may be a proper source of nutrients for lettuce with advantages for yield and no effect on quality. Therefore, its use as an alternative to commercial fertilizers can allow farmers to profitably exploit a waste product and, at the same time, reduce the costs for fertilization, thus achieving environmental and economic benefits.

Flusulfinam, a novel amide herbicide to control weed in rice fields, targets 4-hydroxyphenylpyruvate dioxygenase

Wang, HZ; Sun, H; Yu, S; Lian, L; Jin, T; Peng, XG; Wang, JX; Liu, WT. Pesticide Biochemistry and Physiology (2025) 208: 106240.

Flusulfinam, a post-emergence (POST)-applied herbicide in rice fields to manage annual weeds, has been proven to be safe for various rice strains of japonica and indica. The study confirmed its mechanism of action by inhibiting 4-hydroxyphenylpyruvate dioxygenase (HPPD). The POST application of flusulfinam led to noticeably bleaching symptom in leaves of *Echinochloa crus-galli* within 3 to 7 days and plant mortality by 10 days. In *Echinochloa crus-galli*, flusulfinam induced a significant rise in level of phytoene content, while there was a significant decrease in levels of carotenoid and chlorophyll contents. Meanwhile, the increase in levels of chlorophyll content in *Spirodela polyrhiza* treated with flusulfinam after homogentisic acid (HGA) addition was

observed, suggesting that flusulfinam inhibited HGA production, likely by suppressing HPPD activity. Flusulfinam significantly diminished the catalytic activity of recombinant *Arabidopsis thaliana* HPPD that was expressed by *Escherichia coli*, exhibiting an inhibitory efficacy approximately 16-fold higher than the HPPD-inhibiting mesotrione. Additionally, the rice with overexpression of *Oryza sativa* HPPD showed higher tolerance to flusulfinam than rice of wild type. Furthermore, molecular docking analyses revealed that flusulfinam formed effective bonds with the HPPD active site via the nitrogen atom of the oxadiazole ring and the oxygen atom of the amide group, with distances of 2.0 & Aring; and 2.4 & Aring, respectively, which engaged in bidentate coordination with the Fe^{2+} ion, with a binding energy of-8.7 kcal mol^{-1}, and HPPD-flusulfinam complex showed low root-mean square deviation values of less than 2 & Aring in molecular dynamics tests. This study provides the first evidence of the molecular targets of flusulfinam.

Biochemistry

A preharvest finishing procedure for *Lemna* to produce high levels of zeaxanthin that is retained post-high-light exposure

López-Pozo, M; Adams, WW III; Mcnamara, M; Demmig-Adams, B. Future Foods (2024) 10: 100517.

Development of a productive, nutritious, and low-input food source is needed for humanity's expanding population on Earth and for long-duration crewed space missions. The exceptionally protein- and micronutrient-rich members of the aquatic plant family Lemnaceae are uniquely suited for both purposes. We here introduce a protocol to accumulate large quantities of the carotenoid zeaxanthin that are retained post-high-light exposure. The function of the essential human micronutrients zeaxanthin, lutein, vitamin E (alpha-tocopherol), and pro-vitamin A (beta-carotene) are briefly reviewed. Attention is given to the role of zeaxanthin in opposing oxidants (reactive oxygen species), the production of which is further enhanced by space radiation. We report a new pre-harvest finishing procedure - sudden transfer of *Lemna* plants grown in low light (200 µmol photons m^{-2} s^{-1}) to high light (1000 µmol photons m^{-2} s^{-1}) for six hours, which rapidly increased and stabilized zeaxanthin content. Unlike terrestrial crops, *Lemna* possessed a uniquely high protein content when grown in low light and did not experience any adverse effects of the abrupt five-fold increase in light intensity. These findings provide additional justification for Lemnaceae as food crops with applications on Earth as well as for future long-duration human space missions.

Biotechnology

Synergistic effect of nutrient starvation and abscisic acid on growth and starch accumulation in duckweed

Cao, Q; Zhang, YQ; Tan, AJ; Li, Z; Yang, GL. Planta (2025) 261: 108.

Nutrient starvation and ABA promote starch accumulation in duckweed fronds and turions, enhancing ADP-glucose pyrophosphorylase activity and starch grain size. Duckweed has excellent development potential due to its fast growth, high starch content, and low cellulose content. Nutrient starvation or abscisic acid (ABA) are known to affect growth and starch accumulation in duckweed. However, the joint effects of nutrient starvation and ABA on growth and starch accumulation in duckweed remain unknown. This study analyzed the response of duckweed (*Spirodela polyrhiza*) to nutrient starvation and ABA and the synergistic treatment of the two to examine the growth and starch accumulation of duckweed. Nutrient starvation and ABA can promote starch accumulation in both the frond and turion of duckweed as a result of the key enzyme ADP-glucose pyrophosphorylase activity that was significantly increased in the starch synthesis pathway of duckweed. Nutrient starvation and ABA can induce the formation of turions with high starch content, which reached up to 38.3 ±1.9% (dry weight). Further studies on the ultrastructure of the frond and dormant cell of duckweed found that nutrient starvation and ABA increased the number and size of starch grains in duckweed fronds and dormant cells. These results will provide a theoretical basis for duckweed and its turions as a potential renewable energy crop.

Duckweeds for plant molecular farming: Advances, challenges, and future directions

Kang, J; Kim, K; Do, THT; Han, M; Lee, Y. Journal of Plant Biology (2025) DOI10.1007/s12374-025-09458-8.

Plant molecular farming (PMF), or "pharming," leverages plant cells or whole plants as expression systems to produce recombinant proteins for pharmaceuticals and other applications. This approach has emerged as a viable alternative to traditional platforms like *Escherichia coli* and mammalian cell lines, offering distinct advantages such as low production costs, high protein stability, and human-like post-translational modifications. However, the reliance on terrestrial plants as bioreactors poses challenges, including competition with food crops for agricultural resources and the risk of contaminating the food supply. As a result, identifying new host platforms for efficient recombinant protein production is a critical priority for advancing PMF. In this review, we highlight duckweeds -small, fast-growing aquatic monocots in the family Lemnaceae- as a promising alternative. Duckweeds offer advantages such as rapid growth, high biomass yield, and a rich metabolic profile, making them an attractive platform for recombinant protein production. We summarize recent developments in the use of duckweeds for PMF, including advancements in tissue culture, transformation techniques, and the expanding availability of genetic resources. Finally, we discuss remaining challenges and propose future directions for establishing duckweeds as a robust host platform in synthetic biology.

Machine learning-driven discovery of bioactive peptides from duckweed (Lemnaceae) protein hydrolysates: Identification and experimental validation of 20 novel antihypertensive, antidiabetic, and/or antioxidant peptides

Cournoyer, A; Bernier, ME; Aboubacar, H; de Toro-Martin, J; Vohl, MC; Ravallec, R; Cudennec, B; Bazinet, L. Food Chemistry (2025) 482: 144029.

Duckweed, a sustainable, protein-rich aquatic plant, has recently emerged as a promising source of bioactive peptides. However, their identification remains limited and challenging in such complex mixtures. Following duckweed hydrolysis with pepsin, chymotrypsin, trypsin and papain, and a centrifugation step producing two fractions: supernatant (DS) and pellet (DP), interesting half-maximal inhibitory concentration (IC50) for dipeptidyl peptidase (DPP)-IV and angiotensin-converting enzyme (ACE) inhibition were obtained for DS fractions, especially with pepsin (IC50=0.7 and 0.07mg/ mL, respectively). Using partial least squares-discriminant analysis (PLS-DA) combined with quantitative structure-activity relationship (QSAR) models, five new DPP-IV inhibitors (most active: API, IC50=126.88 μM), eleven new ACE inhibitors (most active: FAR, IC50=13.54 μM) and four new antioxidants (>200 μM) were identified. Two sequences were active across all three tested bioactivities, revealing promising multi-target peptides. These findings highlight the potential of duckweed-derived peptides to support health and metabolic balance.

Plastidic glutamine synthetase (GS2) enhances nitrogen assimilation and protein production in duckweed using urea nitrogen source

Hu, ZB; Chen, S; Wei, CC; Jin, YL; Zhao, LY; Liu, YQ; Gao, Y; Fang, Y; Zhang, Y; Yi, ZL; Wang, SH; Zhao, H. International Journal of Biological Macromolecules (2025) 307: 141701.

Despite urea being the most widely used nitrogen fertilizer, many plants show reduced growth and protein content when urea is the nitrogen source. The underlying causes of nitrogen metabolism imbalance in urea supply remain unclear. In this study, we discovered that plastidic glutamine synthetase (GS2) can improve the physiological traits and protein production of duckweed cultivated with urea. By comparing the urea utilization processes between two duckweed strains, LpZH0104 and Lp9595, which exhibited marked differences under the same urea treatment, we found that GS2 significantly impacts nitrogen metabolism. Overexpressing the GS2 gene in an efficient genetic transformation system of duckweed enhanced nitrogen-carbon metabolism, leading to a 10 % increase in biomass and a 24 % increase in protein content in the overexpression lines (OE lines). Protein productivity and PPPN (protein production per unit of applied fertilizer N) increased by 47 % and 32 %, respectively. Conversely, silencing the GS2 gene reduced these traits. Additionally, transforming GS2 gene into rice, tobacco, and *Arabidopsis* also enhanced their biomass and protein content under urea cultivation conditions. This study highlights GS2's role in enhancing nitrogen metabolism and physiological

traits in plants cultivated with urea, offering valuable insights for improving biomass and protein production in crops using urea fertilizer.

Duckweed extract-mediated green synthesis of ZnO nanoparticles and its antibacterial, antioxidant, and photocatalytic properties

Shukla, YK; Pandey, P; Pandey, JP; Shukla, A; Kumar, J. Biomass Conversation and Biorefinery (2025) DOI10.1007/s13399-025-06706-2.

An environmentally benign approach was developed where duckweed extracts were employed to synthesize zinc oxide nanoparticles (ZnO NPs). The synthesized ZnO NPs were characterized by UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy dispersive X-ray spectroscopy (EDS). The XRD analysis showed that ZnO NPs adopt hexagonal wurtzite crystal packing with crystallite size within nanometer range. The UV-visible spectrum confirmed the characteristic absorption peak for ZnO NPs at around 378 nm. FTIR analysis revealed the presence of various functional groups present on the synthesized nanomaterial. Electron microscope analysis showed that the synthesized ZnO NPs have irregular, quasi-spherical morphology with the size range of 18-38 nm. The mean diameter of synthesized material was calculated to be 28.61 nm. The antibacterial efficacy of the ZnO NPs was evaluated against Gram-positive and Gram-negative bacteria strains *Bacillus subtilis* and *Salmonella typhi*, respectively, with encouraging results. The antioxidant activity of ZnO NPs was assessed using DPPH assay. The Trolox equivalent antioxidant capacity (TEAC) of this material was found to be 19.84 µmol Trolox/mg, indicating the high antioxidant capability. Further, the ZnO NPs exhibit significant photocatalytic activity against cationic methylene blue dye under solar irradiation, and the activity was retained after multiple cycles.

Optimization of protein extraction from duckweed using different extraction processes

Maag, P; Cutroneo, S; Tedeschi, T; Grüner-Lempart, S; Rauh, C; Karslioglu,OO. Food and Bioprocess Technology (2025) DOI10.1007/s11947-025-03777-x.

Despite its recent commercialization of duckweed, the extraction of high-quality protein presents challenges primarily in the efficiency of the extraction processes. In this study, conventional, ultrasound-assisted extraction (UAE), and ultrafiltration (UF) extraction techniques, were evaluated showing significant enhancements in protein yield, amino acid profile, and chlorophyll extraction. The highest protein release of 80.83% was achieved with a 40% amplitude applied for 10 min. UAE increased the protein yield substantially compared to conventional methods, achieving the highest yield of 41.30% with a moderate protein content of 50.91% in *Lemna gibba* protein concentrate. However, it comes at the cost of reduced protein purity and altered protein profiles due to co-extraction of other plant components. SDS-PAGE analysis revealed that ultrasound methods improved the extraction of chlorophyll-binding proteins (LHCB1) but decreased the efficiency of extracting RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) subunits compared to non-ultrasound methods. Lysine and tryptophan were identified as limiting essential amino acids (EAA), except when ultrasound methods were applied, which raised the levels partially above the recommended amino acid score (AAS) threshold of 1 for adults. The study established a nitrogen-to-protein conversion factor for duckweed at similar to 5.8, aligning with RuBisCO-based calculations and providing a more accurate tool for future nutritional assessments of duckweed proteins.

Kinetic modeling of thermophilic anaerobic digestion of Lemnaceae for biogas production

Lower, L; Qiu, YJ; Sartor, RC; Sagues, WJ; Cheng, JJ. Bioenergy Research (2025) 18: 23.

Anaerobic digestion of sustainably sourced biomass to generate biogas is a vital form of renewable energy that provides significant benefits to the environment. Lemnaceae, commonly referred to as duckweed, has shown great potential as a next-generation biomass feedstock for anaerobic digestion due to its rapid growth rates, low lignin content, and ability to remove nutrients from wastewater. However, research in this area is largely focused on the mesophilic (35°C) anaerobic digestion of duckweed. For the first time, batch thermophilic anaerobic digestion was performed using three different duckweed varieties grown on swine lagoon wastewater to ascertain the biochemical methane potential (BMP) of the biomasses and estimate parameters associated with the kinetics of the digestion process. The BMPs of the three duckweed varieties

were 205±5, 217±5, and 262±7 mL CH_4 g^{-1} volatile solids (VS) for the local variety (OxNC), *Lemna gibba* (8678), and *Lemna gibba* (7741), respectively. Four kinetic models were fitted to the experimental data: first order, modified Gompertz, transference, and logistic function. Unique to this study, inoculum from continuous thermophilic anaerobic digesters processing identical feedstocks was used during the BMP, causing the absence of a lag phase. The first-order model predicted the hydrolysis constant (k) to be 0.205-0.285 d^{-1}, which is similar to the hydrolysis constants reported in the literature for effective anaerobic digestion systems, thereby demonstrating that duckweed biomass has viable degradation rates. In this work, BMP experimentation and kinetic modeling have demonstrated the viability of anaerobically digesting multiple varieties of duckweed biomass under thermophilic conditions.

Optimized bioethanol production from *Lemna minuta* biomass harvested from polluted water via acid and enzymatic hydrolysis

Schutz, ERD; Mignoni, DSB; Michelon, W; Nunes, ED. Biofels-UK (2025) DOI10.1080/17597269.2025.2457817.

The contamination of water bodies through domestic, agricultural, and industrial discharges remains a critical environmental challenge, leading to eutrophication and harmful impacts on aquatic ecosystems and public health. In response, phytoremediation, which utilizes aquatic plants for pollutant removal, have gained attention. This study investigates the potential of *Lemna minuta* biomass, harvested from a polluted pond, for bioethanol production. The research evaluates carbohydrate content and explores the efficiency of acid and enzymatic hydrolysis in converting the biomass into fermentable sugars. The study's findings reveal that *Lemna minuta* exhibits a carbohydrate content of 36.46±1.69%. Acid hydrolysis demonstrated a high conversion efficiency, with optimal conditions achieving up to 99.20% efficiency and 18.09 g L^{-1} total reducing sugars. Enzymatic hydrolysis, while effective, yielded lower efficiencies, indicating the need for further optimization. Fermentation tests using *Saccharomyces cerevisiae* chardonnay resulted in ethanol production of 1.5 g L^{-1}, highlighting the potential of *Lemna minuta* as a sustainable bioethanol feedstock. These findings highlight the potential of *Lemna minuta* as a sustainable feedstock for bioethanol production while contributing to environmental remediation, reinforcing its dual role in renewable energy and ecosystem restoration.

Catalytic pyrolysis of duckweed with phosphoric acid: Products yield and composition

Zhu, YJ; Wu, L; Liu, HH; Yang, W; Zhang, WN; Li, H; Li, Y; Yang, HP; Jin, YL; Zhao, H. Renewable Energy (2025) 240: 122287.

The excessive presence of duckweed has led to the deterioration of water quality, which requires an efficient method for its high-value conversion. Therefore, catalytic pyrolysis of duckweed was conducted to elucidate the effects of H_3PO_4 impregnation on the yield and composition of the gaseous, liquid and solid products. The results showed that after H_3PO_4 impregnation, the solid and gas yields increased by up to 43.67 % and 51.78 %, respectively, compared to the case without H_3PO_4. Both the yields and fraction of H_2 increased remarkably, after H_3PO_4 impregnation, resulting in an increase of H_2/CO ratio. At low temperature of 400-500°C, H_3PO_4 impregnation effectively reduced the oxygenated compounds and facilitated the formation of N-containing compounds. With increasing temperature, the phosphoric acid promoted the aromatization of aliphatic hydrocarbons to form aromatic hydrocarbons and enhanced the production of phenols. Additionally, the introduction of H_3PO_4 increased the carbon retention in solid char by 9.9-19.3 % probably due to the formation of relatively stable phosphate esters via crosslink reactions with the saccharides in duckweed. The optimal temperature and H_3PO_4 impregnation ratio were recommended considering the yield and composition of the products, as well as the energy consumption during pyrolysis.

Ecology

Submerged macrophytes can maintain stable dominance over free-floating competitors through high pH

Szabó, S; Fedor, N; Koleszár, G; Braun, M; Korponai, J; Kocic, A; Hilt, S; Oláh, V. Freshwater Biology (2025) 70: e14363.

Submerged and free-floating macrophytes are the two main vegetation types that can alternatively dominate in small shallow ponds and ditches. Submerged macrophytes provide more aquatic ecosystem services and cause fewer problems with anoxia and greenhouse gas emissions than free-floating plants, but are inferior competitors for light. High pH values have been proposed as a contributor to submerged plant dominance, but threshold values for pH-induced growth reduction of floating plants by submerged macrophytes are not known. In this study, we conducted laboratory experiments to test whether submerged plants (*Ceratophyllum demersum*) can effectively limit the growth of free-floating competitors (*Lemna gibba*) by exceeding a critical threshold pH value. We used field data to test if and when such pH values can be achieved within dense submerged macrophyte stands. The pH values that caused 50% reduction in fresh weight- and chlorophyll-based growth of *L. gibba* were 9.6 and 9.8, respectively, and the growth was negligible above pH 10.0. The photochemical efficiency of photosystem II and chlorophyll content of the floating plants declined rapidly above pH 9 and 9.5, respectively. Similarly, phosphorus concentration decreased and dry matter content increased sharply in *L. gibba* fronds above pH 9.5, respectively. We thus expect a critical pH threshold for *L. gibba* at around 9.5. Our mesocosm and field data show that the critical pH threshold for *L. gibba* growth is regularly exceeded within dense stands of bicarbonate-using submerged macrophytes in lentic water-bodies. Such conditions can be prevalent in the upper water layer during most of the day (12 h) in summer. The literature showed that many duckweed species (and other common free-floating plants) in temperate and tropical water bodies show growth inhibition above pH 8. Therefore, high pH created by submerged macrophytes that use HCO_3^- in photosynthesis can be a major mechanism by which dominance over free-floating competitors is maintained in densely vegetated lentic eutrophic water bodies. We propose that alternative stable states occur between free-floating and submerged macrophytes along a pH gradient. Active management of pH may, thus, help in maintaining the desired state.

Feed & Food

Evaluating the efficacy of duckweed paste as a multifunctional organic binder in fish feed formulation

Deepti, M; Kaur, VI; Ansal, MD; Hundal, JS; Barik, S; Tyagi, A. Aquaculture International (2025) 33: 221.

This study explored the efficacy of two potential duckweed species (*Spirodela polyrhiza* and *Lemna minor*) as organic binders in fish feed as compared to traditional binders. Nine feeds including three control feeds-without binder (WB), cane molasses at 5% w/w (M5), and corn starch at 5% w/w (CS5)-and six experimental feeds-*Spirodela* paste feeds at 5% w/w (DWS5), 10% w/w (DWS10), and 15% w/w (DWS15) and *Lemna* paste feeds at 5% w/w (DWL5), 10% w/w (DWL10), and 15% w/w (DWL15)-were prepared and evaluated over a 360-day storage period in terms of water stability, sinking time, sinking time index, physical characteristics, nutritional composition, mineral content, microbial load, and spoilage. Duckweed-based feeds revealed significantly improved binding efficiency compared to control feeds. Notably, DWS15 appeared as most potent binder (followed by DWL15) with enhanced water stability (45.15-57.23%) and lower sinking time (2.14-2.44-fold) than WB, M5, and CS5, respectively. The feeds were found to be acceptable after overall evaluation of physical characteristics. Significantly higher crude protein content (27.77%) was recorded in DWS15, which was 11.44%, 8.99%, and 8.14% higher than CS5, M5, and WB, respectively. In contrast, the mineral content was significantly higher in DWL15. All duckweed binder feeds showed significant ($p < 0.05$) reduction in microbial load, aflatoxin, free fatty acids, peroxide values, highlighting their antimicrobial, antifungal, and antioxidant properties. Hence, the findings of the study revealed that duckweed paste of *S. polyrhiza* at 15% w/w followed by *L. minor* at 15% w/w served as cost-effective organic binders, offering substantial improvements in feed stability, protein content, and shelf life, making duckweed a valuable and sustainable alternative for enhancing fish feed quality and longevity.

The application of duckweed (*Lemna minor*) and freshwater mussels (*Anodonta cygnea*) as living biofilters integrating with a filtration system to maintain water quality in juvenile trout (*Oncorhynchus mykiss*) rearing using the small scale RAS system

Azhar, MH; Memis, D. Water Environment Research (2025) 97: e70046.

Increasing nutrient concentrations in fish culture systems over time can reduce water quality. However, the nutrient increase can be remediated by pairing organisms at lower trophic levels with a mechanical filtration

system to improve nutrient removal efficiency and water quality for fish culture. This research uses the RAS system to determine the performance of integrating living organisms as biofilters in rearing juvenile rainbow trout (*Oncorhynchus mykiss*) for 56 days. Duckweed (*Lemna minor*) was added to replicate tanks at three treatment levels: T1 (100 g wet weight and 20% area coverage), T2 (200 g wet weight and 40% area coverage), and T3 (300g wet weight and 60% area coverage). The duckweed in each treatment tank was supplemented with 20 freshwater mussels (*Anodonta cygnea*) with an average body weight of 56±1.0 g. Physical and chemical water quality parameters were measured in fish tanks and all ponds in the RAS system. Fish from the rearing tanks were weighed every two weeks. Duckweed biomass was measured weekly; the mussels were weighed at the beginning and end of the study, and the mussels were measured at the beginning and end of the rearing period. The fish was partially harvested every two weeks to maintain constant fish biomass. Using duckweed (*L. minor*) with different biomass weights and areal coverage, coupled with the freshwater mussels (*A. cygnea*) as living biofilters, had a significant effect ($P < 0.05$) on water quality parameters. Ammonium (NH_4), nitrite (NO_2), and nitrate (NO_3) concentrations decreased throughout the study. During the study period, juvenile trout experienced growth with an SGR of 2.62-2.72%/g with a survival rate of 100%. Partial harvesting during the rearing period positively impacted the average body weight of fish growth and duckweed biomass. The best duckweed growth performance was found in treatment T1 (cover area 20% with wet weight 100 g) with a productivity of 9.4 ($g/m^2/d$). Twenty percent duckweed coverage with freshwater mussels achieves optimal nutrient removal in RAS systems, improving water quality efficiently and growth better than other treatments. Combined biofilters (duckweed-mussel) and filtration units reduce operational costs while maintaining high fish survival rates in RAS systems. Integration of living biofilters provides sustainable water treatment without chemical additives, suitable for small-scale aquaculture operations.

Utilization of microalgae and duckweed as sustainable protein sources for food and feed: Nutritional potential and functional applications

Song, YJ; Hu, ZL; Liu, SZ; Luo, SS; He, RM; Yang, XY; Li, S; Yang, XW; An, YX; Lu, YL. Journal of Agricultural and Food Chemistry (2025) 73: 4466-4482.

Aquatic biomass, particularly microalgae and duckweed, presents a promising and sustainable alternative source of plant-based protein and bioactive compounds for food and feed applications. This review highlights the nutritional potential of these aquatic species, focusing on their high protein content, rapid growth rates, and adaptability to non-arable environments. Microalgae, such as *Chlorella* and *Arthrospira* spp., and duckweed, such as *Lemna minor*, are evaluated for their functional food applications, including their roles as protein supplements, bioactive components, antioxidants, and emulsifiers in food formulations. The study also examines their environmental benefits, including wastewater bioremediation, nutrient recycling, and greenhouse gas mitigation, which contribute to a more sustainable agricultural system. Technological advancements in the cultivation, harvesting, and processing of microalgae and duckweed are discussed to enhance their scalability and economic feasibility in food and feed production. The findings suggest that integrating microalgae and duckweed into agricultural and food systems can significantly improve food security, nutritional outcomes, and sustainability. Future research should focus on optimizing cultivation efficiencies, advancing protein extraction techniques, and expanding the applications of aquatic biomass in various food products.

Use of aquatic plants (*Azolla caroliniana* and *Lemna* spp) as a feed source in silkworm culture

Bekcan, S; Yavuzcan, H; Atar, HH. Journal of Agricultural Sciences – Tarim Bilimleri Dergisi (2025) 30: 532-537.

This article focuses on the effects of the use of aquatic plants on the growth parameters of silkworm larvae in their artificial feeding. Due to the difficulty of obtaining mulberry leaves, different feeds and substances to be substituted for this food source were tested in various researches. In this study, the silkworm larvae were fed with the diet containing different proportion of azolla (*Azolla caroliniana*) meal and duckweed (*Lemna* spp.) meal found abundant and easily available compared to mulberry leaves in nature. The study aims at defining an appropriate combination of mulberry leaf meal, azolla meal and duckweed meal and the effects of these artificial feeds on the growth rate of silkworm larvae. Consequently, it was determined that 25%, 50%, 75% substitution of azolla meal and duckweed meal up to the 3rd instar (12th day) did not affect the growth and survival rates of silkworm larvae.

Utility of dried and fermented meal from the duckweed species *Spirodela polyrhiza* as fishmeal-protein replacer in diets for common carp fry

Stadtlander, T; Surber, J; Tschudi, F; Seitz, A; Sigrist, M; Pietsch, C; Kreuzer, M; Leiber, F. Journal of Animal and Feed Sciences (2025) 34: 131-138.

The family of cyprinids comprises some of the most important fish species in global aquaculture. With increasing global fish production also increasing amounts of suitable and sustainably produced high-quality feeds other than fishmeal are needed. Duckweed (*Spirodela polyrhiza*) is a fast growing aquatic plant with the potential of high biomass and protein production in nutrient recycling systems. This study reports the effects on growth, feed conversion and proximate body composition of common carp (*Cyprinus carpio* L.) fry fed graded levels of differently processed duckweed (meals dried and fermented) each replacing 150, 300 or 450 g/kg of dietary fishmeal protein. Comparisons were made to a duckweed-free and fishmeal-based control, containing 400 g/kg of fishmeal, equivalent to 261.9 g/kg of fishmeal protein. Carp fed the highest inclusion rate of fermented duckweed showed a significantly reduced performance (growth and feed conversion rate) compared to all other groups. No differences regarding growth and feed conversion rate were found among all other groups. Whole body crude lipid content was generally lower and crude ash content generally higher in carp fed dried compared to those fed fermented duckweed. Whole body crude protein content was not influenced by treatments. Based on the results of this study, dried *S. polyrhiza* meal could be used to replace up to 450 g/kg of fishmeal protein, while fermented *S. polyrhiza* meal should only replace up to 300 g/kg fishmeal protein in diets for carp fry.

Growth & Development

ALPHA: A high throughput system for quantifying growth in aquatic plants

Robinson, KA; Augoustides, V; Madenyika, T; Sartor, RC. Plant direct (2025) 9: e70048.

The need for more sustainable agricultural systems is becoming increasingly apparent. The global demand for agricultural products -food, feed, fuel and fiber- will continue to increase as the global population continues to grow. This challenge is compounded by climate change. Not only does a changing climate make it difficult to maintain stable yields but current agricultural systems are a major source of greenhouse gas emissions and continue to drive the problem further. Therefore, future agricultural systems must not only increase production but also significantly decrease negative environmental impacts. One approach to addressing this is to begin breeding and cultivating new plant species that have fundamental sustainability advantages over our existing crops. The Lemnaceae, commonly known as duckweeds, are one family of plants that have potential to increase output and reduce the negative environmental impacts of agricultural production. Herein we describe the Automated Lab-scale PHenotyping Apparatus, ALPHA, for high-throughput phenotyping of Lemnaceae. ALPHA is being used for selective breeding of one species, *Lemna gibba*, toward the goal of creating a new crop for use in sustainable agricultural systems. ALPHA can be used on many small aquatic plant species to assess growth rates in different environmental conditions. A proof of principle use case is demonstrated where ALPHA is used to determine saltwater tolerance of six different clones of *L. gibba*.

Varied growth media necessitate different light regimes for indoor duckweed cultivation

Redmond, C; O'Mahoney, R; Blanchard, M; Coughlan, NE. Plants (2025) 397.

Controlled indoor cultivation of duckweed plants can support remediation of wastewaters through generation of plant biomass. Despite numerous advantages, indoor cultivation of duckweeds on agri-food wastewaters remains underexplored. Lighting regimes need to be optimised for duckweed growth and affordability of energy consumption, as it has been shown that the composition of wastewater growth medium can alter light utilisation. In the present study, four duckweed (*Lemna minor*) clones were grown under four different light regimes on either optimised half-strength Hutner's medium or wastewater derived from the liquid fractions of anaerobically digested pig slurry. Cultivation of *L. minor* was assessed for the four light regimes using a commercial hydroponics plant growth medium in a 3.96 m^2 multitiered cultivation system. When cultivated on

optimised half-strength Hutner's medium or diluted pig slurry under laboratory conditions, it appeared that photoperiod rather than light intensity was more important for duckweed growth. Yet, under moderate flow conditions within a larger scale multitiered cultivation system, greater light intensity appeared to support duckweed cultivation irrespective of photoperiod. These findings emphasise the need to move beyond small-scale and static assessments of duckweed before embarking on larger, industry-relevant scales.

Minimal assay detects population-level senescence in the aquatic plant *Lemna minor*

Thwaites, VC; Chmilar, SL; Luzardo, AC; Laird, RA. Botany (2025) 103: 0010.

At the population level, senescence occurs when older individuals have increased risk of death and reduced reproduction compared to younger individuals. We investigated senescence in the aquatic plant *Lemna minor* L. (common duckweed), an important species for plant senescence research. Our objectives were to (1) confirm or refute the presence of population-level senescence in this model species; (2) develop a minimal assay of senescence requiring only once-weekly data collection; and (3) test whether there were appreciable differences in senescence in plants grown in glass compared to polystyrene Petri dishes, with an aim to reducing single-use plastic waste and long-term research materials costs. We found that weekly survival arced downward with age when viewed on a semi-log plot, and weekly production of descendants decreased with age, with both findings indicating population-level senescence that matched previous work using more frequent data-collection (per Objectives 1 and 2). Additionally, we found no noteworthy differences in senescence between plants grown in glass versus polystyrene Petri dishes (per Objective 3). The use of weekly data collection could liberate personnel resources for other research-group functions, and could make the *Lemna* system suitable for senescence- or demography-education exercises. The use of glass dishes could reduce lab waste and expense.

Interaction with other organisms

Effects of norfloxacin on the interaction between duckweed and its growth-promoting bacterial assemblages

Lin, H; Chang, J; Liang, T; Yang, C; Wei, Y; Peng, W; Xu, A; Duan, C; Zhao, Y. Journal of Hazardous Materials (2025) 492: 138249.

The entry of antibiotics into aquatic environments can negatively affect various organisms. However, the specific effects of antibiotics on the interactions between aquatic plants and its growth-promoting bacterial assemblages (GPBA) remain inadequately explored. Thus, this study aimed to examine the performance of mono-culture and co-culture systems, involving duckweed and its GPBA, under varying norfloxacin concentrations (0, 0.05, 0.2, 0.5, and 1 $mg\,L^{-1}$), to elucidate the effects of norfloxacin on the interactions between duckweed and GPBA. The results revealed that norfloxacin concentrations of $\geq 0.5\,mg\,L^{-1}$ significantly inhibited duckweed growth and diminished the growth-promoting abilities of GPBA by reducing the relative abundances of plant growth-promoting bacteria (e.g., Pelomonas, Ensifer, Acidovorax and Sphingomonas). Furthermore, physiological analysis of duckweed suggests that GPBA may enhance the antioxidant capacity of duckweed, thereby alleviating membrane damage induced by norfloxacin. Additionally, norfloxacin concentrations of $\geq 0.2\,mg\,L^{-1}$ significantly reduced nitrogen and phosphorus removal efficiencies in the co-culture system. Overall, norfloxacin exerted a more pronounced negative impact on duckweed growth, microbial community and nutrient removal in the co-culture system compared to the mono-culture system, indicating that norfloxacin poses a heightened risk to the co-culture system by disrupting the interactions between duckweed and its GPBA.

The impact of the herbicide paraquat on the interaction between a macrophyte (*Lemna minor*) and two species of cyanobacteria (*Microcystis aeruginosa* and *Microcystis flos-aquae*)

Dauda, S; Uyovbisere, EE; Lorenzi, AS; Ibrahim, ZA; Samuel, SA; Gadzama, IMK; Chia, MA. Aquatic Ecology (2025) DOI10.1007/s10452-025-10188-9.

Macrophytes like *Lemna minor* and cyanobacteria species (for example, *Microcystis aeruginosa* and *M. flos-aquae*) co-exist naturally in water bodies, competing for nutrients, light and space, which influence their growth. The introduction of external factors such as herbicides into water bodies can influence the interaction between these aquatic organisms. This study examined the impact of paraquat, a widely used herbicide, on the interaction between *L. minor* and *M. aeruginosa* and *M. flos-aquae*. This was done by co-culturing *L. minor* (12 viable colonies) with *M. aeruginosa* and *M. flos-aquae* (both at 3.0 x 10^5 cells mL^{-1}) separately, and with 10 µg L^{-1} of paraquat in BG-11 medium for 5 days. Monocultures of *L. minor*, *M. aeruginosa*, and *M. flos-aquae* were exposed to different concentrations of paraquat (0, 0.01, 0.1, 1, 10, and 50 µg L^{-1}). An increase in paraquat concentration reduced the growth and biomass of *L. minor*, *M. aeruginosa*, and *M. flos-aquae* in single cultures, while decreasing total microcystin production in *M. aeruginosa* but increasing it in *M. flos-aquae*. When co-cultured with either *M. aeruginosa* or *M. flos-aquae*, the growth and biomass of *L. minor* decreased, and its POD activity increased. The presence of *M. aeruginosa* increased the MDA content and GST activity of L. minor, while the presence of M. flos-aque plus paraquat increased its proteins content and GST activity. On the other hand, the presence of *L. minor* plus paraquat decreased the growth and biomass of both cyanobacteria (*M. aeruginosa* and *M. flos-aquae*) and increased their microcystins production, H_2O_2 contents, POD and GST activities, and total proteins. These results demonstrate that the presence of paraquat amplifies the suppression of growth and increased stress of *L. minor* caused by both *M. aeruginosa* and *M. flos-aquae*, and vice-versa. This situation may exacerbate the competitive dynamics in aquatic environments by potentially altering community structures, a situation that could affect overall ecosystem health.

Synergistic microalgae-duckweed systems for enhanced aquaculture wastewater treatment, biomass recovery, and CO_2 sequestration: A novel approach for sustainable resource recovery

Song, YJ; Hu, ZL; Yang, XW; An, YX; Lu, YL. Environmental Research (2025) 274: 121271.

Current aquaculture practices generate nutrient-rich effluents that cause significant environmental pollution. This study presents a novel synergistic microalgae-duckweed system integrating *Chlorella* sp. and *Spirodela polyrhiza* for sustainable wastewater treatment, biomass valorization, and carbon sequestration. Over a 15-day treatment period, the system achieved unprecedented removal efficiencies: 91.25% for NO_3^--N, 98.90% for NH_4^+-N, 100% for total phosphorus, and a 95% reduction in chemical oxygen demand (COD). Concurrently, the system produced 6.67 g/ L of microalgal biomass and 90 g/ m^2 of duckweed biomass significantly higher than those of standalone systems, which showed enhanced protein and lipid contents suitable for bioenergy or feed applications. The dual system sequestered CO_2 at a remarkable rate of 1.65 g/L/day, exceeding standalone treatments. Microbial community analysis revealed enriched functional diversity, promoting optimized nutrient cycling and organic matter degradation. Although the system was tested at a lab scale, it demonstrates promising scalability due to its efficient nutrient removal and biomass production, as well as the robustness of the combined microalgae-duckweed treatment approach. This integrated approach not only addresses water pollution but also advances the circular economy by converting aquaculture waste into high-value biomass and mitigating carbon emissions. These findings position the synergistic microalgae-duckweed system as a scalable and eco-friendly solution for sustainable aquaculture management and environmental conservation.

Creation of a functional duckweed holobiont to reduce nutrient competition with microalgae for high-yield biomass production

Pham, HTT; Kuroda, S; Khairina, Y; Morikawa, M. Bioresource Technology (2025) 421: 132110.

Duckweed has been highlighted as an appropriate biomass for low-carbon industries because of its significantly high production rate and multiple resource value. However, the outbreak of microalgae is a practical issue that decreases duckweed production yield. This study demonstrated that the growth of the duckweed *Lemna aequinoctialis* from factory wastewater was enhanced by colonization with indigenous plant growth-promoting bacteria (PGPB), whereas the growth of a duckweed competitor microalga, *Coelastrella* sp. KC10, from the same wastewater was reduced by indigenous microalgal growth-inhibiting bacteria (MGIB). Finally, a quadruple co-culture of a synthetic duckweed holobiont, *L. aequinoctialis* colonized by both KLaR20 (PGPB) and KLaR16 (MGIB), and *Coelastrella* sp. KC10 successfully recovered the duckweed production level by 117.5% in frond number and 84.5% in dry weight compared to those in the absence of microalgae. This case study demonstrates for the first time that duckweed holobionts can be reconstructed and enforced to antagonize growth competitor microalgae.

Copper-induced transgenerational plasticity in plant defence boosts aphid fitness

Chávez, A; Schreyer, A; Pruesener, P; Schaefer, M; Xu, SQ; Huber, M. Plant Cell and Environment (2025) DOI10.1111/pce.15406.

Transgenerational plasticity in plants is an increasingly recognized phenomenon, yet it is mostly unclear whether transgenerational plasticity is relevant to both the fitness of the plant and its interacting species. Using monoclonal strains of the giant duckweed (*Spirodela polyrhiza*) and its native herbivore, the waterlily aphid (*Rhopalosiphum nymphaeae*), we assessed whether pre-treating plants with copper excess, both indoors and outdoors, induces transgenerational plasticity in plant defences that alter plant and herbivore fitness. Outdoors, copper pre-treatment tended to increase plant growth rates under recurring copper excess. Indoors, copper pre-treatment either increased or decreased plant growth rates under recurring conditions, depending on the plant genotype. Copper pre-treatment induced anthocyanins that protected plants against copper toxicity, and these elevated levels were transgenerationally retained. Copper pre-treatment also transgenerationally increased the levels of 12-oxo-phytodienoic acid (OPDA), a jasmonate precursor. Nevertheless, aphids grew up to 50% better when the plants were pre-treated with copper. The increased aphid growth was likely caused by transgenerationally elevated OPDA levels, as aphids grew better when jasmonates were externally applied to plants. Taken together, this study shows that transgenerational plasticity is relevant to both plant and herbivore fitness, which highlights the role of transgenerational plasticity in plant evolution and species interactions.

Molecular Biology and Genomics

Atypical epigenetic and small RNA control of degenerated transposons and their fragments in clonally reproducing *Spirodela polyrhiza*

Dombey, R; Buendia-Avila, D; Barragán-Borrero, V; Diezma-Navas, L; Ponce-Mañe, A; Vargas-Guerrero, JM; Elias, R; Marí-Ordóñez, A. Genome Research (2025) 35: 522-544.

A handful of model plants have provided insight into silencing of transposable elements (TEs) through RNA-directed DNA methylation (RdDM). Guided by 24 nt long small-interfering RNAs (siRNAs), this epigenetic regulation installs DNA methylation and histone modifications like H3K9me2, which can be subsequently maintained independently of siRNAs. However, the genome of the clonally propagating duckweed *Spirodela polyrhiza* (Lemnaceae) has low levels of DNA methylation, very low expression of RdDM components, and near absence of 24 nt siRNAs. Moreover, some genes encoding RdDM factors, DNA methylation maintenance, and RNA silencing mechanisms are missing from the genome. Here, we investigated the distribution of TEs and their epigenetic marks in the *Spirodela* genome. Although abundant degenerated TEs have largely lost DNA methylation and H3K9me2 is low, they remain marked by the heterochromatin-associated H3K9me1 and H3K27me1 modifications. In contrast, we find high levels of DNA methylation and H3K9me2 in the relatively few intact TEs, which are source of 24 nt siRNAs, like RdDM-controlled TEs in other angiosperms. The data suggest that, potentially as adaptation to vegetative propagation, RdDM extent, silencing components, and targets are different from other angiosperms, preferentially focused on potentially intact TEs. It also provides evidence for heterochromatin maintenance independently of DNA methylation in flowering plants. These discoveries highlight the diversity of silencing mechanisms that exist in plants and the importance of using disparate model species to discover these mechanisms.

Inhibition mechanism of *Microcystis aeruginosa* in co-culture of *Lemna* and *Azolla*: Insights from non-targeted metabonomics

Yang, XB; Lou, YS; Song, LY; Zhang, D; Song, YZ; Liang, JX; Liu, ZK; Wang, C; Zhao, Z. Plant Physiology and Biochemistry (2025) 220: 109529.

Microcystis aeruginosa, a harmful alga in cyanobacterial blooms, damages aquatic ecosystems. Species diversity may control the blooms by increasing ecosystem stability and resource utilization. The growth and photosynthetic systems of *M. aeruginosa* were investigated using the water from monocultures of *Lemna aequinoctialis* and *Azolla imbricata* group, as well as their mixtures. The highest rate of inhibition (84%) of *M. aeruginosa* was observed in the water excretions from the mixture of the two species across the three experimental groups. Greater disruption of cell membranes and a more significant decrease in the maximum

electron transfer rate and photochemical quantum yield of *M. aeruginosa* were observed under mixed conditions compared to the monoculture, indicating the increased disruption of their photosynthetic systems in the mixed group. Liquid chromatography-mass spectrometry identified 479 and 431 differential metabolites in the mixed group compared to monocultures of *L. aequinoctialis* group and *A. imbricata*, respectively. Dihydrocapsaicin and 13-hydroxy-9-methoxy-10-oxo-11-octadecenoic acid, previously known to participate in oxidative stress and induce the secretion of benzoic acid to disrupt the cell membrane, were found to be abundant in the mixed group compared to the monoculture groups of *L. aequinoctialis* and *A. imbricata*. Our results showed that a mixture of *L. aequinoctialis* and *A. imbricata* is a potential novel antialgal agent to inhibit harmful algae.

Herbivory can increase plant fitness via reduced interspecific competition-evidence from models and mesocosms

Boettner, L; Dudenhausen, F; Nouere, S; Malacrinò, A; Schaefer, M; Koene, JM; Huber, M; Xu, SQ. Proceedings of the Royal Society B-Biological Sciences (2025) 292: 20241149.

Herbivores are generally considered to reduce plant fitness. However, as in natural communities they often feed on several competing plant species, herbivores can also increase plant fitness by reducing interspecific competition among plants. In this study, we developed a testable model to predict plant fitness in the presence of an interspecific competitor and an herbivore that feeds on both plant species. Our model allows prediction of the herbivore and competitor densities at which the focal species will benefit from herbivory. This can be estimated by quantifying the effects of the herbivore on the fitness of the focal plant and on its competitor, and by estimating the levels of intra- and interspecific competition in a pairwise fashion, respectively. We subsequently validated the model in indoor microcosms using three interacting species: an aquatic macrophyte (the giant duckweed *Spirodela polyrhiza*), its native competitors (green algae) and its native herbivore (the pond snail *Lymnaea stagnalis*). Additional outdoor mesocosm experiments supported our model under natural conditions. Together, this study provides a conceptual framework to understand how herbivores shape plant fitness in a community context.

High productivity of oxylipin KODA using *E. coli* transformed with lipoxygenase and allene oxide synthase genes of *Lemna paucicostata*

Takagi, K; Yokoyama, M; Beppu, T; Uemori, H; Ohno, H; Murakami, T; Ifuku, O; Tada, Y; Yoshida, S. Plant Biotechnology (2024) 41: 469-472.

KODA, a type of oxylipin, has stimulatory effects on plant growth under limiting conditions of stress, such as promoting flowering, rooting, and resistance to pathogens, for use in agriculture. KODA is released from *Lemna paucicostata* under drought, heat, and osmotic pressure, and is produced from alpha-linolenic acid by a two-step enzymatic reaction with 9-lipoxygenase and allene oxide synthase. In this paper, we report the outstanding KODA productivity of *L. paucicostata*, SH strain screened from 56 Lemna species. We constructed co-expression vectors for 9-lipoxygenase gene (LpLOX) and allene oxide synthase gene (LpAOS) isolated from the SH strain to be transformed into *E. coli*. The productivity (per fresh weight) using *E. coli* is 25.3 mg KODA g-1 as compared to 0.366 mg g-1 from *L. paucicostata*, SH strain, which requires a longer culture time, light irradiation and larger space for culture.

Comment DF: The valid name is *Lemna aequinoctialis*

Morphology

On the plant developmental unit: From virtual concept to visual plantlet

Bai, SN. Plants (2025) 14: 396.

This study introduces the concept of the plant developmental unit (PDU) and validates its application using *Wolffia* Horkel ex Schleid (Araceae) as a model system for exploring fundamental processes in plant morphogenesis. Revisiting long-standing contradictions in plant biology, the author proposes viewing plants as coral-like colonies composed of multiple developmental units rather than as unitary-animal-like organisms. Utilizing the "Plant-on-Chip" culture platform, the research demonstrates *Wolffia*'s minimalist structure as a powerful model for investigating core regulatory mechanisms of plant development. The study emphasizes

the pivotal role of "induction" in morphogenetic processes and highlights *Wolffia*'s potential to facilitate a paradigm shift in plant developmental biology while unlocking its applications in a second agricultural revolution. This work underscores *Wolffia*'s value in bridging fundamental research and innovative agricultural solutions.

Physiology & Stress

Physiological responses of *Lemna minor* to polystyrene and polymethyl methacrylate microplastics

Kospic, K; Vitko, S; Kobelscak, L; Mateskovic, A; Stefanic, PP; Dimitrov, N; Tkalec, M; Balen, B. Current Plant Biology (2025) 42: 100473.

Due to its economic viability, plastic has become an indispensable material whose mass production continues to increase, raising concerns about its impact on living organisms. Its long persistence in the environment and slow degradation to microplastics (MPs) pose a serious problem, as MPs can penetrate plants and animals and interfere with physiological processes. In this study, the in vitro cultured duckweed *Lemna minor* was exposed to 10, 50 and 100 mg L^{-1} polystyrene (PS) and polymethyl methacrylate (PMMA) MPs for 7 days to investigate uptake and effects on growth, photosynthetic performance and oxidative stress parameters. We hypothesized that PS-MPs and PMMA-MPs would have different uptake patterns and effects on the physiology of *L. minor*, due to their different properties. A pronounced agglomeration of PMMA-MPs in the exposure medium correlated with a lower uptake of PMMA-MPs compared to PS-MPs. However, PMMA-MPs induced severe ultrastructural changes in the chloroplasts and a decrease in chlorophyll a and b content, resulting in reduced plant growth. In contrast, treatments with PS-MPs stimulated growth, especially frond area, probably as a result of increased content of photosynthetic pigments and improved photosynthetic efficiency. Both MP types induced mild oxidative stress, which triggered protective responses, but the activation of antioxidant defense was dependent on the polymer type, as PMMA-MPs slightly increased proline content and superoxide dismutase activity, while PS-MPs induced peroxidase activities. In conclusion, PS-MPs seem to be less harmful as they promote growth and photosynthetic efficiency, whereas PMMA-MPs have negative effects on *L. minor* physiology by causing structural damage to subcellular parts and inhibiting their function.

Salinity stress-induced impacts on biomass production, bioactive compounds, antioxidant activities and oxidative stress in watermeal (*Wolffia globosa*)

Yadav, NK; Patel, AB; Priyadarshi, H; Baidya, S. Discover Applied Sciences (2025) 7: 106.

This study investigated the impact of salinity stress on the biomass production, proximate composition and bioactive contents, antioxidant activities, and oxidative stress parameters of *Wolffia globosa*. *Wolffia* was exposed to variable NaCl concentrations (0, 8.56, 17.11, 25.67, 34.22, and 42.78 mM) in nutrient-replete nutrient conditions under natural sunlight in a transparent polyhouse. The net biomass gain (NBG; 131.17 g), crude protein (33.16%) and crude lipid content (4.9%) were significantly high at 8.56 mM NaCl compared to control and most of other treatments. Total phenolic content peaked at 17.11 mM NaCl (551.39 mg GAE g^{-1}), while total tannin content was highest at 25.67 mM NaCl (13.38 mg TAE g^{-1}). Total flavonoid content, vitamin C, oxidative stress enzymes; superoxide dismutase, catalase, and lipid peroxidation (malondialdehyde, MDA) peaked at 42.78 mM NaCl, with values of 140.52 mg QE g^{-1}, 218.69 mg 100 g^{-1}, 28.18 U g_{-1} FW, 288.60 U min-1 g^{-1} FW, and 142.32 mg MDA kg^{-1}, respectively. The control invariably had the lowest values. Total carotenoid content was significantly higher at 17.11 and 25.67 mM NaCl (1812.84 and 1749.10 µg g^{-1}), while Chlorophyll-a (Chl-a) was highest at 17.11 mM (21.33 µg g^{-1}). Chlorophyll-b (Chl-b) peaked at 17.11, 25.67, and 34.22 mM NaCl levels. Salinity stress generally elevated antioxidant activities, assessed via DPPH, ABTS, and FRAP assays reaching their highest values at 34.22 mM NaCl (40.52%), 17.11 mM NaCl (56.71%), and 25.67 mM NaCl (428.83 µmol Fe^{2+} g^{-1}), respectively. FT-IR profiles indicated changes in chemical composition and functional groups due to salt stress. Overall, salinity significantly influenced the proximate composition, antioxidant activities, bioactive compounds and oxidative stress enzyme activities of *Wolffia*.

Phytomedicine

Alterations in gut microbiome and metabolite profiling during in vitro fermentation of duckweed (*Wolffia globosa*) and its extracts by gut bacteria from obese adults

Dhamaratana, S; Methacanon, P; Tunsagool, P; Nakphaichit, M; Mok, K; Honwichit, O; Charoensiddhi, S. Future Foods (2025) 11: 100608.

This study investigated the impact of the digesta after an in vitro gastrointestinal digestion of duckweed powder (DWP) and its polysaccharide (DPS) and protein (DPT) extracts on the modulation of gut microbiome and metabolite production at 24 h in vitro fecal fermentation from obese adults. The findings indicate that all duckweed samples enhanced the growth of beneficial intestinal microbial genera, including *Megamonas*, *Bifidobacterium*, *Phocaeicola*, *Bacteroides*, and *Blautia*, while the DPS and DPT promoted the growth of bacterial genera *Fusobacterium*. Furthermore, all duckweed samples contributed to an increase in total short-chain fatty acids (SCFAs) synthesis compared to the control. DPS showed the greatest promotion of SCFAs, followed by DPT and DWP which played a more important role in metabolite alterations compared to DPS and DPT. Pathway analysis results showed that altered metabolites were mostly related to tryptophan metabolism. Therefore, duckweed and its extracts showed potential for use as a dietary supplement to improve gut health benefits in obese adults.

Phytoremediation

The impact of citric acid on metal accumulation in *Lemna minor*

Mobin, F; Deloya, JM; Guo, L. Water (2025) 17: 830.

Potentially toxic metals contaminate the environment and threaten human health. This study investigated the effect of chelator citric acid (CA) on enhancing metals (Cu, Ni and/or Pb) accumulation in duckweed (*Lemna minor*). *Lemna minor* were cultured in solutions with single or mixed metals (Ni 50 ppm, Cu 50 ppm and/or Pb 10 ppm) added with different levels of CA (0 ppm, 10 ppm, 50 ppm or 100 ppm CA) for 4 weeks, then harvested, dried and digested. For single-metal solutions, duckweed treated with higher levels of CA (50 ppm or 100 ppm CA) accumulated more Ni or Cu; 100 ppm CA increased Cu and Ni accumulation in plants by 96% and 120%. Meanwhile, 10 ppm CA, 50 ppm or 100 pm CA had similar effects on improving Pb accumulation in duckweed, which enhanced Pb accumulation in duckweed by 100%. For duckweed cultured in mixed-metals solutions, 50 ppm and 100 ppm CA still significantly increased the amounts of Cu and Ni in duckweed by 50% and 100%, while Pb sequestration was not enhanced. The role of CA in increasing metal accumulation in duckweed depended on the levels of CA, the concentrations and types of metals. Future studies are needed to further investigate the potential of CA to assist phytoremediation of different metals contaminated environment.

Effect of temperature on the physiology and phytoremediation capacity of *Spirodela polyrhiza* exposed to atrazine and S-metolachlor

Cruz, FVD; Venne, P; Segura, P; Juneau, P. Aquatic Toxicology (2025) 282: 107304.

Environmental toxicity of pesticides to aquatic plants can vary with temperature, as temperature affects plant metabolic processes. We exposed the globally distributed duckweed *Spirodela polyrhiza* to environmentally relevant concentrations (40 µg/ L) of atrazine and S-metolachlor at temperatures typical of surface freshwater in temperate zones (10, 15, and 21°C). Our objective was to assess the effects of low temperatures and herbicide concentration, and their interactions, on growth, photosynthesis, pigments, antioxidant enzymes, and phytoremediation capacity. Lower temperatures (10°C) intensified the adverse effects of both herbicides on the quantum yield of photosystem II in *S. polyrhiza*, with photosynthesis being a more sensitive endpoint than biomass growth rate. Both in the control and herbicide treatments, plants exposed to 10°C exhibited lower concentrations of photosynthetic pigments (chlorophylls and carotenoids) and reduced ascorbate peroxidase activity, which may have contributed to the intensified negative effects on photosynthesis at this temperature. The removal of S-metolachlor was lower at 10 and 15°C (3-8 %) compared to 21°C (17 %), while no difference

was observed between the three tested temperatures for atrazine (2-8 %). Our findings suggest that conducting pesticide toxicity tests at around 25°C may underestimate the contaminants' inhibitory effects on aquatic plants during colder seasons and in temperate regions. Additionally, lower temperatures pose a challenge to the effectiveness of atrazine and S-metolachlor phytoremediation in aquatic environments.

Modelling assisted phytoextraction of heavy metals from tannery origin leachate

Yahya, F; Nazir, A; Ahmad, S; Alomrani, SO; Shafiq, M; Bareen, FE; Alshehri, MA; Ali, S. Chemosphere (2025) 373: 144113.

The toxic tannery solid waste leachate (TSWL) containing heavy metals is generated after the percolation of rainwater in openly dumped tannery solid waste (TSW) which poses a serious threat to the surroundings by leaching down and bioaccumulation. For its management, the phytoextraction potential of *Pistia stratiotes* L. and *Spirodela polyrhiza* L. Schleid. was analyzed by growing them individually and combining them in different dilutions of TSWL (0%, 5%, 10%, 15%, and 20%) for 30 days. The removal efficiency of metal content was 79.04% (Cr), 78.49% (Cd), and 88.11% (Cu) in *P. stratiotes*, 72.55% (Cr), 80.12% (Cd), and 77.70% (Cu) in *S. polyrhiza* while in the mixture it was 88.39% for Cr, 92.57% for Cd, and 90% for Cu. The translocation factor of all the metals for every plant was greater than 1 indicating that all the plants used in the study proved to be hyperaccumulators. The Langmuir model more efficiently described experimental data for Cd and Cu while the phytoextraction of Cr was explained by the Freundlich model. The R-L and 1/n were <1 for the above-mentioned metals indicating the favourable and active absorption of metals in plants. Therefore, based on the modelling-assisted phytoextraction findings it is suggested that the use of hyperaccumulator plants is a cost-effective and sustainable approach for managing the toxic TSWL.

Phytoindication and phytoremediation of water quality using the local duckweed plant *Lemna aequinoctialis*

Munaro, AT; Tsamba, J; Siziba, N. Environmental Quality Management (2025) 34: e70062

Environmental pollution exacerbates global freshwater scarcity. In Harare, sewage pollution drives freshwater pollution, compromising its quality and security. Here, we uniquely explored the dual role of *Lemna aequinoctialis* as a phytoindicator and phytoremediator of freshwater quality. Using a purposive sampling strategy targeting common household freshwater abstraction sites in selected communities of Harare (Tafara and Manyame), 12 water samples and an L. *aequinoctialis* culture were collected from the field and incubated in the laboratory. Biochemical oxygen demand (BOD), electrical conductivity (EC), and potential hydrogen (pH) were determined by probes, while total suspended solids (TSS) and turbidity were analyzed by UV-Vis Spectrometry. Chemical oxygen demand (COD), nitrates (NO_3^-), nitrites (NO_2^-), and orthophosphates were analyzed by colorimetric techniques. An analytical balance measured the plant fresh weights and root lengths were measured via microscopy. Akin to $^+$raw sewage, river B (Tafara) exhibited the highest initial pollutant load; BOD (3.8 mg/L), COD (38 mg/L), TSS (96 mg/L), NO_3^- (0.134 mg/L), NO_2^- (0.058 mg/L), orthophosphates (0.524 mg/L), turbidity (87 FTU), with notably reduced mean fresh biomass (2.9 g) and root length (7.3 mm) which were moderately and negatively correlated with NO_2^- and orthophosphates, respectively. Only EC failed to improve after *L. aequinoctialis* incubation significantly. Despite spatial-temporal limitations, we present limited evidence suggesting a potential real-time, dual utility role that can help track, redress pollution, and suppress algal blooms enhancing public health and transcending the typical role of duckweeds. Long-term applicability of this phytotechnology requires further attention for sustainable freshwater management in resource-limited regions like Zimbabwe.

Physicochemical properties of mesoporous acid activated materials from *Lemna minor* for Bezaktiv Red S-MAX dye removal

Stambouli, GB; Benguella, B; Makhoukhi, B; El-ouchdi, MS; Kamel, AH. Analytical Methods (2025) 17: 2134-2143.

Lemna minor, a cheap and easily accessible substance, was utilized to create activated carbon through acid activation [with H_3PO_4 (ALM-P) and citric acid (ALM-C)]. Brunauer-Emmett-Teller (BET) analysis reveals that the *L. minor* activated materials (LACs) had a surface area of 208.54 $m^2\ g^{-1}$ and 216.11 $m^2\ g^{-1}$ after H_3PO_4 and citric acid activation, respectively. The presence of rich hydroxyl, carboxyl, amide, and phosphate functional groups on the surface of LACs is revealed by Fourier transform infrared spectroscopy (FTIR). This enables easy

Bezaktiv dye, type Red S-MAX (BRSM), binding to the surface through strong chemisorptive bonds or ion exchange. With a maximal mono-layer adsorption capacity (m^2) of 16.86 and 7.69 mg g^{-1} at 25°C, the pseudo-second-order model and Langmuir isotherm provided a good description of the kinetic and equilibrium data. The adsorption was caused in part by the intra-particle diffusion mechanism. With negative Delta G degrees and positive Delta H degrees values, the adsorption process was spontaneous and endothermic. Under optimum conditions of pH 3.0 and 25°C, the maximum % removal for BRSM (90 mg L^{-1}) was reported to be 77.65% within 90 min. These indicated that dye-contaminated water might be treated with the inexpensive LACs as a possible adsorbent.

Optimal cultivation concentration of duckweed for pollutant removal from biogas slurry

Li, JQ; Gao, Y; Xie, YR; Li, JY; Li, JZ; Ran, C. Scientific Reports (2025) 5: 5193.

Duckweed is an important plant for wastewater remediation and a promising alternative protein source for animal feed. This study aims to evaluate the biomass, protein accumulation, and wastewater purification capacity of duckweed under controlled cultivation conditions. Using pig farm biogas slurry as a nutrient source, this study analyzes the purification ability of duckweed at different slurry concentrations (2%, 4%, 6%, 8%, 10%) on chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), and ammonia nitrogen (NH_3-N) in biogas slurry. The study also describes changes in duckweed weight, chlorophyll content, and protein accumulation. Our results showed that a 4% biogas slurry concentration was the optimum condition for the cultivation of duckweed, which corresponded to an NH_3-N concentration of 29.56 mg/L. The dry matter production capacity of duckweed under these conditions was 1.78 g/dm^2, the relative growth rate (RGR) was 0.29 g/ g d, the doubling time was 2.42 days, and the protein content was 36.25% by dry weight. In terms of pollutant removal, duckweed efficiently removed 54.69% of COD, 86.89% of TN, 97.25% of NH_3-N, and 85.22% of TP. The results of this study provide an important reference for the operation of the duckweed production system, which is crucial for the design and operation of pilot-scale and large-scale duckweed production systems moving forward.

Links between two duckweed species (*Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleid.), light intensity, and organic matter removal from the water - An experimental study

Peczula, W. Water(2025) 17: 438.

Duckweeds -a group of floating leaf macrophytes from the family of Lemnaceae- have become a major area of interest in the fields of basic and applied aquatic sciences in recent decades, including their use in water purification. Aiming to fulfil one of the gaps in the role of light intensity in duckweed efficiency in organic matter removal, we carried out a laboratory experiment with the use of two duckweed species: *Lemna minor* and *Spirodela polyrhiza*. Our main finding was that the intensity of light has a positive effect on the process of water purification from organic compounds by *Lemna minor*. However, this was not applicable to *Spirodela polyrhiza* due to the fact that the growth of the species was inhibited by high light intensities.

Phytotoxicity and phytoremediation potential of *Lemna minor* exposed to perfluorooctanoic acid

Noori, A; Corbelli, L; Lincoln, E; Thomas, S; Jones, J; Nason, SL; White, JC; Lewis, R; Haynes, CL. Frontiers in Plant Science (2025) 15: 1493896.

Perfluorooctanoic acid (PFOA) is one of the highly toxic compounds which was phased out of application in consumer products in 2015 due to its harmful effects on human and environmental health. However, this chemical was in use for many years and is still found in water resources. This study focuses on the physiological response of duckweed (*Lemna minor*) exposed to PFOA so as to determine phytotoxicity and the potential of this aquatic species to remove PFOA from the environment. A time-dependent phytotoxicity assay showed that exposure to 0.1 µg/ L PFOA for 14 days resulted in the loss of chlorophyll pigment and 15-25% more chlorosis than in controls. Although exposure to PFOA for seven days resulted in chlorosis, no significant impact on physiological parameters such as photosynthetic pigment or anthocyanin content were detected. The analysis of cellular size on day zero and seven of the experiment showed that the control group showed significantly larger cell size after seven days (213±6.5 µm^2) compared with the day zero group (186±18 µm^2),

while the size of the PFOA exposed group (198±13 μm^2) did not change significantly after seven days compared with the day zero group. The nuclear size increased significantly by 13% upon exposure to PFOA compared with the controls (rho < 0.0001). The concentration of essential elements K, Cu, Fe, Mn, Zn, Mo were reduced in *L. minor* exposed to PFOA compared with the controls by 39.6, 33.4, 42.1, 35.2, 31.9, 40.2%, respectively. Additionally, PFOA accumulated in *L. minor* fronds and roots with an average bioaccumulation factor of 56±7. Overall, while some symptoms of toxicity were observed, this study shows that *L. minor* can tolerate up to 0.1 g/L PFOA, a commonly found concentrations in water bodies, and can remove PFOA from water. This study provides invaluable information regarding the phytotoxicity impacts of PFOA on aquatic species and the potential for aquatic phytoremediation of PFOA.

Sustainable dye wastewater treatment: utilizing duckweed-derived adsorbents for efficient methylene blue removal

Jeffrey, KB; Zheng, ALT; Hii, TT; Seng, KWK; Chung, ELT; Lease, J; Andou, Y. Biomass Conversation and Biorefinery (2024) DOI10.1007/s13399-024-06432-1.

Invasive plants threaten natural ecosystems due to their rapid spread and growth. This study used duckweed (DW) to develop a low-cost and sustainable adsorbent to remove methylene blue (MB) from aqueous solution. The adsorbent was analysed using various techniques. Fourier transform infrared spectroscopy (FT-IR) identified oxygen-containing functional groups on the DW surface that enhanced its adsorption capacity. Optimal adsorption conditions were achieved with an adsorbent amount of 20 mg, a pH of 10, and an initial MB concentration of 10 mg/L. Experimental data matched the pseudo-second-order (PSO) model across all concentrations, including 10 mg/L (Qe = 73.53 mg/g), 20 mg/L (Qe = 183.15 mg/g), and 50 mg/L (Qe = 200.80 mg/g). The Temkin isotherm model fit the data better than the Langmuir and Freundlich models. Adsorption decreased with increasing temperatures (Delta Go = - 7.56 to - 8.06 kJ/mol, Delta Ho = - 4.93 kJ/mol, Delta So = - 6.76 kJ/mol). The primary adsorption mechanisms included electrostatic interactions, hydrogen bonding, and pi-pi electron interactions. The production cost of the DW adsorbent was estimated at RM 14.40 per kg, making it a more affordable alternative. With its high adsorption capacity and wide availability, DW is a promising material for controlling dye pollution in wastewater.

Phytotoxicity

Population-specific responses of *Lemna minor* to silver nanoparticle exposure: Implications for standardizing toxicity assessments

Wang, QQ; Wei, SS; Cheng, DM; Yuan, LY; Li, W; Jiang, HS

Aquatic Toxicology (2025) 283: 107332.

The globally distributed and excellent growth properties of *Lemna minor* make it an ideal model species in ecotoxicology. However, the variability among different *L. minor* populations is often overlooked in laboratory toxicity assessments, which could lead to inaccurate toxicity evaluations, especially for newly emerging pollutants. In this study, we investigated the responses of *L. minor* populations from various regions (Wuhan (WH), South Korea (KR), Yunnan (YN), and Tibet (TB)) to silver nanoparticles (AgNPs), a newly emerging pollutant, at concentrations ranging from 0 to 10 mg L^{-1} over a 72-h exposure period. The results showed a significant increase in silver accumulation in *L. minor* tissues with increasing AgNPs concentration. Concurrently, photosynthetic pigments content (chlorophyll a, b, and carotenoids) and chlorophyll fluorescence parameters exhibited a dose-dependent decline, while malondialdehyde levels increased, indicating that AgNPs induced oxidative stress in different *L. minor* populations. Notably, the populations displayed significant differences in tolerance to AgNPs: the KR population showed the highest tolerance, followed by TB, while the YN and WH populations were more sensitive. Further analysis revealed that the differences in toxicity response among *L. minor* populations were mainly attributed to variations in Ag accumulation capacity. Therefore, it is recommended that, when using *L. minor* from different regions to assess AgNPs toxicity, parameters could be standardized based on the silver accumulated by the plants rather than the externally applied silver. This approach will improve the comparability of results across laboratories and provide a more accurate understanding of AgNPs toxicity in global aquatic ecosystems.

Bismuth accumulation and toxicity in freshwater biota: A study on the bioindicator species *Lemna minor* and *Echinogammarus veneris*

Iannilli, V; Passatore, L; Carloni, S; Massimi, L; Giusto, C; Zacchini, M; Pietrini, F. The Science of the Total Environment (2025) 975: 179263

The heavy metal bismuth (Bi) is attracting increasing interest for its wide range of applications, from industrial processes to medicine. Given the foreseeable increase in its use, the occurrence of Bi in the environment is expected to increase. There is a lack of information on the impact of this metal on biota, especially for the aquatic ecosystem. In this regard, an experimental study was performed under controlled conditions to assess the effects of Bi on two bioindicator species of the freshwater compartment, namely plants of *Lemna minor* L. (Lemnoideae) and individuals of *Echinogammarus veneris* (Heller, 1865) (*Amphipoda*, *Gammaridae*). A 7-day assay in *L. minor* fronds exposed to Bi nitrate in the range of 0-242 mg L^{-1} showed no effects of the metal on biometric and physiological endpoints (spectral reflectance indices and chlorophyll fluorescence parameters). In parallel, *E. veneris* individuals were treated with Bi nitrate (0-242 mg L^{-1}) for 24 h to assess genotoxicity by comet assay. The results showed significant Bi-induced DNA damage in gammarids even at the lowest Bi concentrations tested. The analysis of Bi content revealed the high capacity of both species to accumulate the metal in their tissues, demonstrating the ability of *L. minor* fronds to tolerate the presence of a relevant amount of Bi in solution, whereas *E. veneris* individuals showed a remarkable sensitivity to the presence of the metal. The effects of Bi observed in the two aquatic organisms represent the first evidence of a species-specific toxic action of this metal in the freshwater ecosystem.

Multigenerational toxicity effects and impact of antibiotics exposed to duckweed, *Lemna minor*

Alfarsi, A; Weird, GM; Kumar, A; Nugegoda, D. The Science of the Total Environment (2025) 977: 179324.

The escalating presence of antibiotics in aquatic ecosystems poses substantial risks to public health and ecosystem stability. The objective of this study was to examine the effects of three common antibiotics-ciprofloxacin (CIP), erythromycin (ERY), and sulfamethoxazole (SMX)-on the growth and physiology of *Lemna minor* across three generations (parental (F0), first filial (F1), and second filial (F2)). Specifically, the research aimed to determine how these antibiotics influence frond number, frond area, root area, and photosynthetic pigment content in *L. minor*. Higher concentrations of CIP (50 µg/L, 250 µg/L, and 1250 µg/L) significantly decreased frond numbers (F2>F1>F0), while ERY exhibited the opposite trend, and SMX displayed adaptation in F2. ERY increased frond area at a lower concentration (10 µg/L), while high concentrations of CIP (250 µg/L and 1250 µg/L) and lower concentrations of SMX (10 µg/L and 50 µg/L) reduced it. CIP displayed a biphasic response on root growth, with 10 µg/L decreasing root area by 760 μm^2 and 50 µg/L and 1250 µg/L, increasing it by 2480 μm^2 and 2300 μm^2, respectively. ERY consistently inhibited root growth. The F1 generation showed the most pronounced reduction in green area, particularly under higher CIP concentrations (1250 µg/L). Chlorophyll A (Chl A) and carotenoid contents were resilient to antibiotic stress, while Chlorophyll B (Chl B) exhibited generation-specific responses. This study highlights the need for continued monitoring of antibiotics in aquatic systems and calls for further research on the long-term impacts of antibiotics on aquatic plants and ecosystems.

Extracellular vesicle GABA responds to cadmium stress, and GAD overexpression alleviates cadmium damage in duckweed

Sun, ZP; Qu, ZY; He, YM; Han, YJ; Xing, Y; Liu, SZ; Hu, Y; Jiang, YM; Yu, YQ; Liu, YY; Sun, WB; Yang, L. Frontiers in Plant Science (2025) 16: 1536786.

Cadmium (Cd) pollution lead to ecological problems and cause severe damages to plants. Investigating the signal response to Cd is crucial for improving Cd resistance during phytoremediation. While gamma-aminobutyric acid (GABA) is known to accumulate rapidly under environmental stress, the real-time dynamics of GABA signaling and its mechanistic link to stress adaptation remain poorly understood. In this study, a sensitive GABA biosensor, iGABASnFR, was introduced into plants for the first time to monitor GABA signaling. Additionally, glutamate decarboxylase (GAD), a key enzyme catalyzing the conversion of glutamate (Glu) to GABA, was overexpressed in duckweed. The responses of GABA in extracellular vesicles (EVs) under Cd stress were analyzed using iGABASnFR transgenic duckweed. Cd accumulation, photosynthesis, and antioxidant activity were evaluated in GAD-overexpressing duckweed. (1) GABA in extracellular vesicles of duckweed exhibited a dynamic response to Cd stress, as visualized by iGABASnFR transgenic duckweed. GABA content

in EVs was significantly enhanced under Cd treatment. (2) GAD-overexpressing duckweed demonstrated improved photosynthetic efficiency and enhanced antioxidant capacity during Cd stress. (3) Cd accumulation was significantly increased in GAD transgenic duckweed, as evidenced by Cd^{2+} flux measurements, total Cd content, and Cd staining in protoplasts using FlowSight imaging. This study provides novel insights into the role of GABA in extracellular vesicles during Cd stress and establishes a direct link between GABA signal and Cd stress adaptation. The findings demonstrate that GAD overexpression enhances Cd resistance and accumulation in duckweed, offering a potential strategy for improving phytoremediation efficiency. This work advances our understanding of GABA signaling dynamics and its application in Cd stress.

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

Gjata, I; Tommasi, F; De Leonardis, S; Paciolla, C. Frontiers in Plant Science (2025) 16: 1540266.

Rare earth elements (REEs) are increasingly utilized in modern technologies but are now recognized as emerging pollutants, with limited understanding of their impact on aquatic ecosystems. In this study, the effects of selected REEs (Ce, Nd, Gd, Dy, Yb, Ho, and Lu) in chloride form were evaluated on *Lemna minor* L., a plant species widely used as tool for the biomonitoring of the aquatic environments. Under controlled laboratory conditions, growth parameters, pigment content, oxidative stress markers, total antioxidant capacity, and antioxidant enzyme activities were assessed at millimolar concentrations over different exposure periods. *L. minor* exhibited tolerance to low millimolar concentrations of REEs over short-term exposure. However, prolonged exposure to high concentrations resulted in toxicity, characterized by growth inhibition, chlorophyll degradation, increased lipid peroxidation, and oxidative stress. Particularly, a hormetic response was observed for cerium, with stimulation at low concentrations and inhibition at higher levels, while dysprosium did not significantly affect growth. Other tested REEs induced varying degrees of stress, with holmium and lutetium causing the most severe toxic effects. Changes in antioxidant enzyme activities indicated a differential activation of stress responses depending on the REE type. These findings highlight the necessity for continuous monitoring of REEs in aquatic systems and support the use of *L. minor* as a valuable tool for environmental risk assessment.

Evaluation of ecotoxicological effects of sophorolipids on duckweed, *Spirodela polyrrhiza*

Shao, JQ; Li, GS; Jiang, RY; Jin, ZG; Han, HP; Huo, YF; Xun, SP; Ma, XJ. Journal of Surfactants and Detergents (2025) DOI10.1002/jsde.12845.

This study evaluated the ecotoxicological effects of sophorolipids (SLs), biosurfactants with potential environmental applications, on the aquatic plant *Spirodela polyrhiza* (duckweed). Key growth indicators, including biomass, foliose thalli, and leaf area, were examined under varying SL concentrations. Results indicated that low to moderate SL concentrations (below 100 mg/L) had minimal adverse effects on duckweed growth, whereas higher concentrations (200-300 mg/L) significantly inhibited growth and triggered stress responses. Morphological and physiological assessments revealed that elevated SL concentrations caused substantial frond wilting, root hair damage, and reductions in photosynthetic pigments. Antioxidant responses, such as glutathione (GSH), malondialdehyde (MDA), and catalase (CAT) enzyme activities, increased adaptively at low to moderate SL concentrations but declined at higher levels, indicating severe oxidative stress. Despite these adverse effects, *S. polyrhiza* exhibited a remarkable ability to degrade SLs even at high concentrations, demonstrating the exceptional biodegradability of SLs. Overall, the findings emphasize the relative safety of SLs at low to moderate concentrations in aquatic environments, underscoring their potential as environmentally friendly surfactants when used responsibly.

The dual impact of tire wear microplastics on the growth and ecological interactions of duckweed *Lemna minor*

Putar, U; Turk, K; Jung, J; Kim, C; Kalcíková, G. Environmental Pollution (2025) 368: 125681.

Tire wear microplastics (TWMs) are continuously generated during driving and are subsequently released into the environment, where they pose potential risks to aquatic organisms. In this study, the effects of untreated, hydrated, and aged (in stream water) TWMs on the growth, root development, photosynthesis, electron transport system (ETS) activity, and energy-rich molecules of duckweed *Lemna minor* were investigated. The results indicated that untreated and aged TWMs have the most pronounced negative effects on *L. minor*, as

evidenced by reduced growth and impaired root development. In contrast, the effects of hydrated TWMs were less pronounced compared to untreated and aged TWMs. The negative effects associated with untreated and hydrated TWMs are primarily attributed to the abrasive nature of these particles, which physically damage the plant tissue. On the other hand, aged TWMs showed a different mode of action as they serve as transport vectors for algae. Once introduced into a new environment via aged TWMs, these algae competed with *Lemna minor* for available nutrients and space, further impairing the growth, root length, photosynthetic efficiency, and carbohydrate content of *L. minor*. This study revealed the dual threat posed by TWMs: direct physical damage from newly released particles and indirect ecological disruption from aged particles that facilitate the spread of algae.

Global sensitivity analysis of the harmonized *Lemna* model

Guisnet, C; Reichenberger, S; García, EA; Voss, F. Ecologial Modelling (2025) 501: 111016.

Mechanistic effect modelling is becoming increasingly important for environmental risk assessment in the framework of pesticide authorization. For instance, the European Food Safety Authority (EFSA) has judged the model for the aquatic macrophyte test organism *Lemna* as "ready for use." Nevertheless, national regulatory authorities are still hesitant to accept mechanistic effect modelling studies. In order to increase the confidence in the *Lemna* model, in this study we performed a two-step global sensitivity analysis (GSA) of the harmonized model. GSA notably allows identifying and ranking the importance of i) toxicokinetic (TK) and toxicodynamic (TD) parameters, ii) physiological and ecological parameters, iii) environmental driving variables, and iv) initial conditions. In a first step a Morris sensitivity screening was conducted to filter out non-influential input factors. In a second step, a true variance-based GSA was carried out with the Sobol method. The GSA was conducted for four different concentration levels and three different exposure regimes: constant, pulsed and realistic. Moreover, two different sets of input distributions of TKTD parameters were examined. The target variables were the effects of the pesticide on *Lemna* biomass and average growth rate. Both Morris and Sobol GSA showed that for a specific substance three physiological parameters (optimum and minimum growth temperature, maximum photosynthesis rate) and the initial biomass BM0 were more important than the five TKTD parameters. Hence, for predictive applications of the model outside a laboratory context, BM0 must be chosen carefully, and uncertainty in the main physiological parameters must be reduced to a minimum.

Effect of phosmet toxicity on some physiological traits in duckweed (*Lemna gibba* L.)

Yilmaz, S; Bozlar, E; Dogan, M. Aquatic Sciences and Engineering (2025) 40: 18-21.

Pesticides, one of the chemicals that adversely affect the environment and human health, can have effects on non-target organisms due to their chemical properties and wide range of use. Therefore, in this study, the toxic effects of phosmet insecticides on *Lemna gibba*, an aquatic macrophyte, were determined as a non-target organism. The study was carried out in a climate cabinet under controlled conditions. It was determined that the photosynthetic pigments and total carbohydrate content of the macrophyte decreased with increasing phosmet concentration. Similarly, decrease in total phenolic contents were found. A significant and positive correlation between non-protein sulfhydryl groups (NP-SH) and H_2O_2 contents may indicate their role in antioxidant defense mechanism. Besides, increases in malondialdehyde (MDA) and H_2O_2 contents showed that caused oxidative stress in *L. gibba* tissues.

Protective effects of hesperidin and salicylic acid on *Lemna minor* L. exposed to Evercion yellow textile dye

Akbulut, G; Turhan, D; Kivilcim, N; Gueltek, A; Yigit, E. KSU Tarim Ve Doga Dergisi - KSU Journal of Agriculture and Nature (2025) 28: 351-363. DOI10.18016/ksutarimdoga.vi.1501836

Hesperidin (HES) is a flavonone glycoside from the flavonoid family that is present in citrus species. It has potent anti-oxidant and anti-cancer properties. In times of stress, the phenolic chemical salicylic acid (SA), also known as a plant hormone, functions as a signal molecule, controlling the plant's reaction and maintaining its survival. For the removal of numerous harmful chemicals, phytoremediation, sometimes referred to as green reclamation, is an efficient, affordable, environmentally benign, and simple procedure. Duckweed (*Lemna minor* L.) is an important bioindicator species in phytoremediation study. Following the application of 75 ppm, 150 ppm, and 300 ppm reactive dye Evercion yellow 1X, the effects of 0.5 mM SA and 0.5 mM hesperidin on duckweed (*L. minor* L.) were examined in this study. The use of 0.5 mM SA against stress boosted the

activities of peroxidase (POD), ascorbate peroxidase (APX), glutathione S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT). Additionally, total glutathione (GSH), total chlorophyll, and carotenoid content were altered by SA treatment. Similar to the SA application, the application of HES was effective in lowering stress. Lipid peroxidation content measured as malondialdehyde (MDA) content was found to be higher than the control groups. Results suggest that SA plays a positive role in *L. minor* against Evercion yellow 1X.

Some physiological effects of bisphenol A on *Lemna gibba* L., a free-floating aquatic macrophyte

Dogan, M; Yilmaz, S; Yigit, S. KSU Tarim Ve Doga Dergisi - KSU Journal of Agriculture and Nature (2025) 28: 20-24.

The present study was carried out to evaluate the effect of bisphenol (BPA) on *Lemna gibba*, a free-floating aquatic macrophyte, in a climate cabinet under controlled conditions. *L. gibba* was collected from natural water sources in Gaziantep (Türkiye) and acclimatized for two weeks containers containing 10% nutrient solution. Macrophytes were treated with 1.5, 17.2, and 50 mg/L BPA for 96 hours. Chlorophyll a, chlorophyll b, carotenoid, protein, and total soluble carbohydrate contents were declined following BPA application. Contrary to this, an elevation in contents of NP-SH, H_2O_2, and malondialdehyde were detected. Correlation analyses showed that the changes may be related to BPA-induced oxidative stress.

Comparative efficacy of melatonin and brassinolide in mitigating the adverse effects of cadmium on *Wolffia arrhiza*

Chmur, M; Bajguz, A. International Journal of Molecular Sciences (2025) 26: 692.

Melatonin (MT) and brassinolide (BL) are phytohormones that regulate various physiological processes in plants. This study investigates their effects on *Wolffia arrhiza* when exposed to cadmium (Cd). Plant hormones were quantified using liquid chromatography-mass spectrometry, while photosynthetic pigments and phytochelatins (PCs) were analyzed through high-performance liquid chromatography. Protein, monosaccharide levels, and antioxidant activities were also spectrophotometrically measured. The findings reveal that MT and BL treatment decreased Cd accumulation in *W. arrhiza* compared to plants only exposed to Cd. MT was particularly effective in reversing Cd-induced growth inhibition and reducing stress markers more significantly than BL. It also enhanced antioxidant activity and maintained higher levels of photosynthetic pigments, proteins, and sugars. Although BL was less effective in these aspects, it promoted greater synthesis of glutathione and PCs in Cd-exposed duckweed. Overall, both MT and BL alleviate the negative impact of Cd on *W. arrhiza*, confirming their crucial role in supporting plant health under metal stress conditions.

Taxonomy & Geobotany

A survey of duckweed species in Southern Italy provided first distribution records of the hybrid *Lemna x mediterranea* in nature

Romano, LE; Braglia, L; Iannelli, MA; Lee, Y; Gavazzi, F; Morello, L. Perspective in Plant Ecology Evolution and systematics (2025) 67: 125863.

Interspecific hybridisation and polyploidization are two main driving forces in plant evolution, shaping genomes and favouring evolutionary novelty and ecological adaptation. Recent studies have demonstrated hybridisation within the genus *Lemna* (Lemnaceae Martinov) as well as triploid accessions. *Lemna x mediterranea*, a recently described hybrid between *Lemna minor* and *Lemna gibba*, was identified only among long-lasting germplasm collections of in vitro propagated plants, originally collected at different times in the Mediterranean area. We report the first distribution record of *L. x mediterranea* in the nature, in the Campania region of Southern Italy, the same area where *Lemna symmeter* was described as a new species about 50 years ago, confirming their synonymy. Eight specimens isolated from five different sampling sites over an area of about 4200 km^2 showed identical genetic profiles by Tubulin-Based Polymorphism (TBP) analysis, suggesting their common origin from the same hybridisation event, followed by clonal dispersal. The *L. x mediterranea* population of Campania is genetically different from any of the previously analysed clones, suggesting that recurrent hybridisation between the parental species may occur. The natural hybrid clone is

triploid, with *L. gibba* as the plastid donor, and remarkably similar to it by morphology, although the typical gibbosity of this species becomes evident only upon in vitro flower induction. Flowers are protogynous and self-sterile. Ecological factors including competition with parental and invasive species, niche and climate change adaptation, stability in time and space likely played a role in the successful establishment of *L. x mediterranea*.

Contributing to the knowledge of the distribution of the world's smallest angiosperms in the Amazon: first record of *Lemna minuta Kunth* (Araceae) in the state of Pará, Brazil

Soares, WCR; Comassetto, TP; Fidanza, K. Acta Amazonica (2025) 55: e55bc24248

Lemnoideae is a yet little known taxon in Brazil in taxonomic and distributive terms, given the few records for the country, especially in the Amazon region. Here, we present the first record of *Lemna minuta* Kunth for the state of Para, providing a description of the species, its occurrence in Brazilian territory, and additional comments. This contributes to expanding the knowledge of the species' distribution in the Amazon.

Population structure analysis and molecular characterization of duckweed genotypes using iPBS and ISSR markers

Coskun, OF; Aydin, D. Bangladesh Journal of Botany (2024) 53: 871-880.

Duckweed belongs to subfamily Lemnoideae, considered as potential minor vegetable for human consumption. Genetic diversity and molecular characterization of 67 duckweed genotypes grown in different ecosystems were determined using ISSR (Inter Simple Sequence Repeats) and iPBS (Inter-Primer Binding Site) markers. ISSR and iPBS methods identified 100% polymorphism, with genetic similarity coefficients between *Spirodela* and *Lemna* species ranging from 0.17 to 0.56. Structure analysis conducted according to K=6 calculation revealed that *L. minor* had the most homogeneous and diverse populations. Duckweed genotypes and species displayed a wide range of genetic diversity. Using various marker techniques, the genetic structure of duckweed was determined and characterized it as an alternative, sustainable, and cheap source of protein, vegetables, and biofuel. The results obtained from this study will be useful for understanding molecular mechanisms in future genetic improvements of duckweed genotypes and species.

Instructions to Contributors for the Duckweed Forum

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

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

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

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

- A commonly used word processing program, such as Word, is highly recommended.

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

Title:

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

Authors:

- All authors are responsible for the content of the manuscript.
- Provide the complete names and head-shots of all authors.
- Identify which author will receive correspondence regarding the contribution.
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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