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DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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Lemna trisulca

Cover page

Lemna trisulca: Attached ramets are shown here in a cluster of *Lemna trisulca* to illustrate the successive production of clonal duckweed plants from the pockets of each frond where lies the meristematic tissue. In this duckweed species, the ramets can remain attached without separation for multiple generations and thus produce a mesh of connected plants that stays submerged. For more details, refer to the article by Lam and Bai in this issue at page 151. Photo/image by Eric Lam (Rutgers University, USA).

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:

October 30th, 2025

Dear Duckweed Community,

Greetings to All at the end of the festive season of Diwali, here in India, filling the environment with positivity.

We, the members of the ISCDRA, take great pleasure in presenting this year-end issue of our community newsletter, DUCKWEED FORUM. We are very glad about the increasing interest and quality of the publications with each growing issue. We encourage each one of you to contribute actively to the Duckweed Forum by sharing your views and perspectives with the peers and also by participating in its activities for the growth of the duckweed community as a whole.

The commentaries on the articles chosen as highlights in the previous issue of Duckweed Forum make interesting reads on these topics. The first one by Muller and Bazinet details on how the processing of duckweed protein can increase its functionality, thereby making it a potential competitor in the food industry. The second commentary by Stepanenko and Lee talks about the broader topic of genetic diversity and evolution in duckweeds by focusing on the phylogeny of the section Alatae of the genus *Lemna*. Followed by the commentaries is the sequel of the opinion piece on duckweed nomenclature by Lam and Bai which presents an interesting discussion on the topic based on the suggestions received from some of the members of the duckweed community for the opinion piece published in the previous issue. We have two student spotlights in this issue, one by Marie Lambert from Belgium and the other by Raja Amri from Hungary, encouraging young researchers to step into the huge arena of duckweeds. As always, the database section compiled by the ISCDRA member, Klaus Appenroth, is serving the community to keep up-to-date with the latest in the field.

It is a very joyous moment to see duckweed occupy the front-line of food industry. A report by Lambert et al. of the duckweed workshop focused on advancing water lentils as novel human food, announcement of duckweed-AgTech breakthrough using their cultivation system by Tsipi Shoham and the success of *Wolffia* at the food related expo in Thailand as reported by Meetam and Tira-umphon, all of them point towards the increasing reach of duckweeds in the commercial sector.

Now, in less than a year, we will meet at the 8th ICDRA to be held at Portici, Italy from September 28th to October 2nd, 2026. The organizers are open with the pre-registration. All interested are welcome to pre-register so as to be informed directly about the upcoming schedules. Also, find the announcement welcoming all those interested to attend the duckweed focused workshop organized by Eric Lam and Todd Michael at the PAG 33 conference to be held at San Diego, USA from 9-14 January, 2026.

Enjoy reading !!!

Best regards,

Sincerely,
K. Sowjanya Sree
Chair, ISCDRA

Commentary: Processing matters

How extraction and purification can enhance duckweed protein functionality as a food ingredient

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Duckweed is widely recognized for its exceptional productivity (Ziegler et al., 2015) and balanced nutritional profile (Appenroth et al., 2018, 2017), making it one of the most promising sources of sustainable plant proteins. However, as shown by de Beukelaar et al. (2019), the direct inclusion of whole duckweed in food products is not always well received by consumers due to its appearance and color. A logical next step is therefore to extract and purify duckweed proteins so they can be used as functional ingredients, thereby improving the texture and stability of foods rather than appearing as visible components.

With this goal in mind, I (Dr. Tristan Muller) began my doctoral research at Université Laval (Québec, Canada) under the supervision of Professor Laurent Bazinet in 2021, completing it in 2025. The first stage focused on developing and optimizing a method to efficiently extract and purify duckweed proteins from dried biomass. In a first study, we designed a two-step process combining high alkaline and high temperature extraction followed by acid precipitation to produce a duckweed protein concentrate with maximized protein recovery (Muller et al., 2023). This method produced a protein concentrate containing about 55 % protein with a 60 % recovery rate, which is remarkably high for leaf proteins. The process also generated a saline by-product, rich in minerals but still containing nearly 20 % protein, opening the door to its valorization rather than waste.

Building on this foundation, we then explored a greener alternative to chemical purification (where we used HCl) using electrodialysis with bipolar membranes (EDBM), an electrochemical process that generates acid and base directly from water under an electric field, thereby eliminating the need for chemical reagents (Muller and Bazinet, 2025a). This approach successfully precipitated duckweed proteins while simultaneously demineralizing the saline by-product. The salt content of the by-product dropped from roughly 60 % to 15 %, while protein concentration nearly doubled, demonstrating that electrochemical purification can achieve both high recovery and environmental benefits.

Once the purification methods were established, we turned our attention to the composition, structure, and functionality of the resulting protein fractions (Muller and Bazinet, 2025b). Proteomic analyses revealed that the native duckweed protein from the initial powder (IP) contained a mixture of proteins, including about 40 % RubisCO, while the purified concentrates obtained by chemical or electrochemical precipitation (PP_{ch} and PP_{ed}, respectively) and their by-products (PS_{ch} and PS_{ed}, respectively) consisted mainly of RubisCO (80–85 %), known for its high solubility and versatile functional properties (Barbeau and Kinsella, 1988). These findings were consistent with previous observations by Nieuwland et al. (2021).

Spectroscopic analyses (FTIR-ATR) indicated a clear protein structural reorganization during purification. Aggregated intermolecular β-sheets present in the native proteins from the IP gave way to more intramolecular β-sheets and α-helices in PP_{ch} and PP_{ed}, while PS_{ch} and PS_{ed} were mostly composed of α-helices. These structural changes reduced aggregation and enhanced flexibility, which are generally associated with improved protein solubility and interfacial behavior (Rawiwan and Quek, 2024).

To connect structure with performances, we then compared the functional properties of all samples, including the native proteins, the purified fractions, and commercial isolates which were egg white (EGW), whey protein isolate (WPI), and soy protein isolate (SPI). While many recent studies have examined one or two functional properties of duckweed proteins (Duangjarus et al., 2022; Nieuwland et al., 2021; Siriwat et al., 2023), such a comprehensive comparison with commercial isolates remain scarce.

We observed that native duckweed proteins from the IP were poorly soluble at neutral pH (pH 7), with solubility below 10 %. In contrast, all purified fractions reached solubilities above 85 %, comparable to EGW and WPI. SPI showed lower solubility, as sometimes observed depending on processing.

In foaming tests, native proteins from the IP displayed minimal ability to form or stabilize foams, likely due to aggregation and low solubility (Figure 1). The purified fractions performed far better: PP_{ch} and PP_{ed} exhibited moderate foam volumes and a moderate stability, which was similar to WPI and SPI. The foam stability of these products was lower than that of egg white (EGW), which remains the gold standard for foaming properties. Meanwhile, PS_{ch} and PS_{ed}, with their looser α-helical structures, produced large yet short-lived foams. The higher flexibility of α-helices favors rapid adsorption at the air–water interface and bubble formation, but the lack of β-sheets reduces network integrity, leading to quick foam collapse. Conversely, the mixed α/β composition of PP_{ch} and PP_{ed} allowed for slower bubble formation but more cohesive, stable foams (Rawiwan and Quek, 2024).

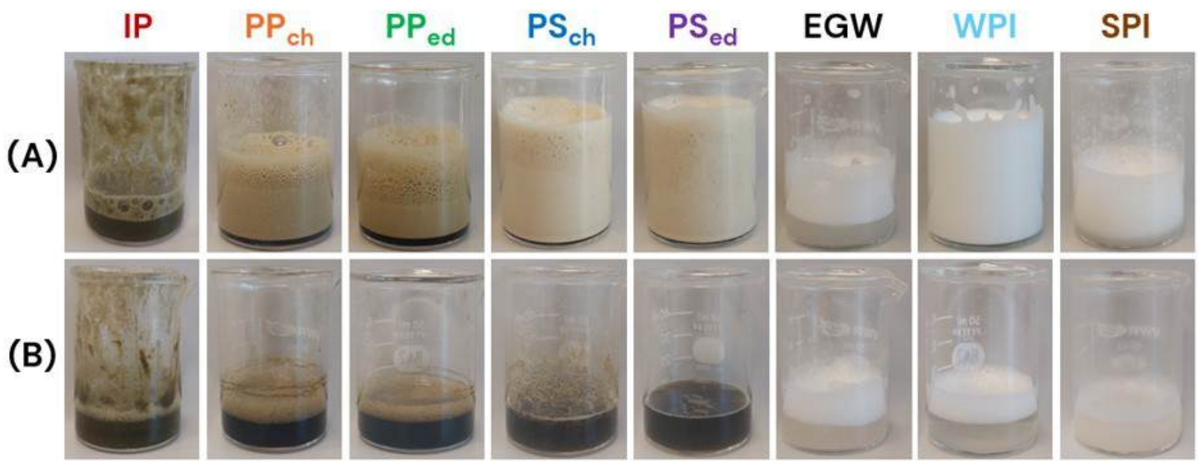

Figure 1. Representative pictures of foams directly after whipping (A) and after 2 hours (B) of the IP, PP_{ch}, PP_{ed}, PS_{ch}, PS_{ed}, EGW, WPI and SPI, from Muller and Bazinet (2025b).

In gelling tests, the IP formed a very viscous suspension rather than a true gel, whereas PP_{ch} and PP_{ed} produced firm heat- and acid-induced gels. Their firmness was slightly lower than that of EGW but higher than SPI (Figure 2). Remarkably, other tests showed that these gels could be formed at only 1.5 % protein, which is much lower than the 10–15 % typically required for commercial isolates. This strong gelling ability likely stems from RubisCO's high gel-forming capacity (Barbeau and Kinsella, 1988; Nieuwland et al., 2021) combined with the enhanced β-sheet content promoting hydrophobic interactions and network formation. By contrast, the loose α-helical structure of PS_{ch} and PS_{ed} prevented sufficient protein aggregation to form gels.

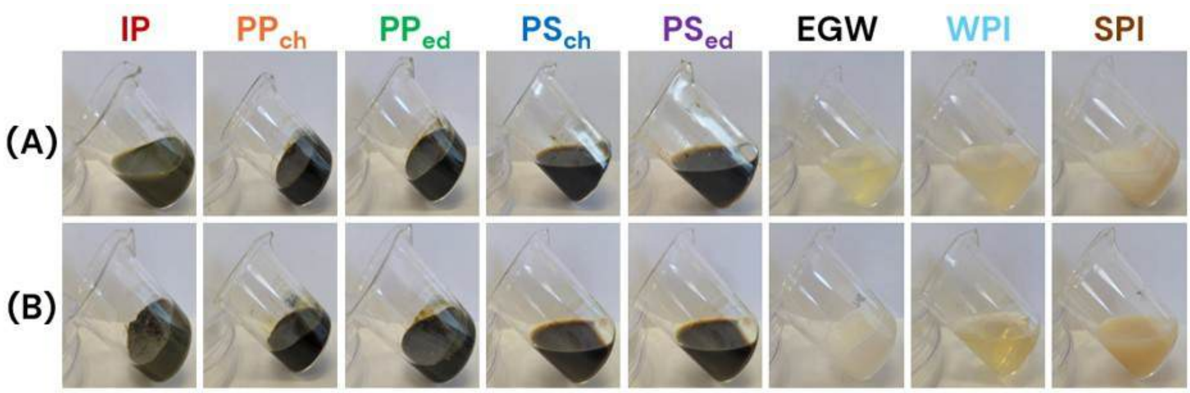

Figure 2. Representative pictures of an acid-induced (A) or heat-induced gels (B) of a 5.0% protein solution (w/v) of IP, PP_{ch}, PP_{ed}, PS_{ch}, PS_{ed}, EGW, WPI and SPI, from Muller and Bazinet (2025b).

In emulsifying tests, PS_{ch} and PS_{ed} again performed best, displaying high emulsifying activities and stabilities comparable to or exceeding commercial isolates. Their flexible α-helical structures favored alignment at the oil–water interface, forming stable emulsions (Rawiwan and Quek, 2024). In contrast, the more compact conformations of PP_{ch} and PP_{ed}, with higher β-sheet content, led to reduced interfacial coverage and lower emulsion stability.

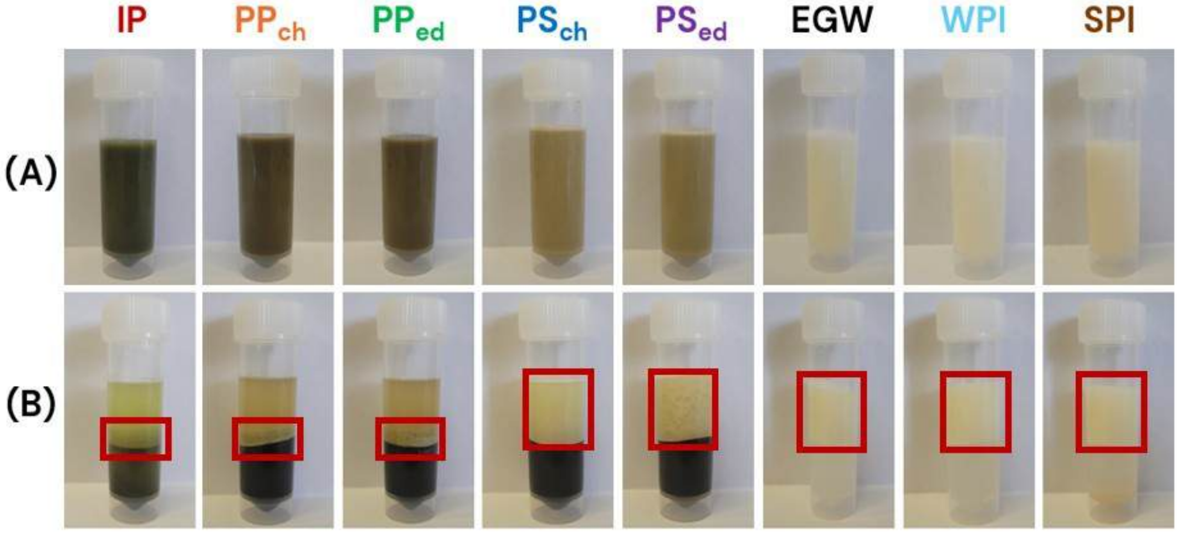

Figure 3. Representative pictures showing the emulsifying activity of 10 mL of a 1.5% protein solution (w/v) of IP, PP_{ch}, PP_{ed}, PS_{ch}, PS_{ed}, EGW, WPI and SPI. These solutions were emulsified with 10 mL of canola oil, with observations taken before (A) and after centrifugation at 1100 x g for 5 minutes (B). The higher the emulsion layer (framed in red), the better the emulsion activity, from Muller and Bazinet (2025b).

With these findings, we hope to demonstrate that extraction and purification can not only improve protein purity but also reshaped duckweed protein structure in ways that enhance functional performance. Despite these promising results, several challenges remain. The purification process darkened the concentrates, which may affect color perception in food. Thus, developing processes that produce lighter powders without compromising protein recovery will be an important next step. In parallel, incorporating duckweed proteins into real food formulations and performing sensory evaluations will be crucial to validate their functional performance and consumer acceptability.

Our research on duckweed at Université Laval continues to progress. Our team has recently published a comprehensive review on the applications of duckweed proteins in food and nutraceuticals (Muller et al., 2025), along with very novel studies exploring their potential as sources of bioactive peptides with antihypertensive and antidiabetic properties (Bernier et al., 2024; Cournoyer et al., 2025). Using machine-learning approaches, we identified 20 novel peptide sequences with experimentally confirmed antihypertensive and/or antidiabetic activities (Cournoyer et al., 2025). We warmly welcome suggestions and collaborations from the duckweed community to continue exploring the remarkable potential of these tiny plants.

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8th ICDRA Announcement

DIPARTIMENTO DI AGRARIA

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Commentary: Genome diversity and evolution in duckweeds

Insights from the *Lemna* Section *Alatae*

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Within genus *Lemna*, the section *Alatae* has drawn scientific attention because of its unusual diversity and unclear evolutionary history. Unlike most duckweeds, which mainly reproduce vegetatively by budding, some *Alatae* species also readily produce flowers and viable seeds, offering opportunities for sexual recombination during reproduction. This reproductive flexibility, combined with their wide geographic distribution, has made *Alatae* duckweeds both fascinating and difficult to classify. Traditionally, two main species in this group have been recognized: *Lemna aequinoctialis* (the lesser duckweed), which occurs worldwide in tropical and subtropical climates, and *Lemna perpusilla* (the minute duckweed), more restricted to North America. A third form, *Lemna aoukikusa*, has been described from Japan, though its taxonomic status has long been debated. Over decades, inconsistent classifications, morphological similarities, and overlapping geographic ranges have led to uncertainty about how these plants are related and how they should be defined as species.

To address these questions, we performed a comprehensive study on 52 worldwide duckweed accessions from the *Alatae* section (Stepanenko et al. 2025), combining plastidic and nuclear markers, genome size measurements, chromosome counts, and genomic *in situ* hybridization to reconstruct the evolutionary relationships within the section.

The study confirmed *L. aequinoctialis* and *L. perpusilla* as distinct diploid species which form the genetic basis of the *Alatae* section. *L. aequinoctialis* is highly variable in morphology and widely distributed, while *L. perpusilla* has a narrower distribution and more limited representation in collections.
Many accessions did not fit neatly into either diploid category. Instead, we identified triploid and tetraploid forms mostly resulting from interspecific hybridization and backcrosses of the hybrids:

•Triploids arose from crosses between diploids where one parent contributed unreduced gametes.
•Tetraploids originated from fusion of unreduced gametes of *L. aequinoctialis* and *L. perpusilla*, or after early tetraploidization of allodiploid hybrids.

The occurrence of mainly vegetative propagation in combination with interspecific hybridization, polyploidization and backcrossing according to Ennos et al. (2005) are features of species complexes, in which individual species cannot easily be delimited. Therefore, we consider the *Alatae* section to represent the '*Lemna aequinoctialis* complex' (including *L. aoukikusa* as an allotetraploid between *L. perpusilla* and *L. aequinoctialis*), because *L. aequinoctialis* contributed to most of the accessions. The ability of *L.* × *aoukikusa* to survive in colder climates likely facilitated its spread into East Asia, where it became established as a stable lineage. Presently, we are still searching for an allodiploid *L.* × *aoukikusa*.

The genomic data indicate multiple, independent hybridization events between the two diploid ancestors. Some hybrids later backcrossed with one of the parent species, generating further diversity. These processes explain why morphological and genetic variations within *Alatae* has been so confusing for taxonomists. Most likely, the primary hybridization events occurred in North America, where both parental species coexist. From there, some hybrids migrated to Asia and adapted to new environments. This explains the presence of similar hybrid lineages on several continents. The findings highlight the importance of hybridization and polyploidy as drivers of diversity in plants. The ability of hybrids to persist, reproduce, and spread across continents illustrates how complex evolutionary pathways shape biodiversity.

Our multiple approach strategy (combining molecular, cytogenetic, and ecological data) serves as a model for resolving phylogenetic relationship in other difficult-to-resolve plant groups, including the duckweed genera *Wolffiella* and *Wolffia*, which also seem to contain cryptic species.

More recently we have shown that, contrary to the described allodiploids and allotriploids in sections *Lemna* and *Alatae* (Braglia et al. 2024, Ernst et al. 2025, Michael et al. 2025, Stepanenko et al. 2025), some natural allotetraploids of section *Alatae* were fertile, retaining the ability to reproduce sexually, while synthetic allotetraploids were not (Lee et al. bioRxiv, 2025). This suggests that natural allotetraploids may become sexually proficient, while the so far studied allodi- and -triploids and synthetic allotetraploids represent evolutionary 'dead ends', i.e. cannot further evolve through sexual recombination.

In summary, our research contributes to clarifying the phylogeny of *Alatae* accessions and the degree of sexual propagation within the genus *Lemna.* In addition, it also provides a framework for exploring biodiversity in other duckweed groups as well as for future breeding effort of this emerging aquatic crop.

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Opinion: Duckweed nomenclature – the Sequel

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Following the publication of our Opinion piece in the previous issue of this forum, we have obtained input from seven duckweed researchers and some of their colleagues who work on a diverse range of basic research topics. Here in Table 1, we like to share a summary of their responses to the questions of whether some of the often-used nomenclature in duckweed research should be updated/revised and if so, what could be a more accurate and functional naming scheme to facilitate the growth of duckweed research. Most of the replies that we received are quite thoughtful as well as informative from a variety of perspectives. We like to thank all the respondents for their time and effort to help us forge some recommendations in this sequel.

For our first question of whether this is an urgent or important issue for the field, 3 of the 7 responses are "no" while the other 4 are "yes". A common feeling, even for some of the researchers that believe it is an important issue, is that changing nomenclature is a difficult endeavor, which we do appreciate and empathize. To that point, we like to explain our motivation for this exercise a bit more in this follow-up discussion. Our perspective is that looking forward to the not-too-distant future, we believe multiple areas of new research frontiers will benefit from a revised nomenclature in duckweed research. For example, novel experimental platforms, such as the Plant-on-Chip set-up (*1*), can enable spatially- and temporally resolved growth and behavior phenotyping of individual plant units in a defined culture system. A systematic and informative system for tracking the relationship between individual plants for such an experimental system would be invaluable. While duckweed plants appear simple morphologically, especially in rootless species from the *Wolffia* genus, application of single-cell or single-nucleus transcriptomics (*1,2*) could reveal the heterogeneous make-up of different regions in the plant body as well as the internal tissue organization that correlates with their functional specification. These complexities could be resolved by spatio-transcriptomics technologies such as combining laser-capture-microdissection with high-throughput transcriptomics (*3*) to describe the transcript landscape for different regions in duckweed tissues. Having more accurate and consistent nomenclature may assist the growth of these new frontiers in duckweed research by providing a unified set of defined terms and tracking system to be used by researchers in the field, should the need arise. The transgenerational research on epigenetics maintenance in the Huber laboratory (*4*) and comparative analysis of ageing and lifespan between duckweed species by Laird and colleagues (*5*) are examples of current research areas that could benefit from this effort.

On the question of whether it is acceptable to use the "mother-daughter" moniker in describing asexual reproduction in duckweeds, most respondents appear to think that this is acceptable even if it may not be accurate in the straightest sense. If used simply to denote the generational relationship, we would agree with this consensus as well. However, as Rob Laird suggests, we also agree with the use of the more neutral terms of "parent-offspring" can improve the accuracy of the description, should that be desirable.

Our previous attempt to apply the usage of "plantlet-clone" for distinguishing the newly formed and still attached duckweed plants from the progenitors was confounded by the potential confusion of these names being used in this context. For example, plantlets could imply juvenile state that may not be true for the attached plants. Instead, a terminology that already exists for asexually propagating plants such as strawberry and poplar is "ramet", with the original, single progenitor plant called an "ortet". The ensemble of the progenitor along with all the offsprings from it, whether attached or detached, will be considered a "genet" (*6*). This established nomenclature could be a better alternative. After examining the various input by our respondents, we agree with this opinion as a more appropriate nomenclature for use in duckweed-related studies and will recommend its adoption for use when accuracy is desired.

Table 1: Summary of community input on duckweed nomenclature.

Name (s)	Date of comment	Importance/urgency of issue	Mother/daughter term acceptable?	Frond definition important to clarify?	Key observations / perspectives	Proposed action (s)
Eric Lam / Shunong Bai	30-Jul-25	Yes	no	Yes	Most duckweeds grow clonally without going through embryogenesis or meiosis, thus mother-daughter relationship is functionally misleading. As technologies rapidly evolve to enable high resolution analysis of organisms at multi-dimensional levels, it will become critical to define a proper set of nomenclature to designate each cells and organs in duckweeds properly to facilitate a consistent description of cell types and clonal relationships in time and space.	Suggested to adopt plantlets (P) and clones (C) to distinguish between attached versus separated and independent duckweed plants, along with a numbering system following the genetic terms used to designate generational relationships.
Ingo Schubert	2-Aug-25	No	yes	plantlet as a synonym for frond	Mother-daughter-granddaughter is similar to parthenogenetically originated progenies (however this does involves embryogenesis and seed formation)	New terminology unnecessary and question its likeliness for adoption
Arturo Mari-Ordonez	4-Aug-25	Yes, but may be difficult to change	While agreeing with Ingo that Mother/daugher are not incompatible with asexual reproduction, Ramet (derived from Ramus in Latin) and Genet may be better	plantlet may be better than frond, since the latter is more associated with undifferentiated thallus	Genet and Ramet nomenclature is commonly used for clonal propagating species (e.g. strawberry, poplar).	Mother duckweed ramet (or plantlet) with its attached daughters and progenies will be a genet.
Meret Huber / Alexandra chavez	4-Aug-25	Yes, especially for studies of epigenetic inheritance	Did not specify, but seems ok to discuss them in terms of "generations"	Yes, is the frond a "fusion" of shoot and leaves?	Prefers to use ramet instead of plantlet to designate newly emerging "offspring", similarly to avoid using the term "clone" and use the consistent terminology of ramet for the new plants being produced asexually.	Suggested to make it less complicated by diffrentiating mature and attached fronds, e.g. by using lowercase instead of capital letters for the attached fronds (see attached figure on the left). Would avoid using "C" for clones, rather to use "R" for ramet, or "G" for generation (see **Figure 1** for an example).
Laura Morello / Yuri Lee	5-Aug-25	Yes, but may be difficult to change	Could be confusing, favor the use of ranking instead to avoid the issue of using Clones (C) terminology	"plantlet" for the developing (attached?) plant and "plant" for the adult (separated) individual	Mother/daugther terminology has also been applied to situations such as yeasts or animal cells (stem cells).	Application of the botanic terminology of ramets (propagules) and genets (clonal population - colonies) for clonal plants.
Rob Martienssen	6-Aug-25	Interesting but not too urgent to change	Fine, pointed to traditonal reference of female for vegetative propagation, such as those for palm trees and grapevines	Happy with fronds and colonies, referring to strains as clones	Ramets could be reserved for special derivatives, such as transgenic clones (?).	Vote to keep the status quo
Robert Laird	2-Sep-25	Yes	Prefers to use "parent" and "offspring", use "ramet" to refer to individual plant/plantlet (physiological individual)	Yes, its current use appears ambiguous; may be important to distinguish between **ramet** (physiological individual) vs. **genet** (a spatially distributed - separated? - genetic individual)	Could use one set of terms and modify that with "a" for "attached" or "d" for "detached".	Suggested a simpler scheme for tracking and naming relationships between individuals in a genet, see scheme in **Table 2**. Thus, R3,1,2,11,2 would be a fifth-order descendant of R (i.e., a great-great-great-grand clonal-offspring) because it has five subscripts. Specifically, working backwards, it would be the 2nd offspring of the 11th offspring of the 2nd offspring of the 1st offspring of the 3rd offspring of R (or R0 representing the ortet), the progenitor. It could even be given an "a" while attached, as in a ramet (R3,1,2,11,2a), and a "d" when detached as in a genet (R3,1,2,11,2d).
Klaus Appenroth / Sowjanya Sree	7-Oct-25	No	Yes	One might refer to Landolt's monograph	Plantlet term for the daughter fronds may be misleading.	No change required

For the finer point of what the definition for "frond" should be, this word can be traced to the Latin word "frons", which can mean foliage or bough (the main branch of a tree). It is commonly used to describe fern and palm leaves that are typically compound, as well as the leaflike thallus of less-differentiated organisms such as lichens and seaweeds. For rootless duckweeds such as those in the *Wolffia* or *Wolffiella* genera, a frond could thus encompass the whole plant, while in more structured genera such as *Spirodela*, the frond would be the part of the plant that is distinct from the true roots which retain defined vasculature with radial symmetry that are typical for this plant organ (*7*).

Finally, what could be a general approach in naming the various ramets should there be a need to differentiate between each of these units to help rationalize the particular question that a researcher may want to address, such as the resetting of biological clocks upon each successive generation? Meret Huber and Alexandra Chavez (Mainz, Germany) suggested one scheme (Fig. 1) while Robert Laird (Lethbridge, Canada) proposed another one that is simpler in structure by only using one letter to denote ramets (Table 2). Both are in fact similar in principle. We believe the usage of ramet to identify each of the plant units in a particular experimental system, such as the *Lemna trisulca* species where ramets stay attached through their stipes (Fig. 2), with the addition of a rank order assignment, can serve the purpose to keeping track of their lineage through successive generations. Adding a lower-case letter of either "a" or "d" to the end of the designation can help distinguish between attached and separated ramets (Table 1). In studies where such a resolution in describing the behavior of the system is neither required nor possible, the numbering system can be omitted, and the ensemble of plants (the genet) are analyzed together with the output measured as an average of all the units.

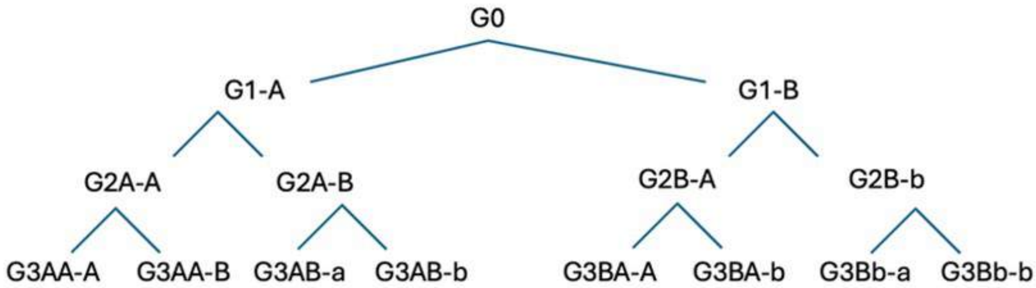

Figure 1. Example of a naming scheme for duckweed ramets starting with a progenitor. When a ramet is still attached, use a lower-case letter to denote it as shown in the diagram. Modified from the suggest by Meret Huber and Alexandra Chavez.

Figure 2. Attached ramets in a cluster of *Lemna trisulca*. New ramets are continuously produced from each ramet and often the stipes do not abscise, thus the ramets stay attached.

Table 2. Example of a naming system to differentiate between various ramets.

Progenitor	Clonal offspring	Clonal grand-offspring	Clonal great-grand-offspring
R	R1	R1,1	R1,1,1
			R1,1,2
			R1,1,3
		R1,2	R1,2,1
			R1,2,2
			R1,2,3
		R1,3	R1,3,1
			R1,3,2
			R1,3,3
	R2	R2,1	R2,1,1
			R2,1,2
			R2,1,3
		R2,2	R2,2,1
			R2,2,2
			R2,2,3
		R2,3	R2,3,1
			R2,3,2
			R2,3,3
	R3	R3,1	R3,1,1
			R3,1,2
			R3,1,3
		R3,2	R3,2,1
			R3,2,2
			R3,2,3
		R3,3	R3,3,1
			R3,3,2
			R3,3,3

R: Ramet; Modified from Robert Laird's original table showing 3 rounds of replication.

As a useful tool for the community, and especially for the benefit of newcomers into the field, we also present here a Glossary for terms to be used in the duckweed research field (Table 3). We hope this could provide a useful reference to help unify the usage of these names in our conversations and publications about duckweed and facilitate communication between researchers and application specialists alike. Of course, further constructive commentaries on this topic by the DF's readers will be welcome. We look forward to hearing from all duckweed enthusiasts.

Table 3. Glossary of terms used in duckweed research.	
Term/name	**Definition**
clone	Clones are genetically identical plants grown from an individual of a strain.
frond	Leaf-like body of duckweed plants that also contain the meristematic tissues. In rootless duckweeds such as *Wolffia* and *Wolffiella* spp., a frond can be the whole plant.
generation	A complete life cycle of reproduction such as the production of an offspring from a progenitor/parent.
genet	An essemble of genetically identical individuals descended from and including their progenitor. Traditionally, this essemble can be referred to as a colony where progenitor and offsprings are still attached or detached.
ortet	The single original progenitor in a genet, traditionally referred to as the generic mother frond, although there can be many mother fronds in a genet or colony, while there is only one ortet in the genet.
parthenogenesis	A form of asexual reproduction in which females produce offsprings with unfertilized eggs via the apomixis process.
ramet	An individual plant produced asexually by a progenitor and genetically identical to other ramets in the same genet. This structure is also traditionally referred to as a daughter duckweed plant.
stipe	A narrow, stalk-like part of the developing ramet which connects it to its progenitor. It usually wilts over time and release the ramet as a free-floating individual. An exception is the duckweed species *Lemna trisulca* (aka star duckweed or ivy-leaved duckweed) where the stipe can persist and thus the ramets remain connected.
strain	A population of plants that are descendants from a pure culture starting from a single frond.

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PAG 33: Announcement

Announcement: *Important Dates for attendees*

Duckweed Research and Applications Workshop

in the

Plant and Animal Genome (PAG33) Conference 2026

January 9-14, 2026

at the Town & Country Conference Center in San Diego, CA, USA



Eric Lam (Rutgers U.) and Todd Michael (Salk Institute) are organizing the annual **Duckweed Research and Applications Workshop** to take place on the afternoon of January 9th during PAG33 in San Diego (**January 9-14, 2026**) and would encourage everyone in the community to attend. For those who are attending PAG33 and would like to present, please contact either one of us as soon as possible. There are still a couple of spot open now! We expect to have an excellent lineup of exciting speakers with new results from diverse studies with duckweed.

Confirmed Speakers and Topics:

Anna O'Brien: "*How to build a microbiome to "breed" better duckweed inoculants*"

Tasmia Islam: "*Highly efficient new approaches for duckweed synthetic biology*"

Todd Michael: "*The Wolffiella genome and the evolution of rootlessness in the Lemnaceae*"

Please register at this link

https://pag.confex.com/pag/33/registration

For Letter of Invitation or a Visa Letter, please go to the "International Visitors" page on the PAG website under the "Attend" tab.

Please also note these deadlines:

Deadline for workshop abstract submission: December 8th, 2025
Deadline for poster abstract submission: November 3rd, 2025
Deadline for posting speakers by organizers: November 15, 2025

Duckweed in the spotlight of AgTech breakthrough

Greenonyx named "Vertical farming company of the year"

Tsipi Shoham, PhD
GreenOnyx CEO & Co-founder
tsipi@greenonyx.biz

QFarming® resets the bar by pairing duckweed, the optimal crop, with a novel highly efficient CEA platform design

GreenOnyx—the company behind the **QFarming®** cultivation platform—was named **"Vertical Farming Company of the Year"** in the **2025 AgTech Breakthrough Awards**. See the formal announcement with quotes and program details [1].

AgTech Breakthrough: The industry benchmark for AgTech excellence

Run by **Tech Breakthrough**, the **AgTech Breakthrough Awards** spotlight top companies, technologies, and products shaping the future of agriculture and food. Each year the program reviews **thousands of nominations** and recognizes winners across categories such as indoor/vertical farming, farm management, AI/IoT, robotics, analytics and more. Winners include global leaders—for example **Bayer's E.L.Y.** (AI-based AgTech Solution of the Year 2025)— underscoring the program's role as a credible industry benchmark and visibility engine for top-tier innovators [2].

Vertical Farming: The Rise… Then the Reset (2016–2025)

Vertical farming indoors promised climate-resilient, local proximate production—less exposed to supply-chain shocks—with sharply lower logistics waste, water and fertilizer use, and cleaner, traceable, pesticide-free produce. A funding surge from 2016 to 2021, backed by blue-chip investors and built ambitious facilities, the sector raised billions. Since late-2022, however, lagging unit economics have driven bankruptcies, restructurings, and strategic pivots. Yet the need for a reliable local supply of fresh food, has not gone away. The next chapter must prioritize (i) crops with superior economics (clear competitive advantages and longer shelf life), (ii) technology engineered for cost and resource efficiency, and (iii) disciplined, resilient go-to-market and capital structures [3,4,5]. QFarming® resets the bar by pairing duckweed, the optimal crop selection, with a novel CEA platform design: a closed, seed-to-pack, sterile system purpose-built to deliver high throughput, low shrink, and ready-to-eat, nutrient-dense freshness. This award recognizes a credible path from resilient local production to viable per-kg economics—so vertical farming can finally scale.

Duckweed – the emerging hero crop for vertical farming

Duckweed brings a rare combination of traits that makes it an **ideal emerging crop** for closed, controlled vertical systems: **Ultra-rapid growth & continuous harvestability** (short doubling times, high harvest index); **tiny, uniform plants** that suit sterile, modular processing with minimal waste; **resource efficiency** at a very small footprint; a new produce category with **mild, neutral taste** that can integrate seamlessly into everyday meals - key for mainstream adoption. These traits directly drive down cost and shrink, while increasing throughput - the levers that make or break indoor-farming economics.

Economics that Work: From Platform to Plate — QFarming® → Wanna Greens®

GreenOnyx's **QFarming®** turns duckweed's biological advantages into a **consumer-ready fresh vegetable** at scale. The closed, sterile, automated system delivers **year-round, local production** and reaches consumers as **Wanna Greens®** -fresh **Wolffia** that needs **no washing or preparation**, has a **delicate flavor** that blends into any

dish, and offers **extended freshness**. It is **outstanding in nutrient density**, delivering a high concentration of essential nutrients while minimizing antinutrients and other nutrients recommended to limit.
The QFarming® process is **100% seed-to-pack in a sterile environment, biomimicking Wolffia**'s native conditions to maximize growth efficiency. The result is **high output in minimal space**, with **significantly less water** and **lower energy** inputs than many CEA setups. That combination - **higher throughput** with **lower resource use**, **lower shrink**, and a **compelling new fresh-produce category**—underpins a stronger **per-kg unit-economics** case for indoor farming.
By securing **FDA, EFSA, and Israeli Ministry of Health** approvals for human **consumption** of **fresh Wanna Greens®,** GreenOnyx, via its **QFarming®** platform, has taken a notable step toward bringing a **fresh aquatic vegetable** into mainstream markets. In the words of **Bryan Vaughn**, Managing Director, AgTech Breakthrough;
*"**Wanna Greens®** uniquely bridges the gap between convenience and fresh nutrition… making fresh greens enjoyable and effortless for all consumers, including children and non-vegetable eaters. Congratulations on winning 'Vertical Farming Company of the Year!'"*

GreenOnyx is **grateful to AgTech Breakthrough for this recognition. GreenOnyx will continue to make healthy fresh greens accessible to everyone, so every spoon tastes like it was picked straight from the field, while pushing the performance ceiling of indoor-farming systems worldwide.**

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From pond to plate – advancing water lentils as novel food

Report of the workshop organised by the European Duckweed Network

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On October 24 some 30 delegates met in Gent, Belgium for a workshop organised by Dr Reindert Devlamynck, Dr Marcella Fernandes de Souza and Ms Marie Lambert. The workshop was organised as part of the activities of the Flemish Research Organisation (FWO) funded European Duckweed Network (EDN)(http://thecharmofduckweed.org/about-EDN).

In recent years, Flanders has witnessed strong growth in duckweed research across universities, research institutions and industry. The FWO-funded European Duckweed Network (EDN) brings together the varied interests in duckweed research and applications in a comprehensive skills and expertise network. EDN covers a range of duckweed activities including ecotoxicological and remediation work, studies of gene-expression and whole genome organisation, and work on large-scale cultivation, biomass processing and economic analysis. As such EDN spans the entire range from pioneering laboratory scale studies to applied, stakeholder-led applications. The Gent workshop zoomed in on one particularly promising area of duckweed research, advancing water lentils as a novel food for human consumption.

The early part of the morning was allocated to three introductory talks. PD Dr Klaus J Appenroth (Friedrich Schiller University Jena, Germany, and ISCDRA steering committee) systematically reviewed knowledge about the suitability of water lentil biomass for human consumption. However, as pointed out in this talk, the metabolic composition of water lentils varies with growth conditions and genotype. For example, the balance between starch and protein accumulation shifts towards starch in plants exposed to stress. Apart from the (highly desirable) amino acid composition of water lentils, Klaus emphasised both the favourable fatty acid profile (high % polyunsaturated fatty acids) as well as the highly attractive content of vitamin B12, which does make water lentils of great interest for those on vegan or vegetarian diets.

Prof. Marcel Jansen (University College Cork (UCC), Ireland and ISCDRA steering committee) emphasised that water lentil biomass for human consumption has to be clean, healthy and nutritious. This places constraints on the cultivation method and emphasises the need for protected cropping. However, any indoor system will need to be space-efficient, and this has driven the development of stacked cultivation systems whereby multiple layers of water lentils can be grown per area of floor space. Marcel detailed some of the operational parameters for such systems, including flow rate, water velocity and turbulence. Poor management of the hydrodynamics can substantially lower water lentil yields. Marcel also presented a novel way of stacked growing, whereby water containing trays are omitted from the stacked design, but instead plants are suspended on thin layers of fabrics exposed to a mist of fine water droplets. Such growth under fog conditions offers scope to massively increase the number of water lentil layers per area of floor space.

Researcher Ms An Callens (VIVES University of Applied Sciences, Belgium) focused in her talk on food processing. An focused on important questions that are rarely addressed, including the question of the shelf-life of water lentils. Heat treatment was found to be essential to keep the total aerobic microbial count of stored water lentils within legal limits (i.e. <5000 CFU/g; https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202500153). However, perhaps surprisingly, storage of water lentils under controlled (75% N_2) atmosphere packaging did lead to discoloration of the water lentils, a discoloration that was not seen when water lentils were packaged using normal atmospheric composition. This is an important consideration given the role of an attractive colour for commercial sales of water lentils. A second part of An's talk focused on the vitamin B12 content of duckweed. Research as part of the LemnaPro project showed that collected Lemna samples contained the active form of vitamin B12. Finally, An presented the development of an innovative vegetarian burger, which contained 45% fresh *Lemna minor*. This kind of product development, including the study of how such burgers can be stored, is an important step towards bringing water lentils to European consumers.

A number of short talks further zoomed in on the development of water lentils for human consumption. Dr. Paul Fourounjian developed a number of economic feasibility scenario's for culturing and processing of fresh water lentils for food production. Interestingly, these models especially emphasise the economic value of wastewater treatment. However, as Paul stated "Every model is wrong, but some models are useful".

One of the major risks of increased duckweed cultivation is an increase in the incidence of pests and diseases. Mr Sai Chand Sabbireddi reported on the very extensive damage caused by the small China-mark moth (*Cataclysta lemnata*), a point illustrated with some excellent video shots. Other potential pests include water lily aphids, clubbed soldier fly and water beetles.

Prof. Viktor Oláh reported on the development of novel proxies for duckweed dry matter and protein content. This very promising approach is starting to reveal that non-destructively measured reflectance and transmittance parameters can be used as indicators for dry matter and especially protein content, creating a useful tool for duckweed growers.

A very informative talk was given by Ms Lynn Verreydt and Ms Katrien Neveux from the organic supermarket chain BioPlanet. They focussed on the drivers for the successful launch of innovative products. Their talk focussed on the readiness of "Product, Price, Place, Promotion and People". The concept of water lentils as a "product" focussed on factors such as taste, vitamin B12 content, water lentil product lines ranging from freshwater lentils to frozen burgers, and packaging. "Place" refers to an awareness of the market, i.e. from supermarkets to restaurant trade, while "People" relates to consumer groups amongst others flexitarians, vegetarians and vegans.

Finally, a series of short poster presentations highlighted some critical research. Mr Christian Perna reported on the cultivation of duckweed using dairy processing wastewater, while Ms Alexandra Katsara used a parallel approach by growing duckweed on fish processing wastewater. Consistently, Dr Muhammad Irfan reported on the environmental sustainability of duckweed protein, especially where wastewater is used. Mr Rinus Minkjan focused on the economic sustainability of duckweed cultivation, including the fact that the price of duckweed protein needs to be comparable to that of soy to be competitive. Finally, Ms Natasha Manyenga reported on the scenario where duckweed is neither suitable for use as food nor as feed. A series of duckweed soil amendment experiments shows great potential for the use of such duckweed as mulch and/or fertilizer.

In the afternoon, delegates visited an innovative duckweed pond operating in a commercial setting (Fig. 1). The pilot pond is located in the extensive greenhouses of azalea growers Mr and Ms Hans and Kathleen Van De Steene. The 180 m² cultivation pond includes an automated sieve-tray harvesting system which is used to capture some 80 kg FW of duckweed per week. The biomass is currently used as feed for fowl but use for human nutrition is a possible consideration. Indeed, some of the harvested duckweed biomass was used by the Hans and Kathleen Van De Steene to serve some excellent duckweed burgers, using a recipe developed by An Callens (Fig. 2). The excellent consistency and taste of the duckweed burger, including the possibility to store prepared raw burgers in the freezer, demonstrates that plans to introduce Lemna species for use as human food in Europe are well advanced.

Concluding statement

The workshop was a resounding success. A key component of the success was that Marie Lambert, Marcella Fernandes de Souza and Reindert Devlamynck brought together highly diverse group of experts (Fig. 3) which had, however, a common focus, i.e. progressing the development of duckweed as human food in Europe. The workshop revealed how these different expertises are in fact complementary parts of a comprehensive product development chain linking cultivation with harvesting, food processing and marketing of products. By bringing together different expertises, Marie Lambert, Marcella Fernandes de Souza and Reindert Devlamynck contributed substantially to the development of water lentils as a product for human consumption.

Figure 1. The 180 m^2 duckweed pond at the farm of Hans and Kathleen Van De Steene. The action of the spray beam halfway gives some gentle water circulation whilst the associated UV-C filter assists with keeping levels of nuisance algae low.

Figure 2 (Left). PD Klaus J Appenroth enjoying an excellent duckweed burger developed by researcher An Callens. **Figure 3** (Right). Delegates at the duckweed farm in Flanders

Wolffia takes center stage as a promising economic crop and future food of Thailand

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For the past few years, our community has observed rapidly increasing interest in duckweed applications. In particular, for those who attended the 7th ICDRA in November 2024, we had a chance to witness first-hand how *Wolffia* (in Thailand *Wolffia globosa*) was rapidly making its way back from the forgotten past into modern cuisine of Thailand. It has been nearly one year since the conference, and we are happy to report that the *Wolffia* trend in Thailand has continued—and even picked up exponential growth in recent months—thanks in part to the greater involvement of several key stakeholders including governmental agencies, private sectors, researchers, and more importantly, local farmers. The widespread adoption of *Wolffia* was clearly evident in the recent event "**Pham Expo 2025: From Local Superfood to Future Green Economy**," the country's first dedicated public trade exhibition for *Wolffia*, held from August 1–3, 2025, at Phoenix Mall in the heart of Bangkok's Pratunam district.

Wolffia—known locally in Thailand as **pham** or **khai-nam**—has long been consumed in some regions of Thailand and neighboring countries, as clearly documented by the pioneering work of **Krachang Bhanthumnavin** and **Michael G. McGarry**, published back in 1971 [1]. Yet, in past decades, it became underappreciated by the younger generations and nearly disappeared from Thailand's food scene. Following the global recognition of duckweed as a high-protein food source in the early 2010s, the Thais quickly recognized the potential of their own heritage food. A major turning point came in 2016 when **Klaus J. Appenroth**, Germany, and **K. Sowjanya Sree**, India, were invited to Thailand by **Metha Meetam** to give a workshop on Duckweed Biodiversity and Applications at Mahidol University, and to visit a small demonstration facility at the Department of Agricultural Extension in Nakhon Ratchasima province. There, **Chuenduangjit Sopat** and **Arak Tira-umphon** developed a program to teach local and young children to grow *Wolffia* for their school lunches. Following this event, **Metha Meetam** co-founded the biotech startup company **Advanced Greenfarm** in 2019 to research large-scale *Wolffia* cultivation and successfully launched their products in early 2024 under the brand **Flo Wolffia** [2], with a core emphasis on nutritious, safe, and sustainable products. The products—including fresh *Wolffia*, dried powder, and several lines of ready-to-eat drinks and snacks—were adopted by health-focused restaurants and sold in retailers around Thailand. *Wolffia* was quickly embraced by Thai consumers, who were familiar with their heritage food and further learned about its exceptional nutritional value from various publications [3, 4] and similar products offered by other global startups such as **Hinoman** and **GreenOnyx**, both in Israel. The early interest in *Wolffia* prompted additional Thai companies and local farms to follow suit, including **WolffiaX** and **Kreen Wolffia**. The popularity of *Wolffia* was soon recognized by the Thai government, which sees *Wolffia* as a new economic crop and a solution for food security, health and wellness, and net-zero climate sustainability.

Implemented in August 2025 through coordinated efforts, **Pham Expo 2025** was co-organized by the Thai Chamber of Commerce, Suranaree University of Technology, and the Thai Wolffia-farming network. The event brought together 40 exhibitors and several hundred attendees, ranging from scientists and educators to chefs, entrepreneurs, policymakers, and the general public. The expo featured a rich program of lectures, food product showcases, cultivation technology demonstrations, and technical training workshops. A major highlight was a panel discussion on the national policy driving *Wolffia* commercialization, joined by representatives from the Thai Chamber of Commerce, the Department of Agriculture (DoA), the Office of National Higher Education Science Research and Innovation Policy Council (NXPO), and Suranaree University of Technology. The keynote talk was given by **Dr. Krachang Bhanthumnavin**, who reminded attendees that the vision for *Wolffia* as an affordable protein source—from his original work in 1971—has deep roots in Thai cultural heritage and biodiversity, and is only now beginning to flourish. Several respected Thai researchers and experts from various public and private organizations were invited to share their insights and updates on relevant aspects such as advanced food processing, health benefits, cultivation technologies, and environmental footprint.

The event's highlights, however, were the 40 booths exhibiting *Wolffia* products and technologies by startups, universities, independent developers, and even local farms—both traditional and modern. Innovation showcases included vertical farming systems, organic fertilizers, automated harvesting and digital control technologies, and modular cultivation units designed for various commercial to urban settings. On the culinary side, *Wolffia* received a complete reinvention—from traditional Thai cuisine to contemporary products and international flavors: pham-infused chocolates and ice creams, pham breads and cookies, pham vegan kebabs, and a variety of refreshing pham-blend drinks. A special highlight was "**pham cha**"—*Wolffia* tea—showcase and competition featuring various farms and tea-specialty vendors. These offerings made a compelling case for the plant's versatility—not only as a sustainable agricultural product but as a creative culinary ingredient with broad consumer appeal—and, more importantly, illustrated the potential of *Wolffia*, or pham, to become a mainstream healthy food choice in the near future.

This momentum is not only market-driven—it's also policy-backed. In early 2025, Thailand's Department of Agriculture officially launched **GAqP (Good Aquaculture Practices) standards for *Wolffia* farms for use as food** [9]. This regulatory framework provides clear protocols for safe, hygienic, and sustainable production of *Wolffia*, and will be accompanied by state-supported training programs for farmers across scales—from rural cooperatives to commercial farms. These GAqP standards are expected to expand the supply of safe, high-quality *Wolffia*, which remains a major roadblock for the food industry both in Thailand and worldwide.

Crucially, cultivation technology for *Wolffia* is still in its early phase and is in dire need of future scientific advancements. Similarly, the full range of applications for this remarkable biomass—and validation of its health benefits and climate-saving impacts—remains to be unlocked. The success of **Pham Expo 2025** illustrates what is possible when science, public engagement, and policy align, and may serve as a model and inspiration for other nations to push forward *Wolffia* and other duckweed species as new flagships of our future green economy and sustainable living.

Keynote address by Dr. Krachang Bhanthumnavin (Left), being introduced by Dr. Metha Meetam (Right)

Photographs from the Expo

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Student spotlight: Marie Lambert

PhD Candidate at Lab for Bioresource Recovery, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University & Provincial Research and Advice Centre for Agriculture and Horticulture (Inagro vzw), Belgium

(Email: mmlamber.lambert@ugent.be)

I have always been fascinated by nature and sciences. During my studies in Bioscience Engineering at Ghent University, I specialised in Environmental Technology within the master's programme. Growing up, I often played outdoors and came across duckweed floating in small creeks, but my first *real* encounter with this interesting tiny plant was during a presentation at our university by Dr. Reindert Devlamynck, who was then completing his PhD on duckweed at Ghent University. His presentation immediately caught my attention, and I decided to join Professor Erik Meers' research group for my master's thesis in the academic year 2019-2020. The title of my thesis was "Valorisation of agricultural wastewater through the production of high-quality duckweed proteins."

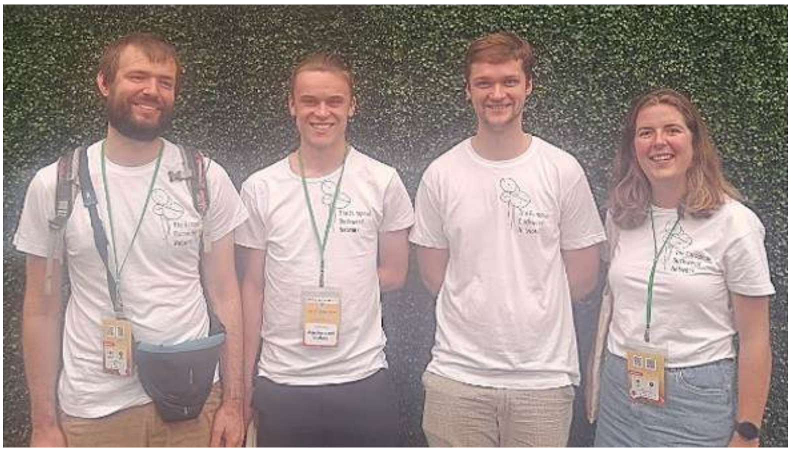
After graduating, I spent a year working elsewhere, but the fascination with duckweed never left me. I soon returned to the university to start my PhD, again on duckweed, this time within the LemnaPro project, which aims to scale up duckweed cultivation as a new crop in Flanders (Belgium). Throughout this project, duckweed was cultivated at six different locations in Flanders. These included, for example, ornamental plant nurseries, pig farms, and calf farms. Working closely with farmers on site at each pilot location was particularly valuable, as it provided insight into practical challenges and helped identify realistic solutions that laboratory setups alone could never reveal.

(Left) Two duckweed cultivation systems fertilised with pig manure effluents; (Right) Duckweed cultivation pond at azalea nursery, fertilised with synthetic nutrients

Two examples of our on-farm research include: first, an experiment using duckweed to recover nutrients from diluted pig manure, which was recently published in the Duckweed: Research Meets Application (2nd edition) special issue of Plants [1]. This study demonstrated that duckweed can effectively remove nitrogen and phosphorus from pig manure effluents (removal capacities of 1.2 g N/m^2/day and 0.13 g P/m^2/day were reported) while maintaining high biomass productivity and protein content. Second, at an azalea nursery in Flanders, part of a greenhouse was converted into a duckweed cultivation pond. Over four project years, duckweed was grown, fertilized, and harvested using an automated system, providing valuable data on long-term cultivation management and system stability.

Building on these pilot-scale studies, my PhD focuses on two main themes. The first is the cultivation of duckweed on agricultural wastewaters. I evaluate duckweed systems for their treatment capacity and biomass production, while also studying the effects of salt accumulation in setups with continuous fertilizer addition and how this impacts duckweed growth and nutrient uptake [1,2]. The second theme concerns the processing and preservation of duckweed biomass. Because fresh duckweed has very high water content, it is difficult to transport or store for long periods. I therefore investigate several processing options such as ensiling, extrusion, and protein extraction. Preliminary results indicate that duckweed cannot be ensiled on its own; a co-substrate is needed to increase the dry matter and sugar content. Maize silage, grass silage, and sugar beet pulp proved to be suitable materials for co-ensiling. In extrusion trials, it was technically feasible to add fresh duckweed biomass directly to the process. At inclusion levels of around 20–25 m% (fresh weight), good expansion was achieved and pellets of acceptable quality were produced. Protein extraction studies are still ongoing and will be further explored in the coming months.

I was very happy to attend the last two ICDRA conferences. In Bangkok, I had the chance to share some results from our pilot experiment on nutrient recovery using duckweed. Together with colleagues from Ghent University and VIB, we also introduced the European Duckweed Network (EDN) and officially launched its website, thecharmofduckweed.org, during the conference. The EDN aims to connect duckweed researchers, companies, and enthusiasts across Europe, and to promote collaboration and knowledge exchange. As part of this initiative, we are currently organizing a Duckweed Workshop in Ghent (October 24th) focusing on duckweed as a novel food source. The website and the EDN LinkedIn page are collaborative efforts by several Belgian and European researchers. The website is still a work in progress, and anyone interested in contributing or sharing content is very welcome to reach out.



Picture taken at the 7th ICDRA conference, from left to right: Quinten Bafort, Lukas Audenaert, Ewout Crombez and me all wearing our EDN tshirts!

I am now in the final stage of my PhD, working on processing and integrating the collected results into my thesis, which I plan to defend next year. After finishing, I hope to continue working on duckweed-related research projects, but we will see what the future brings.

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Student spotlight: Raja Amri

Department of Botany, Faculty of Science and Technology, University of Debrecen, Debrecen, Hungary

Doctoral School of Biological Sciences, Szent István Campus, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Ibn Khaldun, a pioneering figure in sociology, asserted in his seminal work The Muqaddimah: "History, on its surface, is no more than reporting; but at its core, it is contemplation and investigation" (Ibn Khaldun 1967). This principle extends beyond the study of history: In my humble opinion, duckweeds, a globally distributed aquatic plant family comprising 36 species, exemplifies this duality. Easy accessibility for both laboratory and field research, positions them as significant scientific model organisms and offers potential for diverse applications across multiple disciplines.

In my country Tunisia, the use of duckweed as a source of nutrition for humans or animals is not common. Therefore, I had only information about these tiny aquatic plant species from published studies or scientific documents. Today, however, I have the chance to work with them as part of my PhD research. These organisms can open up new horizons, hypotheses, and questions due to their distinct, or unexpected responses amongst duckweed species and even within the same species.

Another benefit of these plants is that they save time for the investigators. I always want to see the results of my experiments quickly, but this was not possible in previous stages of my studies when I worked with other terrestrial plants, such as *Marrubium vulgare*, *Teucrium polium, Globularia alypum* and *Salvia officinalis*. However, this has become completely different with duckweed. I can rapidly collect basic experimental data, and I can say that they are simple in appearance, easy to handle, quick to respond to changes of growth conditions, capable of producing large amounts of biomass within a short time, and they do not take up much space in the laboratory. Due to their simplicity and rapid growth, they are ideal candidates for solving even complex biological questions.

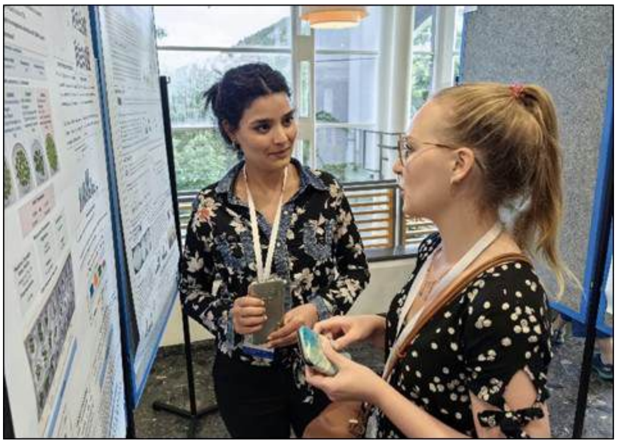
At the start of my PhD research, I presumed that duckweeds would exhibit slow growth comparable to traditional plant models and present laboratory challenges. I specifically anticipated rapid microbial contamination leading to culture loss, necessitating repeated experiments. However, I realized that after proper sterilisation and removal of microorganisms from duckweed samples collected from natural habitats, they have not been infected and can be cultured in an axenic growth medium such as autoclaved Steinberg medium (Environment Canada 2007) even without any additives. This plant's ability to resist contamination, a trait not shared by many other plants, has been crucial for maintaining the integrity of my experiments.

Earlier, during my master study, I wanted to conduct my research on Spirulina (*Arthrospira platensis*), because I wanted to understand more about these organisms, which are simple in form but rich in nutrients and bioactive compounds that can enhance human health and promote sustainable nutrition. However, fate did not allow me to do so. Today, as a PhD student, I study duckweeds which are another group of highly promising aquatic organisms. After arriving at the Department of Botany, Faculty of Science and Technology University of Debrecen, Hungary, with Prof. Ilona Mészáros as my main supervisor we worked out a project that appeals to my interests in duckweed stress physiology. We focus mainly on the productivity and physiological activity with clones of the giant duckweed (*Spirodela polyrhiza* (L.) Schleid.) collected from different geographical locations. This species stands out among duckweeds because of its unique response to stress factors. In fact, scientists often study *S. polyrhiza* for its ability to efficiently remove excess nutrients and contaminants from water, making it useful for wastewater treatment and other ecological applications (Ng and Chan 2019)

In my PhD research, I work with senescent cultures of *S. polyrhiza* clones that were selected according to their unique growth response to nutrient deprivation, that is the turion producing capacity. Even though the cultures of clones were grown under the same laboratory conditions, were the same age, and were in the same medium they did not all behave the same in this respect! Some clones maintained higher frond growth rates, some produced resting buds (turions) sooner, and some did not produce turions at all despite the nutrient deficiency in their aging senescent culture. This has raised several fundamental questions for us.

To reveal how the different clones handle stress, we applied abscisic acid (ABA), a plant stress hormone to their medium. Previous studies reported that ABA is able to trigger turion formation of *S. polyrhiza* accompanied with high starch accumulation. Using 7 day ABA treatments, I studied how quickly the investigated clones initiated and developed turions, and how the overall turion yield differed amongst the clones. Beside the growth traits, we also studied inter-clonal differences in biochemical and physiological responses to ABA, of both fronds and turions (photosynthetic pigments, anthocyanins, protein and starch contents). We have extended our study to 22 different clones of *S. polyrhiza*, originating from different locations and maintained in the departmental Duckweed Clone Collection (Dept. of Botany, Faculty of Science and Technology, University of Debrecen, Hungary). I believe that this inter-clonal approach will help to understand how these plants are able to survive stress and have adapted to their surroundings, while simultaneously identifying the most resilient clones within our collection.

I am always excited to share my research experience and the insights that these tiny green powerhouses continue to offer. Meeting “duckweeders” worldwide, sharing ideas, “speaking duckweed,” and even tasting creative duckweed dishes, makes the experience even more enjoyable and inspiring. I was fortunate enough to present my first results on giant duckweed in a poster at Plant Biology Europe 2025 (24th Congress of FESPB) in Budapest and hopefully will attend the 8th ICDRA in Italy in 2026 and present new interesting results.



Presenting my research on responses of *Spirodela polyrhiza* to ABA at the International PBE2025 Congress in Budapest – Session: Aquatic Plant Biology and Algal Biotechnology

At the end, I like to thank the entire Plant Ecophysiology group at Debrecen, and especially the “duckweeders” for their constant support, encouragement, and collaboration. I am also very grateful to my colleagues at the Department of Botany for welcoming me to this research environment and for sharing their knowledge, expertise, and good humour throughout this process. Working with such dedicated, passionate scientists has enriched my understanding of duckweed and overall knowledge of plant ecophysiology and made this journey enjoyable and inspiring. It has been a real privilege to have this opportunity, and I deeply appreciate the trust and resources that allowed me to carry out my work and grow, both scientifically and personally.

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From the Database

Highlights

Structural and functional properties of bacterial communities associated with rootless duckweed (*Wolffia globosa*) and their effect on the *Wolffia* growth

Saimee, Y; Kuwai, K; Ishizawa, H; Inoue, D; Thamchaipenet, A; Ike, M. (2025) Environmental Microbiome 20: 102.

Rootless duckweed, *Wolffia globosa*, is emerging as a high-protein and starch biomass resource for various applications. However, the microbiomes and functional properties associated with *Wolffia* across a wide range of microbial sources remain largely unexamined. This study investigates the structure and functioning of the *Wolffia* microbiome and its impact on plant growth. A co-cultivation experiment with axenic *W. globosa* and bacterial communities derived from various sources revealed varied effects, that municipal wastewater-derived bacterial communities had a more pronounced positive effect on growth of *W. globosa* compared to those from pond water. 16S rRNA amplicon sequencing found that Beijerinckiaceae, Caulobacteraceae, Comamonadaceae, Methylophilaceae, Rhizobiaceae, and Sphingomonadaceae were consistently conserved and identified as core taxa in the *Wolffia* microbiome. Functional profiling indicated that genes related to bacterial colonization and adaptation to the rootless morphology contribute to selective microbiome recruitment, with enriched functions in motility, chemotaxis, flagella assembly, quorum sensing, and ABC transporters. In addition, it was found that Bdellovibrionaceae, Beijerinckiaceae, and Sphingomonadaceae may act as "hub microorganisms" and "keystone taxa," shaping community structure and directly or indirectly influencing *Wolffia* growth. Collectively, the results of this study unveiled the robust core taxa and functional profiles of the *Wolffia* microbiome across diverse microbial sources, with certain taxa differing from those in rooted duckweed. This study comprehensively characterizes the *Wolffia* microbiome and enhances understanding of it, providing insights for developing efficient biomass production systems.

Duckweed-based edible vaccine confers complete protection against avian infectious bronchitis virus by inducing robust mucosal and systemic immunity

Tan, X; Guo, L; Chen, S; Fang, Y; Liu, PH; Hu, ZB; Jin, YL; Yi, ZL; He, KZ; Li, X; Zhao, LY; Wang, HN; Zhao, H. (2025) Plant Biotechnology Journal DOI10.1111/pbi.70218.

Molecular farming using edible plants offers a cost-effective strategy for vaccine production because plants serve as both bioreactors and oral delivery systems. Owing to its rapid clonal propagation and high protein content, duckweed represents a promising platform for this purpose. Here, we developed an edible duckweed-based vaccine targeting avian infectious bronchitis virus (IBV), a highly contagious respiratory pathogen in poultry, and directly compared its efficacy to that of a commercial live-attenuated H120 vaccine. Transgenic duckweed expressing the IBV antigen peptide EpiC was orally administered to chickens, followed by immunological assessment and viral challenge. Without an adjuvant, the EpiC duckweed vaccine elicited robust systemic IgG and mucosal sIgA responses, conferring 100% protection against lethal IBV challenge - which is comparable to or exceeds the efficacy of the H120 vaccine. Strikingly, co-administration with duckweed expressing IL-17B as an adjuvant synergistically enhanced immune responses, including elevated IBV-specific IgGs in serum ($P < 0.001$), increased IBV-specific sIgAs in the trachea and kidney ($P < 0.05$), and reduced tracheal viral loads ($P < 0.05$). Transcriptomic analysis revealed the upregulation of B-cell receptors and enrichment of the IgA production pathway, indicating that sIgA-mediated mucosal immunity is a key protective mechanism. Histopathological analysis confirmed protection against IBV-induced tissue damage, whereas a reduced viral load in critical organs demonstrated systemic efficacy. By outperforming a licenced vaccine in head-to-head comparisons, this work highlights the readiness of duckweed for real-world adoption, offering transformative potential for combating mucosa-associated infections in the poultry industry and beyond.

Conference Report

7th International Conference on Duckweed Research and Applications: Depicting an era of advancing research translation toward practical applications

Appenroth, KJ; Olah, V; Ishizawa, H; Sree, KS. (2025). Plants 14: 2143.

Duckweeds are aquatic monocotyledonous plants known to be the smallest and the fastest growing angiosperms. The 7th International Conference on Duckweed Research and Applications (7th ICDRA) was held in Bangkok, Thailand, from 12th to 16th November 2024. The conference drew young and experienced scientists from across the world who presented their research in varied fields. This conference report presents the highlights of the advancements in the field of duckweed research and application in the sections: Genomics and Cell Biology; Diversity, Ecology, Evolution; Physiology, Reproduction, Metabolomics; Microbiome and Interactions; Applications; and Future Outlook. The next conference, 8th ICDRA, will be held in Naples, Italy, in 2026.

Aquaculture/ Agriculture

Duckweed's effects on rice yield and quality varied with fertilizer applications

Zhao, YP; Shi, GZ; Luo, JS; Zhao, XY; Hu, SW; Hu, TT; Yang, LX; Wang, YX (2025) Plants 14: 2850.

The incidence of duckweed (*Lemna minor* L.) outbreaks in paddy fields has increased in recent years, but how it impacts rice production is still under debate. This study assessed duckweed's effects on rice yield and quality under different fertilizer regimes: organic fertilizer (OF), chemical fertilizer (CF), a mix (one-third OF and two-thirds CF based on nitrogen content, COF), and no fertilizer (NF) as a control. For each fertilizer regime, two duckweed treatments were applied: duckweed coverage (Duckweed) and no duckweed coverage (Control). A light wet-dry alternate irrigation method was used in the experimental field. Averaged across all fertilizer treatments, duckweed coverage in paddy fields increased grain yield by 8.3%, mainly due to increased panicle density. Duckweed coverage increased chalky grain percentage by 17.0% under NF, but decreased it by 33.7% under CF, with nonsignificant changes under COF and OF conditions. Similar fertilizer-by-duckweed interactions were also found for chalkiness degree, white degree, breakdown and setback values of the starch rapid visco analyzer (RVA) profile, palatability index, and protein and amino acid concentrations. Duckweed coverage decreased protein and amino acid concentrations but improved the taste of cooked rice under NF, while the opposite trend was observed under CF. Duckweed coverage significantly decreased copper and zinc concentrations in milled rice, which may aggravate the "hidden hunger" risk for rice consumers.

A pilot-scale evaluation of duckweed cultivation for pig manure treatment and feed production

Lambert, M; Devlamynck, R; de Souza, MF; Vermeir, P; Raes, K; Eeckhout, M; Meers, E. (2025). Plants 14: 2680.

Livestock-intensive regions in Europe face dual challenges: nutrient surpluses and a high dependency on import of high-protein feedstocks. This study proposes duckweed (Lemnaceae) as a potential solution by recovering nutrients from manure-derived waste streams while producing protein-rich biomass. This study evaluated the performance of duckweed treatment systems at a pig manure processing facility in Belgium. Three outdoor systems were monitored over a full growing season under temperate climate conditions. Duckweed cultivated on constructed wetland effluent showed die-off and low protein content, while systems supplied with diluted liquid fraction and nitrification-denitrification effluent achieved consistent growth, yielding 8 tonnes of dry biomass/ha/year and 2.8 tonnes of protein/ha/year. Average removal rates were 1.2 g N/m^2/day and 0.13 g P/m^2/day. Growth ceased after approximately 100-120 days, likely due to rising pH and electrical conductivity, suggesting ammonia toxicity and salt stress. Harvested duckweed had a high protein content and a total amino acid profile suitable for broilers, though potentially limiting in histidine and methionine for pigs or cattle. Additionally, promising energy and protein values for ruminants were measured. Although high ash and fibre contents may limit use in monogastric animals, duckweed remains suitable as part of a balanced feed. Its broad mineral profile further supports its use as a circular, locally sourced feed supplement.

Enhanced growth and productivity of useful metabolites by indole-3-propionic acid treatment in *Lemna aequinoctialis* culture

Lee, YB; Cho, JY; Jeong, Y; Park, S; Choi, HK (2025) BMC Plant Biology 25: 1063.

Lemna aequinoctialis is a floating plant with potential uses as food, feed, biofuel, biomass, and pharmaceutical resources. In this study, we examined the effect of various concentrations of indole-3-propionic acid (IPA) on the growth, metabolomic, and transcriptomic profiles of *L. aequinoctialis* cultures. IPA significantly improved the growth of *L. aequinoctialis* culture and altered its metabolome and transcriptome profiles. It also increased the cellular production of aspartate, glutamate, glutamine, serotonin, tryptophan, and phytosterols. Transcriptomic analysis revealed that 7,490 genes were significantly altered by the 10 µM IPA treatment. Metabolomic and transcriptomic profiling indicated that the IPA treatment notably altered the major pathways of 'glycerolipid metabolism', 'glutathione metabolism', 'beta-alanine metabolism', 'alanine, aspartate, and glutamate metabolism', and 'starch and sucrose metabolism'. The highest productivities of serotonin, beta-sitosterol, campesterol, and stigmasterol were observed on day 28 following treatment with 10 µM IPA. The results of this study indicate that the application of IPA to *L. aequinoctialis* cultures significantly enhanced the growth and production of useful metabolites by affecting various metabolic pathways. This study is the first to investigate the biological effects of IPA on *L. aequinoctialis*, through an integrated analysis of metabolomics and transcriptomics. This could facilitate the large-scale cultivation of *L. aequinoctialis* for the application in various fields of agriculture and biotechnology including food, feed, and pharmaceutical industries.

Effects of tryptamine on duckweed growth

Di, QQ; Han, WQ; Han, YJ; Liu, SZ; Hu, Y; Qu, ZY; Jiang, YM; Sun, WB; Qiu, T; Yang, L. (2025). Frontiers in Plant Science 16: 1625939.

Plant growth regulation involves complex biochemical and signaling pathways. Tryptamine (Try), a polyamine derived from tryptophan, has been implicated in plant growth and stress responses, yet its specific regulatory mechanisms have not been fully understood. This study investigates the physiological and molecular effects of Try on *Lemna turionifera* 5511, focusing on its role in growth regulation, photosynthesis, and hormonal balance. Our findings reveal that Try content increases in overgrown duckweed, suggesting its involvement in aging and stress responses. Exogenous Try application at concentrations ranging from 50 to 200 µM resulted in dose-dependent growth inhibition, with 150 µM Try significantly reducing growth rate, leaf area, and chlorophyll content. The Chlorophyll a (Chla) and Chlorophyll b (Chlb) levels were decreased by 37.5% and 40.43%, respectively. Try treatment also negatively impacted photosynthesis, as evidenced by reduced chlorophyll fluorescence parameters and downregulation of 16 photosynthesis-related genes. Additionally, Try induced oxidative stress, increasing reactive oxygen species (ROS) and peroxidase (POD) activity by 9.17% and 10.11%, respectively. While modulating endogenous hormone levels, particularly increasing abscisic acid (ABA) and decreasing cytokinin (CTK) content by 23.58% and 17.55%. Moreover, transcriptomic analysis revealed an upregulation of auxin (IAA) metabolism-related enzymes by Try addition. Meanwhile, changes in the expression of genes related to the tryptophan metabolism pathways indicate a metabolic change associated with aging. These results highlight the complex role of Try in regulating duckweed growth and stress responses, suggesting its potential as a regulatory molecule in plant development. Further research is needed to elucidate the molecular mechanisms underlying the influence of Try and its applications in agriculture and environmental management.

Enhancing *Wolffia globosa* cultivation: Feasibility of aerated suspended-growth systems as alternative cultivating option and the influence of abiotic factors on biomass production, CO_2 capture, and biochemical composition

Sucunthowong , K; Motlagh, S.R; Plengsakul, J; Powtongsook, S; Nootong, K. (2026) Process Biochemistry 160: 31-40.

This study evaluated the feasibility of well-mixed suspended-growth systems for cultivating *Wolffia globosa* and investigated the effects of abiotic factors on growth, CO_2 capture, and biochemical composition of *Wolffia*.

Continuous aeration at 0.4 and 0.8 vvm significantly improved biomass productivity compared to static water cultivation systems, while continuous stirring at 600 rpm caused extensive frond damage and lower biomass concentrations. Under optimized aerated conditions (500 mg/L initial biomass, 80 mg N/L initial nitrate, and 402 µmol photon/m2·s light intensity), the maximum biomass productivity reached 199.94 ± 25.88 mg/L·d, representing 83–277 % increase over static water cultivation. The corresponding CO_2 fixation rate (283.71 ± 36.73 mg CO_2/L·d) was comparable to those of Chlorella, highlighting the potential of *W. globosa* for biological CO_2 mitigation. Protein contents under optimized condition was 21.5 % of dry weight, with nitrate availability positively influencing protein accumulation. Key carotenoids, including lutein, β-carotene, neoxanthin, and violaxanthin, were detected, with no significant difference ($p > 0.05$) between suspended growth and static water systems. Furthermore, due to large frond size, biomass harvesting can be effectively conducted using filtration with low-cost, locally available materials.

Biochemistry

Duckweeds: Model organisms for research on plant sterols and steroids

Klein, J; Appenroth, KJ; Sree, KS (2025) Plant Biology DOI10.1111/plb.70095

More than two centuries since the birth of the botanist Matthias Jacob Schleiden, who first described many duckweed (Lemnaceae) species, interest in these small aquatic monocots is still alive. Lemnaceae have high biomass production capacity and can be used as animal feed and in human nutrition. Efficient transformation protocols and available genome data for several Lemnaceae species make them an ideal model system for research into biosynthesis and physiology of sterols and/or steroids in plants, especially monocots. Here we emphasize how studies using duckweed species can address current problems in plant physiology, with a strong focus on sterol and steroid biology in monocots. Further, we discuss how this knowledge can be translated to solve agricultural and industrial problems.

GC-MS, FTIR, XRD, UV-Vis, and HPLC profiling of the natural bioactive constituents of *Lemna minor* L. extract and its in-vitro antimicrobial and anti-parasitic activities against pathogenic organisms

Shaheen, S; Abbas, M; Siddique, R; Khalid, Z; Alyas, T; Khalid, S; Alzahrani, J; Ghani, N; Sarwar, S; Kamal, A; Nazish, M; Lackner, M. (2026) Kuwait Journal of Science 53: 100469.

The current study focuses on *Lemna minor* L. (common duckweed) through proximate analysis, qualitative and quantitative phytochemical analysis by UV spectroscopy (UV), gas chromatography and mass spectroscopy (GCMS), Fourier-transform infrared (FTIR) spectroscopy, and X-ray diffraction analysis (XRD). The phytochemical investigation of the fresh sample of *L. minor* was compared with the powdered sample extracted using different drying procedures. The highest moisture content was recorded in fresh samples (87.94 %), while maximum percentages of ash, fiber, and protein were found in oven-dried powder as 14.81 %, 24.01 %, and 34.92 %, respectively. The freeze-dried powder contained the greatest percentages of fat (8.48 %) and carbohydrates (17.5 %). The qualitative phytochemical analysis detected variable concentrations of bioactive compounds. Quantitative analysis revealed that tannins were found in the maximum concentration across all solvents, while nonpolar or polar steroids were extracted the least. In terms of mineral content, the freeze-dried plant samples contained the maximum levels of potassium (54.51 mg/100g), followed by phosphorus (31.750.25 mg/100g), magnesium (18.25 f 0.25 mg/100g) and sodium (4.4 f 1 mg/100g). The maximum antibacterial potential was achieved against E. coli, that was 21.4 mm zone of inhibition (ZOI). Similarly, the maximum fungal inhibition was observed against *A. niger*, which was 21 mm ZOI at the highest concentration of 75 µ l. The parasite's promastigote and amastigote forms each had a significant death rate of 75.40 f 1.16 and 60.15 f 0.12 at 150 g/ mL, respectively.

Bioenergy

Development and utilization of duckweed starch as an emerging starch resource: A review

Han, D; Li, L; Jin, D; Zhang, P; Cheng, L; Hong, Y; Gu, Z. (2025) International Journal of Biological Macromolecules 147931; DOI10.1016/j.ijbiomac.2025.147931.

Starch resources are highly valued in food processing for their superior functional properties. The source of starch greatly affects food quality. Duckweed exhibits superior resilience, minimal maintenance requirements, rapid biomass accumulation, and high starch yields, positioning it as a sustainable solution to address shortages of traditional starch resources under extreme conditions. However, research on duckweed starch has primarily focused on a limited number of species and methods for inducing starch accumulation and energy production. Meanwhile, systematic research on structural and functional characteristics is lacking. This review discusses starch accumulation methods and the progress in research on starch extraction of duckweed. Additionally, it presents the structural and functional characteristics of duckweed starch while discussing advanced perspectives for its application. Understanding the abiotic stresses and structural traits of duckweed starch will facilitate its resource utilization, thereby diversifying the global starch resource reservoir.

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

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

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

Harnessing green synthesis for bioethanol production: insights from duckweed (*Lemna minor*)

Vyas, M; Bhandari, K; Sharma, JG. (2025). Biofuels-UK DOI10.1080/17597269.2025.2545034

Bioethanol is a renewable liquid fuel derived from the fermentation of sugars obtained from plants or animal biomass. The manufacturing process, types, and commercial applications of bioethanol from various plant sources, including duckweed, are reviewed in this paper. The manufacturing process involves the collection of feedstock, pre-treatment, fermentation using microorganisms, and purification. The duckweed (*Lemna minor*, Family-Lemnaceae) has been highlighted as a feasible feedstock due to its rapid growth rate and adaptability in various aquatic environments. Its biochemical makeup, characterized by high starch content and a low lignin content, allows for effective fermentation and saccharification. Duckweed offers numerous advantages, including minimal land use, considerable biomass output, and wastewater treatment. The paper highlights the potential of duckweed, specifically *L. minor*, as a sustainable source of bioethanol. In the future, there is a need for research regarding genetic advancement, process optimization during the manufacturing process, and profitability, which are essential for fully harnessing *L. minor*'s potential.

Revolutionizing sugar feedstocks: Starch enhancement in duckweed (*Lemna minor*) using nitrogen-free Hoagland media

Vyas, M; Bhandari, K; Sharma, J. (2025) Letters in Organic Chemistry DOI10.2174/0115701786385014250 630070914

Bioethanol production from duckweed (*Lemna minor*) plants is a promising approach to producing renewable energy. Due to its advantageous characteristics, such as being a non-food feedstock, the fastest-growing angiosperm, globally adaptable to various climates, capable of high starch accumulation under nutrient modifications, and possessing low to no lignin content, *L. minor* is a promising candidate for bioethanol production. This research aimed to produce starch-enhanced *L. minor* under conditions of nutritional starvation. The starch enhancement technique was standardized by performing experiments sequentially with organic manure, nitrogen-free Hoagland media, and full-strength Hoagland media. Initially, the starch quantity was found to be 7%. However, it was observed that during nitrogen stress, high starch accumulation occurred in *L. minor*, and on the 9th day, it reached its maximum, at 26% with a standard deviation of ± 0.3. It was also observed that the protein, glucose, and fructose composition dropped during this experiment. One-way ANOVA analysis was performed for statistical analysis. It was found that during the starch enhancement experiment, the starch level was significantly ($P < 0.05$) higher in nitrogen-free Hoagland media compared to the organic manure. Therefore, *L. minor* may be used as a substitute for commercial food feedstocks in bioethanol production.

Biotechnology

Rediscovering duckweed's value: Uniting to advance its research system and applications

Yang, J; Ishizawa, H; Hou, H (2025) Journal of Experimental Botany DOI10.1093/jxb/eraf409

Advocate for substantial investment in and the establishment of an efficient duckweed research system to advance its basic research and applications in multiple fields including resources, energy, environment, and space.

Ecology

A multi-tiered workflow for constructing a quantitative adverse outcome pathway network for gamma radiation-induced reproductive inhibition in *Lemna minor*

Xie, L; Tollefsen, KE. (2025) Ecotoxicology and environmental safety 304: 119129.

While numerous qualitative adverse outcome pathways (AOPs) have been developed to describe the progressive toxicity of ionizing radiation, quantitative AOPs (qAOPs) to detail how effects quantitatively propagate from the initial molecular interaction to an adverse outcome remains limited. This study addresses this gap by proposing a multi-tiered workflow for developing a qAOP network (qAOPN) to characterise the detailed relationship between deposition of ionizing energy and decreased population growth rate in aquatic macrophytes, based on published quantitative dose-response (DR) data for gamma (Co-60) radiation-induced reproductive inhibition in the aquatic plant *Lemna minor*. First, a preliminary AOPN was assembled for the effects of ionizing radiation on plants captured in established AOPs #386, #387, and #388 (www.aopwiki.org). Weight of evidence (WoE) assessment from the AI-informed tool AOP-helpFinder and a narrative review were then applied to validate the confidence of individual key events relationships (KERs). Further quantification of the KERs was performed using a combination of point of departure (PoD) analysis via benchmark dose (BMD) modelling, structure equation modelling (SEM) and multiple nonlinear regression modelling (MNLRM). This approach effectively identified the most sensitive events within the AOPN and determined which linear AOPs were most relevant for low-dose radiation exposure. The resulting multi-tiered analysis enhanced the understanding of the underlying toxicity pathways, identified data gaps, and demonstrate how quantitative qAOPNs can be used to predict adverse (apical) impacts. The study presents a generic workflow for constructing qAOPNs that can expand the utility of mechanistically-informative AOPs towards quantitative models applicable both to chemical and non-chemical stressors.

Hormonal profiles in summer and winter shoot apices of five aquatic plant species

Adamec, L; Placková, L; Dolezal, K. (2025) Physiologia Plantarum 177: e70527.

Hormonal profiles in winter organs of aquatic plants, formed in response to temperature decrease and short days, are largely unknown. We compared the hormonal levels in summer and winter shoot apices/fronds of five aquatic species with insufficiently known overwintering strategies with their dark respiration rates as a criterion for dormancy. Miniature samples of shoot apices/fronds were extracted and analyzed by UHPLC-ESI-MS/MS. Dark respiration of summer and winter shoot apices was measured using a Clark-type oxygen sensor. A simple growth test was conducted on *Lemna trisulca* and *Ricciocarpos natans* to ascertain their strength of dormancy. Variable respiration rates of turions and winter apices/fronds revealed marked interspecific differences in levels of dormancy. The winter total cytokinin (CK) content usually resembled the summer one or was significantly lower, with great interspecific differences. Active CKs decreased by about two times in winter. Also, the content of the active auxin IAA was usually lower than in summer, but in *L. trisulca* it rose 4.5 times. Increased abscisic acid (ABA) winter content (11-3124 nmol kgDW -1) confirmed its significant accumulation in four species studied, except for *Utricularia inflata*. Generally, mature dormant turions have low dark respiration rates, in comparison with non-dormant winter shoot apices/fronds, at least on a dry-weight basis. Deviations from the rule correlate with the power of turion dormancy, which is variable among species and represents a continuous gradient from dormancy to quiescence. Concerning overwintering strategies in aquatic plants, the hormonal regulation of the formation of either turions or non-dormant winter apices is not universal.

Feed & Food

Proteins derived from green biomass: Alfalfa (*Medicago sativa* L.) and water lentil concentrate (*Lemna minor* L.) in the focus as stabilizers for emulsions

Müller, M; Holderer, O; Schwärzer, K; Wiese-Klinkenberg, A; Förster, B; Förster, S; Kohlbrecher, J; Wood, K; Wu, BH; Hauschild, S; Frielinghaus, H; Heiden-Hecht, T. (2025) Food Hydrocolloids for Health 8: 100233.

Green crop biomass is a sustainable protein source, which can support solving global food challenges as well as improving the bioeconomy for novel food utilization. Two green biomasses -alfalfa and water lentil- known for a high protein content and their value as feed and food components were investigated for their potential in stabilizing emulsions. For interfaces in complex food systems like emulsions, the proteins from the green biomasses act similarly to other plant proteins and even to some extent to whey proteins. The extracted green biomass protein concentrates were composed of a diverse mixture of proteins: RuBisCo as the main component, enzymes like ATP synthase subunit, chlorophyll a-b binding proteins, histones and cytochromes, which all were associated as protein aggregates or protein-polyphenol complexes with sizes from 33 up to 85 nm. These multicomponent and complex green protein components were obtained in an extraction process, and proved their capability to stabilize emulsions. Key parameters are a narrow oil droplet size distribution with rather small oil droplets with a median of about 3.5 μm, a low interfacial tension with around 15 mN/m and an elastic interfacial layer with an elastic modulus of about 45 mN/m comparable to functional whey or plant proteins. The oil/water interface of the emulsion revealed a more pronounced structural rearrangement for alfalfa protein compared to water lentil proteins as quantified by small-angle neutron scattering (SANS), indicating the relevance of plant protein source to the final structure. Green biomass proteins from alfalfa and water lentil represent a novel protein source to stabilize emulsions.

Duckweed as a sustainable protein source: extraction methods, functional properties, and applications in food systems

Habeeb, F; Majid, D; Makroo, HA; Castagnini, JM; Barba, FJ; Dar, BN (2025) Food Chemistry 493: 145854.

Duckweed has gained significant attention as a sustainable protein source due to its rapid growth, high protein content (up to 45% dw), and well-balanced amino acid profile. Its potential as alternative to traditional plant-based proteins is explored, focusing on the extraction and purification methods that enhance protein yield and bioactivity. Techniques, including ultrasound-assisted extraction, high-pressure processing, and pulsed electric field, are highlighted for their ability to improve protein efficiency and functionality. Duckweed proteins exhibit valuable properties such as emulsification, foaming, and gelling, along with potential to produce bioactive peptides with health-promoting benefits. However, challenges such as heavy metal contamination, allergenicity, and the need for pure production remain barriers to large-scale commercialisation. A systematic search was carried to write this review article using PRISMA. Future research is essential to develop bioengineering solutions and optimise cultivation methods to fully explore duckweed's potential in contributing to sustainable food systems and the circular economy.

Emerging potentials of duckweed (Lemnaceae): From composition to protein uses in food and nutraceuticals-A review

Muller, T; Cournoyer, A; Bazinet, L. (2025) Food Research International 219: 116777.

Duckweed, a rapidly growing aquatic plant, is gaining significant attention as a sustainable protein source. However, fully unlocking its potential requires a comprehensive evaluation, from its nutrient composition to its applications in both food and nutraceutical products. This review provides a holistic approach, beginning with the factors influencing duckweed's nutrient composition, including growth conditions and environmental variables. The challenges of protein extraction from duckweed are reported in comparison to other leafy plants, highlighting current obstacles and innovative methods. The functional properties of duckweed proteins after extraction-including solubility, emulsification, foaming, and gelling-are critically assessed for their relevance in food product development. Duckweed protein digestibility in animal and humans is then thoroughly examined, underscoring duckweed's prospect as a high-quality protein source. Additionally, the bioactivities of duckweed-derived compounds, particularly peptides, are explored for their potential health benefits, including antimicrobial, antihypertensive, antidiabetic, anticancer and antioxidant properties. This review also addresses consumer acceptance and real-world applications based on existing literature, commercial developments, and patents. Regulatory and safety considerations are reported, highlighting both the successes and challenges encountered in recent regulatory efforts. Overall, while duckweed has significant potential as a sustainable food source, research and commercial interest are still in their early stages. Its future role and identity in the food system, whether as a whole food, functional protein source, or source of bioactive compounds, remains to be defined.

Nutritional value, health properties, safety considerations, and consumer acceptance of Lemnoideae as human food

Ziec, G; Michalski, O; Konieczna-Molenda, A; Dera, T; Tkaczewska, J. (2025) Nutrients 17: 3026.

The growing demand for sustainable and nutrient-rich food has drawn attention to aquatic plants, particularly those of the Lemnoideae subfamily, commonly known as duckweed. These fast-growing plants are rich in high-quality protein and offer an attractive alternative to traditional plant and animal protein sources, especially in the context of targeted nutrition and sustainable diets. This review is a critical assessment of Lemnoideae potential as a functional food ingredient for various population groups, including vegans, diabetics, the elderly, and individuals with specific dietary needs. Their amino acid profile, protein digestibility, allergenicity, and bioactive compound content are evaluated in the paper. Also examined are health-related outcomes and applications, including clinical and space nutrition, alongside current barriers such as limited consumer acceptance and regulatory hurdles. Lemnoideae demonstrate a favorable amino acid profile, high digestibility, and low allergenic potential. They are a source of bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties, and show promising effects in managing metabolic disorders such as type 2 diabetes and cardiovascular disease. Legal approvals in the EU and U.S. mark a shift towards broader acceptance. While sensory attributes and consumer unfamiliarity remain challenges, the findings allow us to highlight Lemnoideae as a promising next-generation plant protein source that could contribute to sustainable food systems and support the development of novel functional foods tailored to specific dietary needs.

Optimizing formulation of fried rice-based snack supplemented with watermeal (*Wolffia arrhiza* (L.) Wimm.) and rice protein isolate powders using an I-optimal design: enhanced protein content and functional properties

Patcharabudsarakumkul, K; Liamlaem, S; Hemathulin, S; Kapcum, C; Itthivadhanapong, P; Somkhumphee, Y; Piayura, S (2025) Frontiers in Sustainable Food Systems 9: 1661446.

This study aimed to optimize a fried snack formulation comprising Tubtim Chum Phae rice flour (RF; 60-79%), watermeal (*Wolffia arrhiza*, WM; 11-30%), and rice protein isolate (RP; 5-10%) to enhance protein content and functional properties, including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AOA). Response Surface Methodology (RSM) with an I-optimal design was employed to predict the effects of ingredient ratios on all responses. The optimized formulation was validated through experiments, and its microstructure was analyzed using SEM. The amino acid profile was also determined. The RSM models accurately predicted the effects of ingredient ratios on all responses. Numerical optimization identified an optimal formulation consisting of 60.91% RF, 29.70% WM, and 8.39% RP, with validation experiments confirming strong agreement between predicted and actual values. The optimized snack exhibited 20.68% protein and improved functional properties. It showed a hardness of 14.04 N and a porous, non-uniform microstructure under SEM analysis. In addition, the amino acid profile, particularly in essential amino acids, was markedly enhanced compared to that of rice flour alone. These findings demonstrate that incorporating

WM and RP using RSM is an effective strategy to improve the nutritional and antioxidant properties of rice-based snacks.

DF comment: The authors did not show convincingly that they worked with *Wolffia arrhiza* and not with *W. globosa*. Does *W. arrhiza* exist in Thailand?

Testing an insect-plant circular system based on black soldier fly and duckweed

Arce-Valdés, LR; Mahdjoub, H; Bianco, L; Vilanova, J; Bafti, NH; Moncivais-Gómez, AK; Khelifa, R. (2025). Journal of Insects as Food and Feed 11: 1689-1702.

Optimizing the circularity of insect farming by reusing and repurposing its by-products is a priority to reach global sustainable goals that benefit both nature and people. The frass of the black soldier fly (BSF) has been used as fertilizer for various crops, but its potential in promoting the growth of aquatic plants with high-protein content has not been investigated. Here, we assess the potential of using BSF frass to produce duckweed (*Lemna minor*, a fast-growing aquatic plant), and then use duckweed as a nutritional supplement for BSF larvae. First, we performed a set of laboratory experiments assessing duckweed growth under different concentrations of frass (with and without the presence of algae). Then, we quantified various life history traits (survival rate, larval growth rate, adult body mass and life span) of BSF across different treatments with increasing levels of duckweed supplementation (fresh and dry) in the standard BSF diet (0%, 25%, 50%, 75% and 100%). We found that the optimal concentration of frass that provided the fastest duckweed growth rates was between 4 and 6 g/L, exceeding that recorded for the traditional Hoagland's E media. BSF larvae growing in a diet supplemented by fresh and especially dry duckweed did not improve growth rate, survival rate, body size, nor lifespan. Higher duckweed contents tended to reduce the performance of BSF. Our results suggest that BSF frass could be used as a cheap fertilizer to produce duckweed, but the latter is not an optimal supplement for BSF, and thus should be used for other applications.

Effect and optimal level of dietary dried watermeal (*Wolffia globosa*) supplementation on the production performance of two-spotted crickets (*Gryllus bimaculatus*)

Mitchaothai, J; Grabowski, NT; Lertpatarakomol, R; Trairatapiwan, T; Lukkananukool, A. (2025) Animals 15: 2052.

Watermeal (*Wolffia globosa*) is an emerging sustainable feed ingredient, valued for its rapid growth, high protein content, and rich nutrient profile, offering great potential to improve the efficiency and sustainability of edible cricket production systems through efficient resource use and reduced environmental impact. This study aimed to evaluate the effects of replacing commercial cricket feed with dried watermeal and to determine the optimal substitution level for the production performance of two-spotted crickets (*Gryllus bimaculatus*). Seven dietary treatments were tested, namely 0% (control; T1), 10% (T2), 25% (T3), 50% (T4), 75% (T5), 90% (T6), and 100% (T7). Crickets were reared for four weeks in an open system with 6 replicates per treatment, with each containing 120 nymphs. Body weight, feed intake, and survival rate were recorded weekly, and a production index was calculated at the end of the trial. Results showed average growth rates ranging from 7.40 to 28.20 mg/day, feed conversion ratios (FCR) between 1.03 and 1.68, survival rates of 29.28% to 69.73%, and production index values from 24.00 to 128.30. A significant decline in production efficiency ($p < 0.05$) was observed at substitution levels of 75% and above, with the 100% watermeal group showing the lowest production efficiency ($p < 0.05$), likely due to nutrient imbalances and indigestible compounds. Segmented regression analysis identified 36.7% as the optimal level of watermeal supplementation. In terms of body composition, crickets fed diets containing up to 50% watermeal (10%, 25%, and 50%) showed crude protein, crude fat, crude fiber, and ash contents comparable to those fed the commercial diet. This finding suggests that dried watermeal can replace up to 50% of the commercial cricket feed without negatively affecting growth performance, survival, or nutritional quality, with 36.7% identified as the optimal replacement level for maximizing production efficiency.

Innovative integration of duckweed proteins: Enhancing the nutritional and textural properties of snack bars, roll breads, and muffins enhancing baked goods with duckweed protein

Akyüz, A; Ersus, S. (2025) Journal of Food Science 90: e70374.

Duckweed, a fast-growing aquatic plant rich in high-quality protein, has emerged as a sustainable and alternative vegan protein source for food applications. This study aimed to evaluate the effects of duckweed protein concentrate (DPC) incorporation on the physical, chemical, and textural properties of snack bars, roll breads, and muffins. Based on preliminary optimization studies, DPC was added at levels of 12.5% in snack bars, 5% in roll breads, and 10% in muffins. Its inclusion significantly decreased the lightness values of all products, with the most pronounced reduction observed in roll bread crumb (54.5%) and snack bars (34.8%). Protein contents increased by 41%, 28%, and 12% in snack bars, roll breads, and muffins, respectively, improving their nutritional profiles. Chlorophyll content rose substantially-up to 18.8-fold in roll breads-along with notable increases in total phenolic and flavonoid contents, enhancing antioxidant capacity. A significant increase in hardness was observed in all products, especially in snack bars. These results suggest that DPC can be effectively used as a functional ingredient in bakery and snack products to boost their nutritional and functional value. Duckweed protein concentrate can be used to enhance the nutritional profile of bakery and snack products by increasing protein, chlorophyll, and antioxidant contents. Its incorporation offers a sustainable plant-based protein alternative, making it a promising ingredient for the development of functional food products.

Dietary bile acid supplementation alters the gut transcriptome and metagenome and contributes to herbivorous diet adaptation in juvenile allodiploid hybrid fish

Zhou, ZX; Yuan, Y; Liu, YY; Chen, SD; Li, YC; Miao, Y; Wang, S; Shen, ZY; Zeng, L; Ren, L. (2025) Aquaculture 604: 742506

The aim of this study was to explore the effects of dietary bile acids (BAs) supplementation on herbivorous diet adaptation in allodiploid hybrid fish derived from blunt snout bream (female) x topmouth culter (male). Three experimental diets were formulated: a commercial basal diet (CG group), a basal diet supplement with duckweed (H group), and a basal diet supplemented with duckweed and 600 mg kg^{-1} BA (T group). After 56 days of feeding, the growth parameters increased, and greater intestinal cellulase activity was detected in the T group. Moreover, the T group fish presented a longer and denser small intestinal villus. In general, the characteristics of the H group were intermediate between those of the other two groups (T > H > CG). Compared with CG group fish, 711 specific differentially expressed genes (DEGs) were identified in the intestine of T group fish. The upregulated DEGs were primarily enriched in MAPK signaling pathway, protein digestion and absorption, and mTOR signaling pathway; the downregulated DEGs were predominantly enriched in steroid hormone biosynthesis, fatty acid metabolism and primary bile acid biosynthesis. Besides, several upregulated genes, such as jak, irs, egfr, prss, and cpa which related to cell proliferation and differentiation, digestion, and metabolism, are associated with the changes in intestinal histomorphology. These changes could promote the intestinal adaptation of an herbivorous diet. The metagenome sequencing results revealed that exogenous BAs significantly changed the structure of the gut microbiota; decreased the abundance of potentially pathogenic bacteria or fungi such as Proteobacteria, Mucoromycota, and Pseudomonas; and increased the abundance of probiotics such as the cellulose-producing bacteria Micromonospora. In addition, functional analysis revealed that the abundance of some enzyme families related to cellulose degradation was significantly greater in the T group. These findings suggested that exogenous BAs influences host intestinal gene expression and the structure of the gut microbiota via the gut microbiota-bile acid pathway, which in turn contributes to herbivorous diet adaptation in juvenile allodiploid hybrid fish.

Growth & Development

Salicylic acid-triggered flowering in *Lemna gibba*: evidence of a stress-mediated pathway via NO and ROS

Chauhan, S; Chatterjee, D; Peer, LA; Mir, BA; Babbar, SB (2025). Journal of Biochemistry and Biotechnology DOI10.1007/s13562-025-01004-4

Salicylic acid enhances flowering in *Lemna gibba* L. (a long-day monocotyledonous plant), leading to earlier initiation of flowering under long days and induction of flowering under non-inductive conditions. The nitric oxide scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide partially suppressed salicylic acid-induced flowering, indicating the involvement of nitric oxide. The nitrate reductase inhibitor, sodium tungstate, and nitric oxide synthase inhibitor, N-omega-nitro-L-arginine-methyl ester hydrochloride, significantly reduced salicylic acid-enhanced and induced flowering under both light regimes. Salicylic acid-treated fronds showed higher nitrate reductase activity than the controls under both light conditions, suggesting nitric oxide synthesis via the nitrate reductase pathway in the presence of salicylic acid. These findings are further supported by the observation of higher nitric acid concentrations in salicylic acid-treated fronds before flowering under both light regimes. The reactive oxygen species scavengers, dimethyl thiourea, and N-methyl L-cysteine, significantly negated the effects of salicylic acid on flowering in both long and short days. The combined application of nitric acid and reactive oxygen species scavengers had an additive effect, reducing flowering in salicylic acid-treated fronds to control levels under long-day conditions and completely repressing it under short-day conditions. Additionally, salicylic acid-treated fronds exhibited increased malondialdehyde content and decreased antioxidant enzyme activities under both light regimes, indicating oxidative stress. Statistical analyses, including Kruskal-Wallis and bootstrap resampling, confirmed the robustness of the results. These findings suggest that flowering in *L. gibba* is induced by salicylic acid through a stress-mediated signaling pathway involving nitric oxide and reactive oxygen species.

Nitric oxide and cyanide enhance flowering in *Lemna gibba* through a stress-mediated pathway under inductive conditions

Chauhan, S; Chatterjee, D; Peer, LA; Mir, BA; Babbar, SB . (2025) Journal of Plant Growth Regulation DOI10.1007/s00344-025-11820-6

Nitric oxide serves as a pivotal signaling molecule that governs diverse physiological processes in plants, including the regulation of flowering. This study elucidates the interplay between nitric oxide and cyanide signaling in modulating floral induction in *Lemna gibba*, a model long-day plant, under photoperiodic inductive conditions. Through a systematic investigation of three distinct nitric oxide donors, sodium nitroprusside, S-nitroso-N-acetyl-DL-penicillamine, and 3-morpholinosydnonimine, we demonstrated concentration-dependent flowering promotion, with maximal responses observed at donor-specific optimal concentrations. The complete reversal of this stimulation by the nitric oxide scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide established nitric oxide as the central mediator. Notably, cyanide-induced flowering exhibited identical dependence on nitric oxide signaling, as evidenced by both chemical inhibition and direct measurement of elevated endogenous nitric oxide levels. Physio-biochemical investigations revealed that nitric oxide donors activate endogenous synthesis pathways, as flowering is suppressed by inhibitors of both nitrate reductase and nitric oxide synthase. The involvement of oxidative stress signaling was confirmed through multiple lines of evidence: flowering induction correlated with increased lipid peroxidation, alterations in antioxidant enzyme activities, and partial suppression by reactive oxygen species scavengers. Our findings establish a novel stress-mediated flowering pathway, wherein nitric oxide and cyanide converge through shared oxidative signaling mechanisms. These results provide fundamental insights into plant reproductive control and open new avenues for manipulating flowering timing in agronomically important species through targeted modulation of nitric oxide signaling networks.

Clonal parental effects on competitive interactions between two duckweeds

He, LX; Jin, Y; Zhang, XM; Luo, FL; Xue, W; Lei, JP; Liu, H; Yu, FH. (2025) Frontiers in Plant Science 16: 1587907.

Parental environments can influence offspring fitness via clonal (asexual) propagation, and such clonal parental effects may vary among plant species and depend on offspring environments as well. Consequently, clonal parental effects may alter competitive interactions between plant species, and such impacts may vary with offspring environments. We conducted a two-phase experiment with two clonal floating duckweeds, *Spirodela polyrhiza* and *Lemna minor*. In the parental phase, *S. polyrhiza* and *L. minor* were grown separately under two distinct nutrient conditions and produced offspring ramets. In the offspring phase, the ramets produced from the parental phase were grown with or without a heterospecific neighbor under the same two nutrient conditions. In the first phase, parent ramets of both species produced more biomass and offspring ramets under high nutrient availability than under low. In the second phase, nutrient availability experienced by the parents significantly affected the competitive ability of offspring in both species. Specifically, the offspring of *L. minor* suppressed those of *S. polyrhiza* more strongly when the parent of *L. minor* had been grown under high than low nutrient availability, although such clonal parental effects did not vary with nutrient availability experienced by the offspring. In contrast, the offspring of *S. polyrhiza* suppressed those of *L. minor* more strongly when the parent of *S. polyrhiza* had been grown under high rather than at low nutrient availability, but this effect occurred only under high nutrient availability for the offspring and diminished under low nutrient availability. These results suggest that clonal parental effects can influence competitiveness of plants and may vary depending on offspring environments. Our findings highlight the potential role of clonal parental effects in regulating interspecific interactions, which may further influence species composition and productivity of plant communities.

Interaction with other organisms

Novel cyanobacterial strains modulate morphophysiological traits of *Lemna trisulca*: Insights from short-and long-term interactions

Kaminski, A; Lelito, B; Panou, M; Pappas, D; Panteris, E; Gkelis, S. (2026)Marine Pollution Bulletin 222: DOI10.1016/j.marpolbul.2025.118632.

Cyanobacteria produce a diverse array of bioactive secondary metabolites, encompassing both harmful and potentially beneficial compounds. This study evaluated the effects of five cyanobacterial strains with uncharacterized metabolomes, including the new species *Komarekiella chia*, *Nodularia mediterranea*, and *Iphianassa zackieohae* - on *Lemna trisulca* plant. Both short-term (exposure of plant to cyanobacterial extracts for 24 h) and long-term (2-week co-cultivation) experiments were conducted. In short-term assays, *Jaaginema* sp. extract transiently inhibited photosynthesis within 7 min, while all extracts produced an increase in respiration after 24 h. Ultrastructural analysis revealed that co-cultivation with J*aaginema* sp. and *Trichormus variabilis* resulted in chloroplast membrane abnormalities and absence of starch grains. *K. chia* and *N. mediterranea* induced distinct ultrastructural modifications, whereas *I. zackieohae* caused only minimal changes. During the long-term experiments, co-cultivation with *I. zackieohae* initially stimulated plant growth, although this was not sustained after 14 days. The remaining strains caused a slight inhibition of biomass accumulation. Notably, long-term exposure to *K. chia* and *N. mediterranea* led to increases in plant protein content by 33 % and 44 %, respectively. Only *I. zackieohae* did not affect chlorophylls content, while other strains reduced chlorophyll a and b by 48 % and 53 %, respectively. Reactive oxygen species imaging revealed no signs of oxidative stress during either exposure period. Finally, HPLC/MS-MS analyses ruled out the common cyanotoxins anatoxin-a, cylindrospermopsin, and microcystin-LR production in all tested strains. These findings identify promising candidate species for further investigation of novel bioactive metabolites and underscore the need for additional studies to elucidate plant-cyanobacteria interactions.

Seasonal differences in effects of microbial communities on duckweed traits

Kinerson, E; Trott, A; O'Brien, AM. (2025.) Ecology and Evaluation 15: e72211.

Host-associated microbiomes shape the expression of a diverse suite of host traits, ranging from longevity in animals to flowering time in plants. Despite their importance to host traits, the composition of host-associated microbiomes can fluctuate, and microbiomes of differing composition often have differing impacts on hosts. Fluctuations in microbiome composition can include seasonal shifts that correlate with seasonal phenotypic changes in hosts. Yet, identifying whether these correlated seasonal changes in hosts and microbial communities are causally linked remains difficult because it requires separating the effects of the abiotic environment and host or tissue age from the effects of seasonally altered microbial communities. Here, we used microcosm experiments simultaneously reinoculating axenic duckweeds with microbial communities cultured from different seasons to compare their effects on duckweed growth and trait expression. We found seasonal differences in the effects microbial communities had on duckweeds. Microbial communities cultured from duckweeds in the summer shifted duckweed fronds to be more aggregated (by 5.7%) and slightly more green (by 0.7%) than microbial communities cultured from duckweeds in other seasons, and nonsignificantly increased duckweed growth rates. Inoculation with microbial communities cultured from summer collections also resulted in more total microbial growth in microcosms. These findings enhance our understanding of the influence of seasonality on the complex relationship between microbes and their hosts, with potential future applications in agriculture and medicine.

Duckweed as an emerging model system for plant-microbiome interactions

Ishizawa, H; Saimee, Y; Sugiyama, T; Kojima, T; Inoue, D; Ike, M; Thamchaipenet, A; Morikawa, M (2025) Environmental Microbiology 27: e70181.

Understanding the processes through which plant-associated microbiomes influence host physiology and fitness is a central goal of plant-microbiome interaction research. While traditional model plants such as *Arabidopsis thaliana* have provided foundational platforms to examine these processes, alternative model systems may address certain bottlenecks in current research. In recent years, duckweeds (Lemnaceae) have emerged as a unique model plant offering several experimental advantages owing to their small size, simple morphology, aquatic habitat, and two-dimensional clonal growth. These features facilitate the establishment of highly tractable and reproducible model systems that facilitate robust investigations and high-throughput screening platforms, enabling multifactorial massive parallel experiments. This review provides an overview of the recent studies that have applied the advantages of using duckweed in the field of plant-microbiome interactions to highlight how duckweed-based systems have enabled unique experimental approaches that are difficult in conventional systems. We have also discussed the emerging directions in duckweed-microbiome research, including elucidation of the co-evolutionary processes mediated via metabolic exchange and bottom-up explanation of community structure and functions using synthetic bacterial communities. Together, this review underscores the potential of duckweed to serve as a distinctive model for advancing plant-microbiome interaction research.

Aphid herbivory on macrophytes drives adaptive evolution in an aquatic community via indirect effects

Schäfer, M; Malacrinò, A; Walcher, C; Spaak, P; Sárazová, MS; Käser, S; Bulas, T; Dambone-Bösch, C; Dexter, E; Hottinger, J; Böttner, L; Vorburger, C; Ebert, D; Xu, SQ. (2025). Proccedings of The National Academy of Sciences USA 122: e2502742122.

Indirect ecological effects occur when the impact of one species on another is mediated by a third species or the shared environment. Although indirect effects are ubiquitous in nature, we know remarkably little about how they may drive ecoevolutionary processes across community boundaries. Here, we show that insect (aphid) herbivory on macrophytes (duckweed) drove the adaptive evolution of a planktonic crustacean (*Daphnia magna*) in large outdoor aquatic mesocosms via indirect ecological effects. Aphid herbivory reduced duckweed growth and increased the nutrient and light availability in the water column, which promoted phytoplankton growth and boosted the abundance of *D. magna* that feed on phytoplankton. Whole-genome pool-sequencing and phenotypic assays revealed aphid-herbivory-mediated evolutionary changes to Daphnia population. Transplant experiments indicated that these evolutionary changes were adaptive. Furthermore, aphid-herbivory-mediated biotic and abiotic changes in the aquatic community increased the performance of the macrophytes and aphids. These results demonstrate that indirect ecological effects can shape ecoevolutionary interactions between seemingly independent species in natural communities.

Endophytic bacterium *Paenibacillus illinoisensis* Y11 promotes duckweed growth and enhances cadmium pollution remediation

Chen, ZQ; Tan, AJ; Wan, YF; Dong, YH; Yang, X; Yang, GL. (2025) Current Microbiology 82: 447.

As Cd pollution increases, ecological security becomes increasingly endangered, highlighting the significance of exploring and optimizing sustainable remediation technologies. Phytoremediation, recognized as a promising strategy for environmental restoration, is enhanced when combined with microorganisms, particularly endophytes. Duckweed, known for its high Cd accumulation capacity, holds great potential for remediating Cd pollution. However, the impact of endophytic microorganisms on duckweed's remediation of Cd pollution remains unclear. This study focuses on the endophytic bacterium *Paenibacillus illinoisensis* Y11 to investigate its effects on duckweed (*Lemna minor*) growth and duckweed's capacity to remediate Cd pollution. The results indicate that strain Y11 can successfully colonize duckweed under Cd stress, and the colonization by strain Y11 can significantly promote duckweed growth and increase its Cd removal rate. Strain Y11 was evaluated in vitro for plant growth-promoting properties, revealing the capacity to synthesize indole-3-acetic acid and siderophores, solubilize phosphate, and produce 1-aminocyclopropane-1-carboxylate deaminase, all of which contribute to plant growth. This study not only provides valuable microbial resources to enhance the Cd pollution remediation capacity of duckweed, but also establishes a theoretical foundation for the widespread application of endophytic microorganisms in plant-microbe combined remediation techniques.

Molecular Biology and Genomics

Duckweed Evolution: from Land back to Water

Fang, Y; Tian, X; Jin, Y; Du, A; Ding, Y; Liao, Z; He, K; Zhao, Y; Guo, L; Xiao, Y; Xu, Y; Chen, S; Che, Y; Tan, L; Wang, S; Li, J; Yi, Z; Chen, L; Zhao, L; Zhang, F; Li, G; Li, J; Xiong, Q; Zhang, Y; Zhang, Q; Cao, XH; Zhao, H. (2025). Genomics, Proteomics Bioinformatics DOI10.1093/gpbjnl/qzaf074

Terrestialization is an important evolutionary process that plants experienced. However, little is known about how land plants acquired aquatic growth behaviors. Here we integrate multiproxy evidence to elucidate the evolution of the aquatic plant duckweed. Three genera of duckweed show chronologically gradual degeneration in root structure and stomatal function and decrease in lignocellulose content, accompanied by gradual contraction in the number of relevant genes and/or their transcriptional decline. The gene numbers in the main phytohormonal pathway are also gradually decreased. The co-action of genes involved in auxin and rhizoid development causes a gradual decrease in adventitious roots. The significant expansion of the flavonoid pathway is also related to the adaptation of duckweed to floating growth. This study reconstructs the evolution history of the duckweed habitat from land back to water, reversing that of early land plants.

Physiology & Stress

Lemna gibba clones show differences in phenotypic responses to the light environment

Xuan, PTH; Amri, R; Bach, NP; Irfan, M; Bog, M; Appenroth, KJ; Sree, KS; Jansen, MAK; Szabó, S; Meszáros, I. (2025) Plants 14: 2840.

Duckweeds are aquatic plants with a worldwide distribution that can thrive under very contrasting ambient conditions due to their diversity and high phenotypic plasticity. In this study, we analyzed and compared the responses of four clones (i.e., distinct accessions) of *Lemna gibba* to two different light intensities. The clones represented different geographical origins and, in addition to two diploid cytotypes, included one tetraploid mutant and a triploid interspecific hybrid. We hypothesized that clonal origin had an effect on light acclimation. We studied growth, morphological (frond size and shape, mass-to-area ratio), and photosynthetic (pigment composition, chlorophyll fluorescence induction) traits of these clones to test whether light acclimation was a conserved process or whether clone-specific strategies could be found. We also analyzed frond-level photosynthetic adjustment during ontogenesis to track how light acclimation evolved in developing fronds. Our results confirmed that even clones of the same duckweed species and a hybrid of closely related species followed partially different strategies in acclimation to ambient conditions. This acclimation involved various morphological, physiological, and biochemical adjustments but happened in a very short time window at the early life stage when young, still-developing fronds could flexibly achieve an optimized phenotype. In addition to explaining the worldwide success of duckweeds in colonizing very contrasting habitats, our results also highlight the importance of approaching duckweed responses at the frond level, where the actual acclimation takes place.

Physiological and transcriptomic analysis reveal the response mechanisms to nutrient deficiencies in aquatic plant *Spirodela polyrhiza*

Zhao, XY; Li, XZ; Sun, ZL; Li, GJ; Guo, WJ; Chen, Y; Xia, ML; Chen, YM; Wang, XY; Li, YX; Luo, KS; Ji, MF; Duan, PF; Yang, JJ; Hou, HW (2025) Plant Molecular Biology 115: 107.

Macrophytes are critical primary producers in freshwater ecosystems and offer potential as crop resources to support the growing human population. They are also widely used to mitigate eutrophication. Aquatic plants adapt themselves to the more complicated, changeable, and unstable conditions compared to terrestrial plants, especially the fluctuating nutrient environments. Nitrogen (N) and phosphorus (P) are the key nutrient elements for plants, and their biogeochemical cycles have been significantly disrupted by anthropogenic activities in diverse ecosystems. However, there is still a lack of comprehensive understanding about the adaptive mechanisms of N and P stress in aquatic plants. In this study, the response mechanisms in the macrophyte *Spirodela polyrhiza* under various nutrient conditions were analyzed. *S. polyrhiza* showed universal changes under nutrient deficiencies at the physiological level, including enhanced root growth, lower Chl content, higher root-frond ratio, and starch content. Genes involved in nutrient acquisition and remobilization, carbon metabolism, transcriptional regulation, hormones, and antioxidant systems were identified. Physiological and transcriptional changes revealed that the macrophyte *S. polyrhiza* adopts a nutrient acquisition-prioritization strategy under nutrient deficiency conditions, employing strategies similar to those observed in terrestrial plants. Post-transcriptional regulatory networks also highlighted the critical role of non-coding RNAs nutrient stress responses. Overall, *S. polyrhiza* employs integrated physiological and molecular strategies to cope with nutrient deficiency in aquatic environments. This study provides comprehensive insights into its adaptive responses and offers a valuable genetic resource for further novel gene discovery and functional analysis.

Phytoremediation

Screening of selenium-tolerant duckweeds and evaluation of their selenium removal capability

Ascencio-Damian, JE; Inoue, D; Ike, M. (2025) Environmental Technology and Innovation 40: 104486.

Soluble forms of selenium (Se), selenite [Se(IV)], and selenate [Se(VI)] are recognized as important pollutants in the aquatic environment. This study attempted to identify Se-tolerant duckweed species as potential agents for sustainable Se(IV)/Se(VI) phytoremediation and examined their Se removal capacity. Among six tested duckweed species, *Landoltia punctata* (LAp) and *Lemna aequinoctialis* (La) demonstrated relatively high tolerance against Se(IV) and Se(VI) in the presence of both high- and low-sulfate concentrations. Both LAp and La could grow in healthy states and increasingly remove substantial amounts of Se(IV) and Se(VI) at typical low and high Se concentrations commonly found in contaminated sites, though they failed in removing Se(VI) under high-sulfate conditions. Major portions of removed Se accumulated in the duckweed biomass and could be recovered as a usable micronutrient. These results indicate that LAp and La are promising candidates as agents for the phytoremediation of Se-contaminated aquatic environments, while La is particularly preferable due to its ability to remove up to 40 % of both Se(IV) and Se(VI). Additionally, Se accumulation in the biomass of La reached 0.35 and 0.99 mg-Se/g dry weight for Se(IV) and Se(VI), respectively, which was higher than LAp.

Effects of duckweed species diversity on physiological responses and removal efficiency under cadmium stress

Cao, Q; Tan, AJ; Lan, Y; Zou, WB; Yang, GL (2025). Water Air and Soil Pollution 236: 820.

Cadmium (Cd) contamination threatens aquatic ecosystems and human health. Duckweeds are ideal for toxicological studies due to their rapid growth and high metal uptake. While interactions between single duckweed species and Cd has been extensively studied, the impact of duckweed diversity on Cd tolerance remains unknown. This study examines three duckweed species (*Lemna minor, Landoltia punctata*, and *Spirodela polyrhiza*) under Cd stress, assessing growth, chlorophyll content, Cd accumulation, removal efficiency, and metabolic adaptations in monoculture and mixed-culture systems. The results showed that the growth of duckweeds was inhibited across all cultivation modes under 10 mg L^{-1} Cd stress, with growth rates remaining below 0.1 g d^{-1} and experiencing more than 80% inhibition. Moreover, chlorophyll content declined with increasing Cd concentration under all cultivation modes, exceeding 64% inhibition under 10 mg L^{-1} Cd stress. Cd accumulation peaked in *L. minor* monoculture at 10 mg L^{-1} Cd (1601.33 mg kg^{-1}, with bioenrichment coefficients exceeding 50 in all systems. The combination of *S. polyrhiza* and *L. minor* exhibited the highest Cd removal efficiency, achieving 50.60% at a Cd concentration of 1 mg L^{-1}. However, the Cd removal rate of all culture modes was below 30% when the Cd concentration increased to 10 mg L^{-1}. Elevated antioxidant enzyme activities (POD, CAT) and malondialdehyde (MDA) levels indicated enhanced oxidative stress responses. This study provides support for the application of duckweed polycultures in water contaminated with heavy metals.

Toxicological efficiency evaluation of the ASEC technology for contaminated mining water using *Lemna minor*

Conradi, M; Sanchez-Moyano, E; Bonnail, E; DelValls, A; Riba, I. Water 17: 2175.

The Adiabatic Sonic Evaporation and Crystallization (ASEC) technology was developed as a disruptive zero-liquid discharge system to treat contaminated mining effluents. This study evaluates its ecotoxicological efficacy using *Lemna minor*, a freshwater macrophyte, as a sensitive bioindicator. Acute growth inhibition tests were conducted using OECD Guideline 221. *Lemna minor* was exposed for 7 days to untreated and treated effluents from the Tharsis mine and the Tinto River in southern Spain. The results revealed 100% inhibition of frond growth and biomass in untreated samples (pH < 2.6), indicating acute toxicity. In contrast, effluents treated with ASEC showed growth and biomass accumulation statistically indistinguishable from the control, confirming the system's efficiency in reducing toxicity and restoring water quality. These findings support the environmental viability of ASEC technology for mine and port effluent treatment.

Enhanced phytoremediation of galaxolide using *Lemna minor*: Mechanisms, efficiency, and environmental implications

Sokól, A; Karpinska, J. (202) International Journal of Molecular Sciences 26: 6636.

This study aims to evaluate the potential of *Lemna minor* (common duckweed) for the removal of galaxolide (HHCB) from polluted water, a compound commonly used in consumer products such as perfumes and detergents. The focus was to identify the optimal conditions for removal, determine the removal efficiency, and elucidate the mechanisms involved. The experiment was conducted by cultivating *L. minor* using as a cultivation medium synthetic sewage and laboratory solutions (MilliQ water) containing galaxolide at two levels of concentration (1034 μg L^{-1} and 2326 μg L^{-1}). The plants were exposed to light for 16 h a day and grown at pH 5. Removal efficiency was assessed through liquid chromatography (HPLC) with fluorescence detection (FLD). Kinetics of observed process was modelled using a pseudo-first-order equation. The study of the HHCB decay mechanism included determining the contributions to the final effect of the following processes occurring simultaneously: sorption on the plant surface, photodegradation, and uptake by *Lemna*. The removal efficiency (RE%) of galaxolide by *L. minor* was 99.7% when aqueous standard solution was used as the cultivation medium after 14 days, and between 97.8% and 98.6% in the case of wastewater samples. Sorption onto plants surface, photodegradation, and uptake by the plants were identified as the primary mechanisms for HHCB removal. Toxicity studies revealed that galaxolide exposure adversely affected *L. minor* growth, altering photosynthetic pigments (chlorophyll and carotenoid) levels.

Phytotoxicity

Comparative physiological responses of *Lemna aequinoctialis* and *Spirodela polyrhiza* to mercury stress: Implications for biomonitoring and phytoremediation

Ruamsin, C; Sonjaroon, W; Khumwan, S; Thamchaipenet, A; Roongsattham, P. (2025) Plants 14: 2859.

Duckweeds are commonly used as standard ecotoxicological test species and are excellent candidates for phytoremediation due to their adaptability to diverse aquatic conditions. This study investigated the response of two duckweed species, *Lemna aequinoctialis* and *Spirodela polyrhiza*, to mercury-contaminated environments, specifically focusing on their growth rates and photosynthetic performance under mercury stress. Duckweeds were treated with $HgCl_2$ at various concentrations (0, 0.1, 0.2, and 0.3 mg/L) in 10% Hoagland's medium for seven days, after which growth parameters, pigment contents, and chlorophyll fluorescence levels were evaluated. The results showed that increasing mercury concentrations significantly affected growth and photosynthetic pigments in *L. aequinoctialis* and *S. polyrhiza*. Chlorophyll fluorescence analysis indicated that, under higher mercury concentrations, Fv/Fm and Y (II) decreased, while NPQ increased. The findings revealed that *L. aequinoctialis* was more susceptible to mercury toxicity than *S. polyrhiza*. Therefore, *L. aequinoctialis* may serve as a more sensitive species for mercury biomonitoring, whereas *S. polyrhiza* is more resistant and might, for this reason, be more useful for phytoremediation of mercury-contaminated soil.

Exploring aquatic toxicity of diverse pesticides against *Chironomus riparius* (harlequin fly) and *Lemna gibba* (swollen duckweed): Applications of QSTR, i-QSTTR, and novel q-RA approaches

Nath, A; Ojha, PK. (2025) The Science of the Total Environment 1000: 180384.

Organic pesticide molecules pose toxicity risks to aquatic species such as *Chironomus riparius* and *Lemna gibba*. However, limited toxicity data and resource-intensive laboratory tests impede comprehensive assessment. To overcome these obstacles, computational techniques like Quantitative Structure-Toxicity Relationship (QSTR) offer an efficient and effective approach. In this study, we performed QSTR modelling by employing these two species. Also, interspecies-QSTTR models have been developed, which aid in predicting one absence response data for one species by employing another available response data for another species. The models demonstrated strong statistical reliability, as indicated by robust internal validation metrics (R^2=0.711-0.806 and Q_2LOO=0.619-0.709) and consistent external validation results (Q_2F1=0.75-0.788 and Q_2F2=0.736-0.75). Furthermore, a new "quantitative read-across (q-RA)" method improved the accuracy of predictions. The study also identified key structural features of chemicals that contribute to their toxicity. Finally, we screened a large number of chemicals to predict their toxicity levels and to identify the ten most and ten least harmful ones, along with the structural patterns responsible for their toxicity. These insights play a vital role to design safer pesticide molecules towards a sustainable management of aquatic ecosystems.

Sulfonamides impede duckweed growth by inducing an excessive accumulation of salicylic acid

Ma, AL; Liu, SQ; Du, ZX; Zhu, LF; Feng, GP. (2025) Environmental and Experimental Botany 237: 106224.

Sulfonamides, owing to their therapeutic properties and cost-effectiveness, are widely employed in clinical settings and the animal husbandry industry. However, the misuse of these compounds has resulted in severe water pollution, thereby posing significant threats to the growth and development of aquatic plants such as duckweed. Our controlled experiments revealed that sulfonamide exposure disrupts the plasma membrane integrity in duckweed root tip cortex cells and inhibits the root growth. Utilizing ESI-HPLC-MS/MS, we identified that sulfonamide exposure elicits an immune response in duckweed, characterized by the generation of substantial amounts of salicylic acid (SA). The exogenous application of SA exacerbated the sulfonamide-induced growth inhibition, whereas the addition of the SA synthesis inhibitor 1-aminobenzotriazole (ABT) mitigated the stress induced by sulfonamides. A loss-of-function mutant of ICS1, a key enzyme in the SA biosynthesis pathway, exhibited complete insensitivity to sulfonamide exposure. Furthermore, transcription factors SARD1 and CBP60g, which regulate ICS1 gene expression, were induced by sulfonamides, and their double mutant showed insensitivity to sulfonamide exposure. Reactive oxygen species (ROS) detection indicated abnormal accumulation of ROS in duckweed roots under sulfonamide exposure, which is likely to induce SA synthesis. These findings suggest that sulfonamide-mediated growth inhibition in plants is dependent on the ICS1-catalyzed SA biosynthesis pathway, thereby enhancing our understanding of the effects and mechanisms of sulfonamide exposure on aquatic plants.

DF comments: The duckweed used in this work appears to have been collected from a local water source in China and may not have been purified and identified in terms of species. This makes the work difficult to be replicated by others in the field. In addition, some of the work reported – such as those with the ics1 mutants - were in fact carried out with the model plant *Arabidopsis thaliana*. While supportive of the authors' interpretations, the conclusions made with these experiments need to be taken with caution.

Microplastic modulates cadmium toxicity in duckweed: Insights into metal uptakes and oxidative stress in the plant

Pawaiya, A; Suthar, S. (2025) Ecotoxicology DOI10.1007/s10646-025-02957-x

Microplastic (MP) are widespread in aquatic environment alongside other toxic pollutants, potentially impacting the uptake and toxicity in aquatic organisms. This study investigated the effect of MP (1,500 ± 50 particles/L) on the removal of Cd (0.5, 1.0, and 2.0 mg/L) using a duckweed (DW)-based batch setup over 24 days. The setup without MP served as an experimental control. Results showed that Cd exposure significantly hindered DW growth (2.0-3.33 g/d), whereas in the presence of MP, growth slightly increased (2.60-3.70 g/d) (t-test: $p < 0.05$). Visible signs of Cd toxicity, such as frond discolouration, decay, and necrosis, were observed. The MP-spiked setups resulted in a 1.89-2.0fold decrease in Cd uptake by DW. Oxidative stress enzyme analysis indicated enhanced tolerance of DW to Cd in setups with MP. Meanwhile, Cd bioaccumulation in DW decreased (35.65-55.30%), while its deposition in the bottom sediments increased (30.60-57.15%). The reduced Cd sensitivity of DW in the presence of MP could be due to mechanisms such as the adsorption of Cd onto MP fibers, leading to sedimentation and decreased bioavailability, and the deposition of MP on DW fronds and roots, which might limit metal transfer. These findings suggest that MP can significantly modulate Cd behaviour in aquatic environment by lowering its toxicity and availability to DW through adsorption and enhanced sedimentation, indicating a dual role in reducing metal stress while raising concerns over microplastic accumulation and associated ecological risks. Nonetheless, nano-sized MP bioaccumulation and its toxic effects in such systems warrant further research.

Fabrication of smart chitosan composite beads for alleviate boron toxicity in a model plant (*Lemna gibba*): Characterization, toxicity assessment, and boron removal

Byakar, A; Tuerker, OC; Cakmak, E; Baran, NY; Baran, T. (2025). Journal of Cleaner Production 519: 146038.

Excessive boron (B) in aquatic ecosystems poses a significant threat to environmental health and biodiversity. In this respect, an attractive strategy should be evaluated to reduce B toxicity in the water environment and protect aquatic organisms. The study aims to reduce B-induced toxicity in a model plant, *Lemna gibba*, using smart chitosan-magnetic composite beads enriched with keratin, and further enhanced with boron-tolerant bacteria (*Acinetobacter* sp.). We tested different chitosan-magnetic composite beads for their B adsorption capacity, focusing on a specific type enriched with keratin for the first time in the literature. The effects of adding chitosan-magnetic composite beads in a test solution containing B mine effluent to alleviate B toxicity on *L. gibba*'s growth parameters (frond number, biomass production, and EC_{50} value) were detailly evaluated in the experiment period. Accordingly, the chitosan-magnetic composite beads with keratin (Mag-Ch-K) demonstrated high B adsorption, with a maximum loading capacity of 2.875 mg/g at pH 7. The relative growth rate of *L. gibba* in a reactor containing Mag-Ch-K beads was measured to be approximately 2-fold (0.2065) higher than that of the control reactor (0.1212) without composite beads at 64 mg L^{-1} B concentration. More importantly, Mag-Ch-K bead significantly increased the plant's tolerance against B in the reactor matrix, as indicated by an EC_{50} value of 44.18 mg L^{-1} compared to 17.17 mg L^{-1} in the control. This study provides a promising approach to mitigate B toxicity in water bodies, offering a practical operation, high growth production, and preventing B pollution shock via modified bead with *Acinetobacter* sp. High B removal (76 %) was also achieved from reactors containing Mag-Ch-K-D through the high B-loading capacities and plant uptake. These dual benefits encourage designers to design chitosan and duckweed-based treatment systems for ecological conservation and pollution management in B-rich waters, such as B mine effluent pollution.

Dual acute effects of tire microplastics and their leachates on *Spirodela polyrhiza* and phyllosphere bacteria

Osman, DM; Yuan, W; Nyaga, MP; Monikh, FA; Yang, Y. (2025). Water Research 287: 124362.

Tire microplastics (TMPs) significantly contribute to global plastic pollution, entering aquatic environments primarily through runoff, where they pose potential threats to aquatic ecosystems. Beyond the particles themselves, TMPs-derived leachates further exacerbate environmental risks. Despite growing concern over plastic pollution, the influences of TMPs and their leachates on aquatic systems remain largely understudied. To address this gap, we systematically evaluated how TMPs and their leachate (01 % w/v) affect the growth, physiochemical responses, and phyllosphere bacterial community of model aquatic macrophyte duckweed (*Spirodela polyrhiza*), using natural particulate wood as a control. Our findings reveal that both unleached TMPs and leachates exerted detrimental effects on the growth of *S. polyrhiza* and inhibited the production of photosynthetic pigments. Unleached TMPs and leachate exposures triggered oxidative stress, as evidenced by alterations in antioxidant enzyme activities and lipid peroxidation levels. Notably, leached TMPs enhanced plant growth comparably to wood particulates, suggesting their potential to generate favorable conditions for aquatic plants at prevailing concentrations. Phyllosphere bacterial composition exhibited significant differences among treatments; TMPs exposures increased Aquabacterium abundance but induced a dose-dependent decrease in Pedobacter. Also, exposure to TMPs substantially altered the metabolic processes within phyllosphere bacterial communities, particularly affecting key microbial functions linked to nutrient metabolism, xenobiotic degradation, and stress adaptation. The correlations between plant properties and phyllosphere bacterial communities varied depending on the treatments, suggesting that the dual effects of TMPs may be attributed to the dissolved substances in the leachates. This study elucidates the complex interactions among TMPs, aquatic macrophytes, and phyllosphere microbiomes, highlighting the need for further investigation into the ecological consequences of TMP pollution in freshwater environments.

Assessment of waterborne metal contaminants by integrating deep learning with support vector machine and random Forest classifiers through *Lemna minor* phenotyping

Kalist, V; Poornapushpakala, S; Jenitha, JMM; Subramoniam, M; Barani, S. (2025) Journal of Indian Chemical Society 102: 101906.

Water is an important source of life for living beings on the earth. Due to urbanization, growth in industries and various other human activities, this water is polluted by microbes, pesticides, chemical solvents and metal contaminants. Such polluted water may degrade the quality of the crops and yield when utilized for irrigation. This research proposes a novel methodology to analyse the quality of water with respect to metal contaminants such as Arsenic (As), Copper (Cu), Lead (Pb) and Mercury (Hg) using *Lemna minor* as a bio-indicator. *Lemna minor* was grown on known metal contaminated water samples as well as water collected from reservoir. The images of *L. minor* grown on this medium were processed for the study. Image processing and machine learning techniques are integrated to assess the metal contaminants in water. In this research, VGG16 and VGG19 models are attempted initially. Due to over fitting issues encountered with these models, integrating the features of VGG 16 model with multiclass classifiers is attempted. VGG16 with Random Forest classifier produced an accuracy of 98.41 % whereas with VGG16 with SVM classifier produced an accuracy of 99.77 % in assessing the metal contaminants in water. The modified VGG-SVM model is extended for 12 class classification for assessing suitability of water for irrigation produced a classification accuracy of 84 %. Implementation of this hybrid methodology is first of its kind for the identification of metal contamination in water through image processing using the *L. minor* phenotyping.

Discovery of new PSII inhibitors: systematic investigation for herbicidal activity screening, properties, and mode of action

Umurzokov, M; Kim, D; Kim, JS; Cho, KM; Choi, JS; Park, KW; Lim, HN. (2025) Pest Management Science 81: 7219-7231.

The research focused on developing effective photosystem II (PSII) inhibiting triazine-derived herbicidal compounds from the internal chemical library. Initially, the compounds were screened for their herbicidal activity using *Spirodela polyrhiza* (SPIPO) assays. Subsequently, compounds with stronger activity were synthesized on a gram scale and further investigated for their phytotoxicity, mobility, and mode of action. In the SPIPO screenings, two compounds, K-31706 and K-31487, showed superior efficacy at concentrations exceeding 0.4 μmol, surpassing the commonly used PSII inhibition herbicide, atrazine. Both compounds showed promising performance, especially in pre-emergence applications under greenhouse conditions, with the application rate causing 50% biomass reduction below 100 g a.i. ha^{-1}. However, both compounds exhibited strong phytotoxicity on major monocot and dicot crops, particularly when applied pre-emergence. Absorption studies revealed that K-31706 and K-31487 have strong activity in both soil-only and overtop applications, with root-based absorption occurring in monocots like *Digitaria ciliaris*. K-31487 was effective across various application methods on *Abutilon theophrasti*, indicating dual root and leaf absorption in dicots. Nevertheless, leaf excision assays indicated that both compounds act as contact herbicides rather than systemic ones. Chlorophyll fluorescence induction (OJIP) curve and molecular docking analyses confirmed PSII inhibition, which is comparable with simazine and diuron, with suppressed electron transport via interaction with QB site amino acids of D1 protein. Overall, K-31706 and K-31487 are nonselective PSII inhibitors that block the electron transport in PSII, positioning them as potentially useful active ingredients for further investigation.

Evaluating herbicidal risks of the fungicide tebuconazole: differential sensitivity of dicot and monocot macrophytes in freshwater mesocosms

Mohr, S; Antony, M; Contardo-Jara, V; Scholz, U; Bader, S; Polleichtner, C; Arts, G. (2025) Environmental Sciences Europe 37: 119.

The triazole fungicide tebuconazole (TBZ) has plant growth regulatory properties and is used as such in, e.g., oilseed rape crop production. To date, very little is known about the phytohormonal effects of TBZ on macrophytes. There are only data for the monocot macrophyte *Lemna* sp. available in the literature, which indicate a moderate risk for macrophytes. However, dicot macrophyte species are known to generally be more sensitive to growth regulating pesticides, due to the phytohormonal effects of these substances. Therefore, this study aimed to investigate if TBZ may impact monocot and dicot aquatic macrophytes differently and to quantify these effects. Two in situ experiments were conducted as part of a comprehensive study in freshwater mesocosms to evaluate direct and indirect effects of TBZ on five macrophyte species, particularly comparing the dicot species *Myriophyllum spicatum* with monocot duckweed species and the macrophyte standing stock of the mesocosms. For this, six naturally established freshwater mesocosms were dosed once with six different concentrations of TBZ (5-5000 µg/L), two served as controls. The dicot *M. spicatum* was the most sensitive species with the lowest EC^{50} (14 d) of 228 µg/L TBZ. In contrast, the monocot broad-leaved pondweed *Potamogeton natans* and two duckweed species reacted far less sensitive in this study with *Spirodela polyrhiza* being the most sensitive monocot species (EC_{50} (14 d): 1487 mu g/L TBZ). Shoots of *M. spicatum*, which were grown directly as standing stock in the mesocosms, developed strong growth deformities in the form of shorter stems and condensed leaf whorls during the course of the study in the higher TBZ treatments. This effect can be linked to the anti-gibberellic mode of action of TBZ. The results of this study highlight that short-term in situ experiments are a valuable tool that can efficiently be integrated into comprehensive mesocosm studies investigating aquatic communities, in order to gain reliable EC_x data for several non-standard macrophyte species with different growth forms under near-natural conditions. The results of this research underscore that for regulatory risk assessment of fungicides with a phytohormonal mode of action, dicot aquatic macrophytes need to be included in the first tier of the risk assessment.

Tissue-specific responses of duckweed to cadmium stress under nanoplastic co-exposure: differential accumulation and toxicity in roots and fronds

Xue, ZH; Liao, XY; Hou, J; Xu, J; Lin, DH. (2025) Environmental Science-Nano 12: 4235-4246.

Nanoplastics (NPs), characterized by their small size and widespread presence, are known to interact with other contaminants, potentially modifying their environmental behavior and biological impacts. Herein, we explored the response of duckweed (*Spirodela polyrhiza*) to Cd (0, 0.01, 0.1, and 1 mg L^{-1}) stress upon co-exposure to polystyrene NPs (PS-NPs) (0, 0.1, 1, and 10 mg L-1). Cd exhibits significantly higher toxicity to the duckweed compared to PS-NPs. Notably, the presence of PS-NPs exacerbates Cd toxicity in the roots, while mitigating its adverse effects on the fronds. In situ analyses using Laser Ablation Inductively Coupled Plasma Mass Spectrometry and Confocal Laser Scanning Microscopy demonstrate that PS-NPs enhance Cd accumulation in the root tips (by up to 19.3%) but reduce its presence in the basal root regions and further translocation to the fronds by up to 26.8%. Metabolomics and gene expression analyses further indicate that PS-NPs elevate organic acid contents and modify gene expressions in duckweed against exogenous Cd stress, thereby inhibiting the root-to-frond translocation of Cd. The findings enhance our comprehension of the complex interactions between NPs and environmental pollutants in aquatic flora.

Pre-print

Systematic optimization enables near-perfect in vitro transformation efficiencies for *Spirodela polyrhiza* (Greater Duckweed)

Islam, T; Ligaba-Osena, A; Josephs, EA. (2025) bioRxiv : the preprint server for biology

DOI10.1101/2025.09.10.675371

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

Copper resistance predicts heritable transgenerational fitness variation in the clonal duckweed *Spirodela polyrhiza*

Chávez, A; Schaefer, M; Finkemeier, I; Xu, SQ; Huber, M. (2025). bioRxiv DOI10.1101/2025.07.03.662999

Transgenerational plasticity in the absence of genetic change can alter organismal fitness, yet we know little about the genetic basis of transgenerational fitness effects. Here, we explored whether transgenerational fitness effects can be predicted by the rate of vegetative reproduction, plant resistance and defence. Thereto, we exposed monoclonal, single-descendant lineages from 56 globally distributed genotypes of the clonal duckweed Spirodela polyrhiza to five generations of copper excess, followed by five generations without stress, and then measured plant fitness and phenotypes in both environments. Previous copper excess reduced fitness variation within each genotype and elicited heritable and reproducible fitness effects that depended on both the genotype and environment: genotypes that benefited from previous stress under recurring conditions suffered when the stress was absent, and vice versa. These transgenerational fitness effects were predicted best by copper resistance, explaining 30% of the intraspecific variation: the more susceptible a genotype, the more its offspring suffered from ancestral stress in the absence of recurrent stress; however, these offspring became more resistant to copper excess, likely by protecting the photosystem II. Together, these findings show that transgenerational fitness effects are heritable and variable and likely controlled by genes that regulate plant reproduction and defence.

Strain, procedures, and tools for reproducible genetic transformation and genome editing of *Spirodela polyrhiza* (L.) Schleid.

Barragán-Borrero, V; de Santana Lopes, A; Diniz Rodrigues Batista, E; Hoefer, M; Elias, R; Chakraborty, A; Ponce-Mañe, A; Descombes, C; Diezma-Navas, L; Petraki, L.L; Huber, M; Xu, S.Q; Marí-Ordóñez, A. (2025) doi: https://doi.org/10.1101/2025.08.28.672806. This article is a preprint and has not been certified by peer review.

Duckweeds (Lemnaceae) have excellent potential as plant systems for fundamental and applied research due to the ease of cultivation, their small size, and continuous fast clonal growth. However, their usage as model organisms and platforms for biotechnological applications is often limited by the lack of universal genetic manipulation methods necessary for insertional mutagenesis, reporter gene expression, gene editing, and other methods to modify gene expression. Here, we present a robust transformation protocol for a publicly available strain of *Spirodela polyrhiza*, SP162, that we identified to be amenable to *Agrobacterium*-mediated stable transformation via tissue culture. The transformation procedure allows stable expression of different reporter genes and selectable markers and enables CRISPR/Cas9-mediated genome editing. Due to the lack of a small RNA-based silencing response, *S. polyrhiza* SP162 also sustains prolonged periods of transgene activity in Agrobacterium-mediated transient expression assays. Thus, SP162 is an ideal reference strain for developing improved transformation procedures and applications in *S. polyrhiza*. To promote duckweed research and encourage the adoption of *S. polyrhiza* in research laboratories, we have made SP162 (ID#: 5676) and its genome publicly available and provide here detailed procedures for its cultivation and transformation.

Taxonomy & Geobotany

Comprehensive summary of duckweeds in Sri Lanka

Ranasinghe, H; Yakandawala, K; Yakandawala, D; Tippery, NP. (2025) Phytotaxa 708: 23-42.

Duckweeds (Lemnaceae) are a family of floating aquatic plants that include the smallest angiosperms in the world. In Sri Lanka, there have been inconsistent reports about which duckweed species are native, and there have been no comprehensive taxonomic surveys of Lemnaceae in the country. Following the recent discovery of non-native *Landoltia* plants in Sri Lanka, we undertook a comprehensive investigation using extensive sampling, followed by morphological and molecular methods to identify species and document their distributions. Ultimately, we confirmed the existence of the following species in Sri Lanka: *Spirodela polyrhiza, Landoltia punctata, Lemna aequinoctialis*, and *Wolffia neglecta*.

Instructions to Contributors for the Duckweed Forum

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

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

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

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

Title:

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

References:

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

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

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

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

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

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

Authors:

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