

8th International Conference on Duckweed Research and Applications

28 September – 2 October 2026

Department of Agricultural Sciences
University of Napoli Federico II · Portici, Italy

Model plant and novel crop, the thousand faces of duckweed

BOOK OF ABSTRACTS

CONTENTS

Honorary Session — Elias Landolt Centenary - Keynote Speech	3
Cell Biology, Physiology & Metabolisms, Invited Talk	5
Cell Biology, Physiology & Metabolisms I	7
Cell Biology, Physiology & Metabolisms II	19
Genes, Genomes & Evolution, Invited Talk	31
Genes, Genomes & Evolution I	33
Genes, Genomes & Evolution II	41
Microbiome & Interactions, Invited Talk	47
Microbiome & Interactions I	49
Ecology & Diversity, Invited Talk	57
Ecology & Diversity I	59
Applications — Food, Feed & Nutrition, Invited Talk.....	71
Applications I A — Food, Feed & Nutrition	73
Applications I — Food, Feed & Nutrition, Invited Talk	85
Applications I B — Food, Feed & Nutrition	87
Applications II — Wastewater Remediation	95
Applications II — Food, Feed & Nutrition, Invited Talk	107
Applications III — Biotech	109
Posters	115

Klaus J. Appenroth

Department of Plant Physiology, Matthias-Schleiden-Institute, Friedrich-Schiller-University of Jena,

Corresponding author: bka@uni-jena.de

The late Elias Landolt was famous as the unquestionable top scientific expert on the plant family of Lemnaceae, commonly called duckweed or water lentils. He was an honorary member of the Swiss Botanical Society, a Professor at the Swiss Federal Institute of Technology Zurich (“Eidgenössisch Technische Hochschule”, ETH) and director of the Geobotanical Institute until his retirement in 1993. Interestingly, he did not start his scientific career by investigating duckweeds nor was he only focused on duckweed research during his scientific life. He published more than 70 papers on Lemnaceae and more than 150 papers about other topics, mainly related to the flora of Switzerland and of Zurich in specific. He wrote a two volume book “The family of Lemnaceae - a monographic study”, volume 2 together with Riklef Kandeler, with approximately 3000 references, which is available in internet (1, 2). His collection of living Lemnaceae species, which exceeded much more than 1000 clones, remains “the invaluable legacy” also for further duckweed research. (cf. Duckweed Forum 41, 47-54 (2023)). In this year we celebrate the 100th birthday of the father of modern duckweed research.

Keywords

Geobotany · Stock collections · taxonomy

References

(1) Landolt, E. (1986). The Family of Lemnaceae – A Monographic Study. Vol. 1. Biosystematic Investigations in the Family of Duckweeds (Lemnaceae). Zurich: Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübél. (2) Landolt, E., and Kandeler, R. (1987). The Family of Lemnaceae – A Monographic Study. Vol. 2. Biosystematic Investigations in the Family of Duckweeds (Lemnaceae). Zurich: Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübél. <https://www.eperiodica.ch/digbib/view?pid=gbi-002%3A1987%3A95%3A%3A3>

NOTES

Elias Landolt (1926 – 2013), father of modern duckweed research

Anthony Bishopp

Plant and Crop Sciences, School of Biosciences, University of Nottingham

Corresponding author: anthony.bishopp@nottingham.ac.uk

Miniaturisation, or an extreme reduction in body size, is present in both the plant and animal kingdoms. This process has been associated with novel morphological innovations and functional adaptations. Whilst miniaturisation has been studied in a wide variety of animal lineages, we lack an understanding of the morphological changes associated with miniaturisation in plants. The Araceae exhibit a striking evolutionary breadth, encompassing both the smallest flowering plants and some of the largest herbaceous species known, making it an ideal family with which to explore the concept of miniaturisation. We focused on duckweeds, investigating the morphogenesis and homology of their fronds with those of other Aroids. Duckweeds measure just a few millimetres. Their aerial body plan consists of a single frond or thallus, which divides asexually through clonal reproduction. We followed morphogenesis in *Lemna minor* fronds and observed that cell division was tightly constrained to a narrow time window during early organogenesis, suggesting that the early cessation of division constrains final frond size. Transcriptional dissection of the duckweed frond revealed that it comprised distinct zones with homology to either leaves or petioles in Aroids, suggesting that the duckweed frond is a hybrid organ consisting of a fused stem and leaf. Collectively, our data support the hypothesis that while, in animal lineages such as tardigrades, miniaturisation has arisen through the loss of body plan components, in duckweeds, it has arisen through a restriction in cell division and fusion, rather than the loss of organs.

Keywords

Developmental Biology; Transcriptomics; Imaging

NOTES

Living on Less: Miniaturization in Duckweed

Trophic Diversity in Duckweed: Mixotrophy, More Than the Sum of its Extremes

#6

Hongwei Hou^{1,2}; Zuoliang Sun³; Xuyao Zhao²; Yan Chen⁴

¹ State Key Laboratory of Breeding Biotechnology and Sustainable Aquaculture, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, P. R. China; ² Institute of Emerging Agricultural Technology, Shenzhen University of Advanced Technology, Shenzhen, P. R. China; ³ Jia Sixie College of Agriculture, Shandong Provincial University Laboratory for Protected Horticulture, Weifang University of Science and Technology, Shouguang, P. R. China; ⁴ Special Agricultural Resources in Tuojiang River Basin Sharing and Service Platform of Sichuan Province, Analysis and Testing Center, College of Chemistry and Chemical Engineering, Neijiang Normal University, Neijiang, Sichuan, P. R. China

Corresponding author: hongwei@suat-sz.edu.cn

Duckweeds (Lemnaceae) are ideal model plants and valuable biotech resources with diverse trophic strategies, including mixotrophy. This study investigates the mechanisms and value of mixotrophy in *Spirodela polyrhiza* 7498. It demonstrates that mixotrophy endows duckweed with significant phenotypic and metabolic flexibility. Under mixotrophic conditions, duckweed enhances intracellular CO₂ supply through respiration, alleviating photosynthetic limitations and reducing oxidative damage. Crucially, mixotrophy achieves synergistic growth, with biomass yield significantly exceeding the sum of photoautotrophic and heterotrophic growth alone. Experiments confirmed that multiple carbon sources support this growth, with the mixotrophic growth rate reaching 6.22 and 4.98 times that of photoautotrophic and heterotrophic modes, respectively. Besides superior biomass, mixotrophy also promotes protein synthesis and effective nutrient removal from water. Notably, heterotrophic cultivation favors starch accumulation (2.06 times higher than mixotrophy), suggesting a combined strategy for starch-rich biomass production. This work reveals the synergistic advantages of duckweed mixotrophy, providing a theoretical basis for optimizing biomass production, high-value product synthesis, and organic wastewater remediation.

Keywords

Duckweed; Mixotrophy; Photoautotrophy; Heterotrophy

References

1. Zuoliang Sun, Wenjun Guo, Jingjing Yang, Xuyao Zhao, Yan Chen, Lunguang Yao, Hongwei Hou. Enhanced biomass production and pollutant removal by duckweed in mixotrophic conditions. *Bioresour Technol.* (2020) doi: 10.1016/j.biortech.2020.124029.
2. Zuoliang Sun, Xuyao Zhao, Gaojie Li, Jingjing Yang, Yan Chen, Manli Xia, Inhwon Hwang, Hongwei Hou. Metabolic flexibility during a trophic transition reveals the phenotypic plasticity of greater duckweed (*Spirodela polyrhiza* 7498). *New Phytol* (2023) doi: 10.1111/nph.18844
3. Zuoliang Sun; Yan Chen, Fajun Li; Xuyao Zhao; Qingxiang Han; Hongwei Hou. Trophic Diversity in Duckweed: Mixotrophy, More Than the Sum of its Extremes. *Advanced Science.* (2026) doi: 10.1002/adv.75665

NOTES

Trophic Diversity in Duckweed: Mixotrophy, More Than the Sum of its Extremes

Edna Ben-Izhak Monselise

Ben Gurion University, Edna Ben-Izhak Monselise Department of Life Science, Bergmann Campus, Ben-Gurion University of the Negev, Beer-Sheva 8441901, Israel bened@post.bgu.ac.il

Corresponding author: bened@post.bgu.ac.il

Cellular stress responses involve extensive metabolic reprogramming. While alterations in amino acid concentrations under stress have been widely documented, the potential role of amino acid chirality in stress adaptation remains poorly understood. Using ^{15}N NMR spectroscopy, stress-dependent changes in amino acid profiles were investigated in aquatic plant systems, *Landoltia punctata*. These studies revealed the appearance of D-alanine under defined stress conditions (Monselise et al., 2015), providing evidence for stress-associated D-alanine accumulation in duckweed and suggesting that stereochemical remodeling may represent a component of the plant stress response. Subsequent studies in *Escherichia coli** demonstrated stress-associated production and extracellular release of D-glutamate (Monselise, 2019; Monselise 2024). The release of D-glutamate into the surrounding medium suggests that stress-induced D-amino acids may function beyond intracellular metabolism and participate in intercellular biochemical communication. Taken together, these observations from both plant and bacterial systems raise the possibility that D-amino acids constitute conserved elements of stress-response networks across biological kingdoms. These findings support a broader conceptual framework in which stress-induced stereochemical changes contribute to cellular adaptation and signaling. The presentation will discuss the evolution of this research, from duckweed stress physiology to broader questions concerning the role of D-amino acids in cellular stress responses and their possible relevance to the early stages of cellular dysfunction.

Keywords

Cell signaling; Metabolic reprogramming; Stereochemical remodeling

References

Monselise E. B-I., Levkovitz A. & Kost D. (2015). Ultraviolet radiation induces a stress effect in etiolated *Landoltia punctata*, as evidenced by the universal stress signal alanine: an ^{15}N NMR study. *Plant Biology*, 17(1), 101–107. Monselise E. B-I. (2019). D-Amino Acids are Signaling Agents Under Stress that Broadly Impact Preventive Medicine. Preprints, 2019090334. Monselise E. B-I., Vyazmensky M., Scherf T., Batushansky A. & Fishov I. (2024). D-Glutamate Production by Stressed *Escherichia coli* Gives a Clue to the Hypothetical Induction Mechanism of ALS. *Scientific Reports*, 14, 18247. Scientific Session: Cell Biology, Physiology, Metabolisms

NOTES

From Duckweed Stress Physiology to D-Amino Acid Signaling: Insights Across Biological Kingdoms

Halina Gabry; Weronika Krzeszowiec

Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology

Corresponding author: halina.gabrys@uj.edu.pl

Chloroplasts relocate within cells to maximize energy capture under limiting light and/or to minimize exposure to excess light. In higher plants these movements are mediated by phototropins, blue light photoreceptors that control also other light acclimation processes. Chloroplast movements share many similarities between the aquatic angiosperm *L. trisulca* and *Arabidopsis thaliana*. These include fluence rate-response curves in continuous light and fluence-response profiles obtained with strong light pulses. In both species phosphoinositides and Ca^{2+} participate in signalling, with a greater role of calcium released from the internal stores. The signalling pathway leading to light-induced chloroplast movement may involve GLutamate Receptor-like (GLR) channels, previously shown to participate in light signalling in plants. These ligand-gated ion channels, structurally homologous to animal neurotransmitter receptors, are known to generate Ca^{2+} transients inside plant cells. We chose *L. trisulca* to determine the role of GLR channels in chloroplast movements using specific inhibitors. NMDA GLR channels were shown to participate in the control of chloroplast avoidance response in the duckweed. This control occurs in a pH-dependent way, similar to NMDA receptors operating in animal cells. As the avoidance response is activated only by strong blue light, these GLR channels are involved in the phototropin2 signalling pathway. GLR AMPA channels, previously shown to take part in seedling growth, proved inactive in chloroplast movements. Thus, we provided evidence that GLR receptors activated by different agonists have specialised functions in plants. Genetic modification of *L. trisulca* is desired to get further insight into the mechanism of its blue-light-induced chloroplast movements.

Keywords

Chloroplast movements; GLR channels; *L. trisulca*

NOTES

Lemna trisulca: Contribution to the Progress in Understanding the Mechanism of Chloroplast Movements

Lauritsen Markus Dahl; Quentin Geissmann

Center for Quantitative Genetics and Genomics (QGG), Aarhus University, Aarhus, Denmark

Corresponding author: mlauritsen@qgg.au.dk

Duckweed is a uniquely fast and tractable plant model, making it ideal for studying dynamic plant– environment interactions on time scales impossible with conventional crop or model species. However, fully harnessing this in duckweed requires sample-level environmental control paired with dense temporal phenotyping. Standard room-scale or chamber-scale growth systems lack the targeted precision, parallel experimentation and imaging frequency required to impose the environment and monitor the plant's response. We present DWARPH (DuckWeed Automatic Robot for PHenotyping), an open-source hardware and software platform engineered to bridge this gap exact gap. DWARPH integrates programmable, localized control of light intensity and temperature with continuous, high-frequency imaging. The system supports complex environmental perturbations, including rapid step changes, continuous ramps, and cyclic regimes. By automatically synchronizing microenvironmental perturbations with image capture, DWARPH allows researchers to measure phenotypic metrics, quantitative growth dynamics, and short-interval acclimation responses in real time. Because DWARPH relies on accessible 3D-printed components and a modular architecture, researchers can deploy multiple units in parallel to explore broad parameter spaces simultaneously rather than relying on sequential experiments in expensive climate chambers. This presentation will focus on the design and engineering of the DWARPH system. We will present initial data validating system performance and temporal precision for detecting plant responses to microenvironmental perturbations, followed by a brief overview of future experimental applications.

Keywords

High-throughput phenotyping; Environmental response

NOTES

DWARPH: An Open Source, scalable platform for High-Throughput Environmental response mapping in Duckweed

Alexander Ware¹; Freya Persyn²; Anthony Bishopp¹

¹ University of Nottingham

² VIB Single Cell Core, VIB, Ghent

Corresponding author: alex.ware@nottingham.ac.uk

Duckweeds are unusual among angiosperms in being able to acquire nutrients through the shoot rather than the root. We previously showed that their roots are dispensable for this ancestral function, and that they have lost root-biased nutrient transporter expression (Ware et al., 2023). The cellular biology underpinning shoot nutrient uptake, and its evolution, remain unknown. To address this, we generated single-nucleus transcriptomic atlases of shoots and roots from *Spirodela polyrhiza*, *Lemna minor* and *Wolffia australiana*, alongside the aroid *Pistia stratiotes*, which independently colonised the free-floating aquatic niche. Integration of these datasets across species revealed a previously undescribed cell population, conserved across all duckweed shoots, whose identity does not readily correspond to known cell types in terrestrial monocots. This novel cell type expresses a broad suite of nutrient-uptake genes canonically restricted to the root in terrestrial species. In *Lemna minor*, optimisation of whole-mount hybridisation chain reaction FISH has spatially localised this cell type to the underside of the frond, extending one to two cell layers deep into the abaxial frond body. The abundance of this cell type increases across the duckweed genera as the root is reduced, and it is scarce in root-reliant *Pistia*. We propose that this cell population constitutes a dedicated nutrient-uptake tissue in the duckweed frond. Functional characterisation of the cell type, the transporters it expresses, and the evolution of the regulatory network governing its identity is now ongoing.

Keywords

Cell identity; Single-nuclei transcriptomics

References

Ware, A., Jones, D.H., Flis, P., Chrysanthou, E., Smith, K.E., Kümpers, B.M., Yant, L., Atkinson, J.A., Wells, D.M., Bhosale, R. and Bishopp, A., 2023. Loss of ancestral function in duckweed roots is accompanied by progressive anatomical reduction and a re-distribution of nutrient transporters. *Current Biology*, 33(9), pp.1795-1802.

NOTES

Single-nuclei transcriptomics reveals a novel frond nutrient-uptake cell type in duckweeds

Vesna Peršić¹; Jasenka Antunović Dunić¹; Ivana Eržić¹; Anita Ament²; Ondřej Novák²

¹ Department of Biology, Josip Juraj Strossmayer University of Osijek

² Laboratory of Growth Regulators, Faculty of Science of Palacký University Olomouc and the Institute of Experimental Botany of the Czech Academy of Sciences

Corresponding author: vpersic@gmail.com

Salinity primarily reduces plant productivity by disrupting the photosynthetic apparatus, and cytokinins are considered to be context-dependent regulators of its tolerance (1-3). This study aimed to determine if and how 6-benzylaminopurine (1 μ M 6-BA) protects the photosynthetic apparatus of *Lemna minor* under salt stress (50, 100, 150 mM NaCl) and to analyze how it modulates accompanying metabolic responses. Chlorophyll a fluorescence and oxygen exchange were measured to assess plant responses. Simultaneously, we profiled hormone and cytokinin levels and quantified phenolics, antioxidant capacity, pigments, proline, malondialdehyde (MDA), starch, and antioxidant enzymes. The interaction between 6-BA and NaCl was the most significant source of variation in the fluorescence response (PERMANOVA $R^2 = 16.5\%$, $p < 0.001$). 6-BA had a modest cost at low salinity, but it stabilized PSII at high salinity (reversing between 100 and 150 mM), while the PSI acceptor side declined regardless. A similar pattern was observed in cytokinin homeostasis, where 6-BA decreased the endogenous cytokinin pool by half (0.52-fold) and increased it by more than twofold at 150 mM (2.24-fold), with a reversal occurring between 50 and 100 mM. Salinity decreased levels of jasmonates, auxin, phenolics, antioxidant capacity, and pigments but increased levels of ABA, proline, MDA, and starch as expected. 6-BA decreased ABA and MDA at all salinity levels and induced a maximal antioxidant response at 100 mM that was lost at 150 mM NaCl. The results suggest that 6-BA improves salt tolerance of *L. minor* by coordinating stress-level-dependent adjustments of photosynthesis, respiration, hormonal and antioxidant metabolism.

Keywords

PSII biophysics; Phytohormones; Antioxidant defense

References

1. Feng, Y., Liu, J., Zhai, L., Gan, Z., Zhang, G., Yang, S., Wang, Y., Wu, T., Zhang, X., Xu, X., & Han, Z. (2019). Natural variation in cytokinin maintenance improves salt tolerance in apple rootstocks. *Plant, Cell & Environment*, 42(2), 424–436. <https://doi.org/10.1111/pce.13403>
2. Golan, Y., Shirron, N., Avni, A., Shmoish, M., & Gepstein, S. (2016). Cytokinins Induce Transcriptional Reprogramming and Improve Arabidopsis Plant Performance under Drought and Salt Stress Conditions. *Frontiers in Environmental Science*, 4. <https://doi.org/10.3389/fenvs.2016.00063>
3. Yan, Z., Wang, J., Wang, F., Xie, C., Lv, B., Yu, Z., Dai, S., Liu, X., Xia, G., Tian, H., Li, C., & Ding, Z. (2021). MPK3/6-induced degradation of ARR1/10/12 promotes salt tolerance in Arabidopsis. *EMBO Reports*, 22(10), EMBR202152457. <https://doi.org/10.15252/embr.202152457>

NOTES

6-Benzylaminopurine modulates photosynthetic performance and cytokinin homeostasis in *Lemna minor* under salt stress

Tokitaka Oyama; Yu Horikawa; Sho Honda; Tsuyoshi Aiso; Shogo Ito

Graduate School of Science, Kyoto University

Corresponding author: oyama@cosmos.bot.kyoto-u.ac.jp

Duckweeds serve as ideal model plants for whole-plant imaging due to their simple two-dimensional structure and compact size. Using bioluminescent reporters, we have achieved long-term, continuous monitoring of gene expression dynamics at both the whole-plant and single-cell levels (Sci Adv 2016; New Phytol 2021; Plant Physiol 2023). These studies spatiotemporally revealed dynamics of circadian and diel gene expression in duckweed. While these bioluminescence techniques were originally developed for duckweeds, they have recently been applied to other species, such as *Arabidopsis* (Sci Rep 2025). In this presentation, we will highlight the unique bioluminescence behaviors specific to duckweeds (and aquatic plants) and introduce new applications of bioluminescent reporters for monitoring plant physiological processes. Finally, we will discuss the advantages and limitations of duckweeds as a model system.

Keywords

Bioluminescent reporter; Luciferases gene expression

References

Horikawa, Y., Watanabe, E., Ito, S., Oyama, T. Model-based analysis of the circadian rhythm generation of bioluminescence reporter activity in duckweed. *Plant Biotech.* 42: 173-177 (2025) Watanabe, E., Muranaka, T., Nakamura, S., Isoda, M., Horikawa, Y., Aiso, T., Ito, S., Oyama, T. A non-cell-autonomous circadian rhythm of bioluminescence reporter activities in individual duckweed cells *Plant Physiol.* 193: 677-688 (2023)

NOTES

Development of bioluminescence reporter systems using duckweed plants

*Mi Zhang*¹; *Anton Stepanenko*¹; *Michael Möbes*¹; *Anthony Bishopp*²; *Eric Lam*³;
*Hardy Rolletschek*¹

¹ RG Assimilate Allocation and NMR (AAN), Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK)

² Plant and Crop Sciences, School of Biosciences, University of Nottingham

³ Department of Plant Biology, Rutgers the State University of New Jersey

Corresponding author: zhangm@ipk-gatersleben.de

Lemna minor of the Lemnaceae (aka duckweeds) family is a superior model for investigating plant-microbe interactions. We designed a project, which aims to quantify the effects of a synthetic bacterial community across the largest genotype × environmental stress matrix (6,480 combinations) examined in a plant-microbe study to date. Multi-omics (metabolome, transcriptome, ionome, and phenome) studies will be applied to understand how microbes affect stress resilience and growth performance of 30 genome-resolved *L. minor* genotypes. In this report, we present a novel metabolomics workflow for deep metabolome annotation and interpretation in *L. minor*. The workflow integrates complementary LC-MS data processing, statistical prioritization of biologically relevant features, multi-layer metabolite annotation, confidence-level labeling, and pathway-based interpretation. Metabolite annotation combines MS/MS spectral library matching, fragmentation-based annotation, and spectral similarity or metabolic relationship-based approaches. These results are harmonized into an automated confidence-ranking system spanning confirmed structures to unknown features. As a case study, the workflow was applied to two *L. minor* genotypes with contrasting growth performance. Our analysis detected thousands of metabolic features, including a subset of high-confidence annotations. Initial analyses identified broad metabolic differences between the two model genotypes involving amino acid and nitrogen, phenylpropanoid/flavonoid, carbon and energy, lipid, and pigment-associated metabolism. Although the biological interpretation is ongoing, we can conclude that the novel workflow offers a promising approach to automate deep, evidence-based metabolome annotation, as well as providing a flexible foundation for future research into duckweed metabolomics and the plant-microbiome relationship.

Keywords

Metabolomics; multi-stress; metabolite annotation

NOTES

A Novel Workflow for Deep Metabolome Annotation and Interpretation in *Lemna minor*

Yasuyo Yamaoka

The Catholic University of Korea

Corresponding author: yasuyoyamaoka@gmail.com

Duckweeds are among the fastest-growing flowering plants and represent promising aquatic biomass resources for food, feed, bioenergy, and other value-added compounds. However, the endogenous mechanisms controlling carbon allocation and lipid accumulation in duckweeds remain poorly understood. Here, we investigated abscisic acid (ABA)-induced lipid remodeling in *Lemna minor*. Treatment with 1 μ M ABA caused a pronounced and sustained increase in triacylglycerol (TAG), reaching approximately 2.9-fold higher levels than in untreated plants. Time-course analysis showed that TAG continued to accumulate over seven days. Lipidomic profiling revealed extensive remodeling of plastidial and extraplastidial membrane lipids. In particular, reduced monogalactosyldiacylglycerol suggested that membrane-derived fatty acids may contribute to TAG synthesis. ABA strongly induced diacylglycerol acyltransferase genes involved in the terminal step of TAG assembly, while repressing fatty acid desaturase genes, resulting in lower polyunsaturated fatty acid levels and increased proportions of oleic and linoleic acids. Confocal microscopy further demonstrated substantial lipid droplet accumulation in both fronds and chloroplast-containing roots. Notably, sustained ABA-induced TAG accumulation was particularly prominent in *L. minor* and was not observed to a comparable extent in other duckweed species or in *Arabidopsis thaliana* under identical conditions. These findings reveal a species-specific link between ABA signaling, membrane lipid turnover, and carbon storage in duckweed. Building on these findings, we are establishing a Korean duckweed collection to explore natural variation in lipid metabolic traits. We are also developing transformation-based approaches to modify carotenoid and lipid composition. Together, these efforts integrate duckweed biodiversity, stress physiology, and metabolic engineering toward sustainable biotechnological applications.

Keywords

ABA; Triacylglycerol; Metabolic engineering

References

Kim, E., Choi, B.Y., Je, S., Kang, J., Shin, S., Roh, Y., Kim, M., Ito, S., Oyama, T., Lee, Y., Shim, D. and Yamaoka, Y. (2026) Abscisic acid induces triacylglycerol accumulation and lipid remodelling in chloroplast containing green tissues of *Lemna minor*. *Plant, Cell & Environment*, 49, 2464–2479. <https://doi.org/10.1111/pce.70386>

NOTES

A ABA-induced triacylglycerol accumulation and lipid remodeling in Lemna minor toward metabolic engineering of duckweed

Sujeong Je¹; Haneul Seo ¹; Bea Young Choi ²; Seungwoo Shin ¹; Yoomi Roh ¹; Kyungyoon Kim ³; Shogo Ito ⁴; Tokitaka Oyama ⁴; Donghwan Shim ⁵; Yuree Lee ³; Yasuyo Yamaoka ¹

¹ The Catholic University of Korea

² School of Liberal Arts and Sciences, Korea National University of Transportation

³ Seoul National University

⁴ Graduate School of Science, Kyoto University

⁵ Chungnam National University

Corresponding author: sujeongh.je@gmail.com

Duckweeds predominantly propagate vegetatively, and the mechanisms underlying their sexual reproduction and seed biology remain largely unexplored due to the absence of a tractable experimental system. Here, we identified a Korean ecotype of *Lemna aoukikusa* that completes its entire sexual life cycle under laboratory conditions, and established a developmental framework encompassing photoperiod-dependent flowering, seed maturation, germination, and re-establishment of vegetative growth. Flowering reached 100% under short-day conditions, and seeds remained highly viable after storage at 4°C in darkness for up to two years. Mature seeds accumulated triacylglycerol (TAG) as their major storage reserve, containing approximately 481-fold more TAG than vegetative fronds and no detectable starch. Seed maturation was accompanied by induction of conserved seed developmental regulators (LEC1, ABI3, FUS3, LEC2, WRI1, and bZIP67), together with genes involved in fatty acid synthesis and TAG assembly. During germination, seed-derived TAG was mobilized to sustain early frond establishment prior to the attainment of photosynthetic autonomy, revealing a conserved seed reserve utilization strategy in this aquatic monocot. Together, these findings establish *L. aoukikusa* as an experimentally tractable model for investigating seed development and storage lipid metabolism in duckweeds, and provide a foundation for future genetic improvement and domestication efforts in this emerging aquatic crop system.

Keywords

Sexual-reproduction; Seed development; Seed germination; Seed metabolism; Triacylglycerol

NOTES

A complete seed-to-seed cycle beyond vegetative propagation reveals seed development and triacylglycerol mobilization in Lemna aquinoctialis

Meret Huber¹; Gopal Singh ¹; Enrico Diniz ¹; Amanda Lopes ¹; Martin Schäfer ¹; Sandra Irmisch ²

¹ University of Mainz

² Leiden University

Corresponding author: meret.huber@uni-mainz.de

Duckweeds are promising photosynthetic chassis for plant synthetic biology. Yet, it remains unclear whether they can support the heterologous production of complex specialized metabolites and which expression strategies are best suited for multigene pathway reconstruction. Here, I first provide an update on our transformation protocol for the giant duckweed (*Spirodela polyrhiza*). I then show that a six-gene construct driven by an inducible promoter was the most effective strategy to stably produce the complex anti-diabetic flavonoid glucoside mini-Montbretin A in *S. polyrhiza*. Together, these results establish *S. polyrhiza* as a promising chassis to produce complex plant metabolites and highlight the potential of duckweeds for synthetic biology.

Keywords

Metabolic engineering; Specialized metabolites; Montbretin A

References

Barragán-Borrero et al (2026) New Phytologist

NOTES

Engineering a complex plant biosynthetic pathway in the giant duckweed

Mark Polikovsky¹; Asaph Aharoni¹; Jelena Cveticanin¹; Shlomy Arava¹; Nir Shachaf²; Elinor Aviv-Sharon¹; Breno San-Martin¹; Himabindu Vasuki¹; Julia-Eva Fortmueller¹; Dong-Qi Li¹; Inbar Cahana¹; Lu Qian¹; Madlena Ursu¹; Ron; Vunsh¹; Priel Siman-Tov¹; Efrat Weithorn¹; Nadav Elad²; Ziv Porat³; Gil Feiguelman¹; Meital Kupervaser⁴; Maxim Itkin²; Sergey Malitsky²; Ilana Rogachev¹

¹ Department of Plant & Environmental Sciences, Weizmann Institute, Rehovot, Israel

² Department of Chemical Research Support, Weizmann Institute of Science, Rehovot, Israel

³ Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel

⁴ The De Botton Protein Profiling institute of the Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel

Corresponding author: mark.polikovsky@weizmann.ac.il

In the aquatic environment of Lemnaceae (duckweed), the mechanism for transporting hydrophobic bioactive signals through the aqueous medium remains a critical question. While bilayer extracellular vesicles (EVs) are established components in the plant apoplast, in this study, we identified a unique class of secretory nanoparticles (NPs) in duckweed exudate. Structural and omics profiling reveal that this distinct class of NPs represents not passive debris, but a dynamic metabolic hub enriched with lipid-trafficking proteins and enzymes associated with β -oxidation and the glyoxylate cycle. We demonstrate that the NPs' cargo composition is associated with the jasmonate (JA) pathway. Notably, downregulating the JA-biosynthetic gene allene oxide cyclase (AOC) expands the chemical diversity within the NPs, enriching the cargo with bioactive lipids. These NPs sequester approximately 38% of the total exudate lipid diversity, specifically partitioning long-chain triacylglycerols (TAGs) and sphingolipids. Functionally, the duckweed-derived NPs facilitate inter-kingdom crosstalk by promoting the growth of beneficial duckweed-associated bacteria while inhibiting non-beneficial taxa. Together, these findings suggest that non-vesicular NPs are a specialized biochemical strategy for duckweed to target its microbiome and traffic lipids in a complex aquatic rhizosphere.

Keywords

Nanoparticles; Secondary metabolites: Rhizosphere

References

Acosta, K. et al. Return of the Lemnaceae: duckweed as a model plant system in the genomics and postgenomics era. *Plant Cell* vol. 33 Preprint at <https://doi.org/10.1093/plcell/koab189> (2021).
Ishizawa, H. et al. Duckweed as an Emerging Model System for Plant-Microbiome Interactions. *Environmental Microbiology* vol. 27 Preprint at <https://doi.org/10.1111/1462-2920.70181> (2025).
Yu, L., Fan, J., Zhou, C. & Xu, C. Sterols are required for the coordinated assembly of lipid droplets in developing seeds. *Nat. Commun.* 12, (2021).

NOTES

Duckweed Nanoparticles Mediate Bioactive Lipid Trafficking

Arturo Marí-Ordóñez

Gregor Mendel Institute, Vienna

Corresponding author: arturo.mari-ordonez@gmi.oeaw.ac.at

Small RNA pathways play important regulatory and genome defense roles in plants, 21-to-24-nt short interfering (si)RNAs and micro (mi)RNAs guide the silencing of transposable elements (TEs), viruses and endogenous genes, acting both at the transcriptional gene silencing level (TGS), through RNA-directed DNA methylation (RdDM), and post-transcriptionally (PTGS). Duckweeds unique reduced morphology and reproduction, and a wide gradient of genome size and TE content from the TE-poor *Spirodela polyrhiza* (~138 Mb, ~20% TEs) to the TE-rich *Wolffia* (~850 Mb, ~75% TEs), makes them a powerful comparative system to ask how a simplified body plan, a clonal lifestyle and contrasting genome organization shape plant RNA and epigenetic silencing. Several studies have shown that duckweeds run a streamlined silencing machinery with uneven losses. The RdDM and transcriptional-silencing arm is simplified while the antiviral/PTGS arm lacks as well several key silencing factors. In *Spirodela* and *Wolffia*, this reduced toolkit still resolves TEs sharply: silencing and DNA methylation concentrate on young, intact elements, and the contrasting TE loads of the two species reorganize their small RNA and methylation landscapes accordingly. Across these analyses siRNAs of all size classes are frequently produced from the same loci, indicating that the pathways might be wired differently from the canonical pathways established in *Arabidopsis* rather than simply reduced.

NOTES

Small meets smaller: rewiring small RNA silencing in duckweeds

Ingo Schubert¹; Anton Stepanenko¹; Joerg Fuchs¹; Veit Schubert¹; Phuong Thi Nhu Hoang²; Yuri Lee³; Luca Braglia³; Laura Morello³

¹ Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben

² Dalat University, Vietnam

³ Istituto di Biologia e Biotechnologia Agraria (IBBA-CNR) 20133 Milano, Italy

Corresponding author: schubert@ipk-gatersleben.de

The shortest definition of evolution is maintenance and expansion of information by the change of information (mutation) and subsequent selection (by environment). On the biotic (post-cellular) level, evolution led to +/- sexually isolated populations of individuals, called species. While karyotypic mutations can initiate speciation, genic mutations lead to allele diversity and adaptability. The main reasons for karyotype mutations (and thus for speciation) are mis-repaired DNA double-strand breaks. Another important route of speciation is reticulate evolution by fusion of (reduced or unreduced) gametes from different species. Our actual collaborative efforts to resolve the phylogeny of duckweed genera demonstrate how these principles of evolution shaped duckweed diversity in the past and that they are still ongoing.

Keywords

Duckweed genera; Phylogeny, Evolution; Interspecific hybridization; Genome rearrangements

NOTES

Duckweeds – an example for evolution in flux

Cristian Mateo-Elizalde; Evan Ernst; Robert Martienssen

Cold Spring Harbor Laboratory; Howard Hughes Medical Institute

Corresponding author: mateo@cshl.edu

Duckweeds are aquatic monocotyledons including the smallest and fastest-growing angiosperms. They predominantly propagate clonally, making sexual reproduction and floral development poorly explored. The most fertile duckweed species belong to the section Alatae, which includes *Lemna aequinoctialis*, *Lemna perpusilla* and recently described interspecific hybrids including *L. x aoukikusa* (Stepanenko et al., 2025). To investigate the molecular basis of duckweed sexual reproduction, we generated transcriptomic profiles of reproductive tissues from the highly fertile hybrid accession *L. aequinoctialis* 8011. As a relatively recent hybridization event, la8011 constitutes an excellent model for studying flowering because of its highly synchronized floral development and the ability to evaluate the contribution of each subgenome. Duckweed evolution has been associated with gene loss linked to clonal propagation, including components of the RNA-directed DNA methylation (RdDM) pathway (Ernst et al., 2025). This is reflected in the absence of the canonical 24-nt small RNA peak (Ernst et al., 2025; Dombey et al., 2025). Our transcriptomic data reveal activation of genes involved in 24-nt small RNA biogenesis during flowering, consistent with preliminary small RNA-seq findings where these elements are restored. Additionally, we observed a slight increase in CHH methylation in anthers, seeds, embryos and endosperms, supporting activation of the RdDM pathway. These findings suggest that reproductive development is accompanied by finely tuned, cell-specific epigenetic reprogramming that resets epigenetic marks accumulated during clonal propagation. Together, our results provide new insight into the genomic, and epigenetic dynamics underlying natural hybridization and flowering, establishing a molecular framework for future duckweed breeding.

Keywords

RdDM; Transcriptome; Flowering

References

Ernst E, Abramson B, Acosta K, et al. Duckweed genomes and epigenomes underlie triploid hybridization and clonal reproduction. *Curr Biol.* 2025;35(8):1828-1847.e9. doi:10.1016/j.cub.2025.03.013
Stepanenko A, Braglia L, Fuchs J, et al. Genome diversity and evolution of the duckweed section Alatae comprising diploids, polyploids, and interspecific hybrids. *Plant J.* 2025;122(2):e70158. doi:10.1111/tpj.70158

NOTES

Transcriptomic and Epigenetic Insights into Flowering and Sexual Reproduction in *L. aequinoctialis*

Kevin Cox

Washington University in St. Louis - Danforth Center

Corresponding author: coxkevin@wustl.edu

Understanding how plants grow, develop, and respond to their environment requires not only identifying the genes involved, but functionally characterizing what each gene does. To understand how transcription factors, regulate genes across tissues and organs, transcriptional data needs to be placed within a spatial, cellular, and organismal context. *Wolffia australiana* is an attractive system for addressing this fundamental question due to its small body plan, fast clonal growth rates, and compact non-redundant genome. In our lab, we use high-resolution, 3D imaging technologies and transcriptomics to pinpoint the localization of transcription factors and how they globally regulate genes in a whole plant. We used X-ray microscopy to capture the 3D architecture of *W. australiana*, resolving the structure of the meristematic region and laying the groundwork for future cell segmentation and quantification. We further employed expansion microscopy, which enables nanoscale imaging beyond the diffraction limit, achieving roughly 4x physical expansion of intact *W. australiana* and enhanced resolution of nuclei and chloroplasts. Together, these imaging approaches provide a spatial and cellular scaffold for mapping transcriptional activity across the whole plant. To begin applying this framework, we examined the transcriptomic response to early salt stress by generating a bulk RNA-sequencing atlas over a 48-hour timecourse, which identified specific transcription factors and functional pathways underlying the salt stress response. These imaging and transcriptomic approaches together lay the groundwork for localizing transcription factor activity within a defined spatial and cellular context in *W. australiana*.

Keywords

Imaging; Transcriptomics; Salt Stress

NOTES

Building a Spatial and Transcriptomic Framework for Transcription Factor Regulation in *Wolffia australiana*

Ewout Crombez^{1,2}; Quinten Bafort^{1,2}; Yves Van de Peer¹⁻⁴

¹ Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium

² Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium

³ Department of Biochemistry, Genetics and Microbiology, University of Pretoria, 0028 Pretoria, South-Africa

⁴ College of Horticulture, Academy for Advanced Interdisciplinary Studies, Nanjing Agricultural University, 210095 Nanjing, China

Corresponding author: ewout.crombez@ugent.be

Recent discoveries of extensive ploidy variation and natural hybridization have revealed an unexpected level of genomic diversity within duckweeds, particularly in the genus *Lemna*. Naturally occurring cytotypes include auto-triploids, auto-tetraploids, and diploid, triploid, tetraploid and aneuploid interspecific hybrids. This remarkable diversity provides a unique opportunity to investigate how genome duplication and hybridization shape transcriptional regulation. We first focus on the highly variable *Lemna aequinoctialis* x *perpusilla* hybrid species complex to examine how differences in ploidy level and subgenome composition influence the transcriptome. A selection of natural hybrid clones spanning multiple ploidy levels was exposed to salt stress for three weeks and subsequently profiled by RNA-sequencing, enabling us to assess how ploidy affects transcriptional responses to environmental stress. In particular, we examine patterns of expression level dominance, homoeolog expression bias, dosage and odd-even ploidy effects and regulatory divergence across hybrids. Complementing the hybrid study, we compare gene expression in diploid and colchicine-induced autotetraploid lineages of *Spirodela polyrhiza* under a long-term mild salt stress regime, tracking transcriptional changes over many clonal generations. By comparing transcriptional responses at the onset and after prolonged mild salt exposure, we investigate how genome duplication influences the dynamics, stability, and plasticity of gene expression. Together, these studies demonstrate the potential of duckweeds as powerful model systems for investigating the molecular consequences of genome duplication and hybridization, and their roles in shaping plant responses to environmental stress.

Keywords

Polyploidy; Hybridization; Transcriptome; Alatae; Salt stress

NOTES

The Impact of Polyploidy and Hybridization on Gene Expression in Duckweeds

Anton Stepanenko¹; Luca Braglia²; Yuri Lee²; Veit Schubert¹; Jörg Fuchs¹; Manuela Bog³; Hardy Rolletschek¹; Laura Morello²; Ingo Schubert¹

¹ Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, 06466 Seeland, Germany

² Istituto di Biologia e Biotecnologia Agraria (CNR-IBBA) 20133 Milano, Italy

³ Institute of Botany and Landscape Ecology, University of Greifswald, 17489 Greifswald, Germany

Corresponding author: stepanenko@ipk-gatersleben.de

Harnessing plant diversity is essential for modern agriculture. Duckweeds have attracted increasing attention as novel crops with applications ranging from food and feed production to molecular farming. Species complexes, which may involve hybridization and polyploidization events, represent an important source of hidden genetic diversity that can be utilized for crop improvement. Recently, species complexes have been described in the duckweed genus *Lemna* (Stepanenko et al., 2026; Morello et al., 2026), suggesting that a similar pattern may also exist within the genus *Wolffia*. However, evolutionary relationships and species boundaries in *Wolffia* remain poorly understood due to the high morphological similarity among its species. In this study, we used an integrated cytogenetic and molecular approach to investigate the diversity of *Wolffia arrhiza*. By combining flow cytometry, chromosome analysis, genomic in situ hybridization (GISH), morphological traits, chloroplast and nuclear DNA markers, we identified distinct diploid genetic lineages as well as reciprocal triploid hybrids between them. Our findings reveal unexpectedly high diversity within *Wolffia arrhiza*, supporting its recognition as a species complex and highlighting the need for particular attention when describing and identifying accessions within this group.

Keywords

Wolffia phylogeny; Interspecific hybridization; Polyploidy

References

Stepanenko, A., Braglia, L., Fuchs, J., Schubert, V., Hoang, P.T.N., Lee, Y., Chen, G., Gianì, S., Morello, L. and Schubert, I. (2025). Genome diversity and evolution of the duckweed section *Alatae* comprising diploids, polyploids, and interspecific hybrids. *Plant J*, 122: e70158. <https://doi.org/10.1111/tpj.70158> Morello, L., Lee, Y., & Braglia, L. (2026). Exploring Duckweed Diversity at the Dawn of Its Cultivation Era: The Invaluable Legacy of the Landolt Collection. *Plants*, 15(3), 345. <https://doi.org/10.3390/plants15030345>

NOTES

Wolffia arrhiza: A Cryptic Species Complex within the Genus *Wolffia*

Yuri Lee¹; Veit Schubert²; Anton Stepanenko²; Joerg Fuchs²; Luca Braglia¹; Silvia Gianì¹; Ingo Schubert²; Laura Morello¹

¹ Istituto di Biologia e Biotecnologia Agraria (CNR-IBBA) 20133 Milano, Italy

² Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben

Corresponding author: laura.morello@ibba.cnr.it

Duckweeds (Lemnaceae) are emerging as promising crops because of their exceptional biomass productivity and diverse applications. However, progress toward duckweed domestication has been limited by a poor understanding of their sexual reproduction, which is essential to improve genetic information via breeding. Recent genomic studies unexpectedly revealed numerous interspecific hybrids and polyploid lineages in the genera *Lemna* and *Wolffia*, suggesting that sexual reproduction, although rarely observed, has played a much stronger evolutionary role than previously recognized. We systematically investigated the reproductive biology of representative *Lemna* hybrids and their parental species. Furthermore, we performed a comprehensive cytological analysis of meiosis in some selected *Lemna* species to determine the origin and frequency of unreduced gametes associated with polyploids formation. Our analyses revealed remarkable diversity in reproductive traits, including protogyny, homogamy, and variation in self-fertility among species. Except for the naturally fertile allotetraploid *L. ×aoukikusa*, all investigated allodiploid and triploid hybrids were sterile, and artificial whole-genome doubling of natural allodiploid hybrids has failed to restore fertility so far. Cytological analyses combined with fluorescence in situ hybridization showed both dyads and tetrads, representing unreduced and reduced male meiotic cells, respectively. Unreduced gametes occurred at variable, but rather low frequencies (up to 6.85% in *L. turionifera* and 12% in *L. minor*), providing a plausible explanation for the absence of naturally occurring tetraploids in *L. ×japonica* and both parental species, *L. minor* and *L. turionifera*. This study provides keys for elucidating evolutionary pathways of duckweeds and establishes a foundation for future domestication by sexual crossing of this emerging crop.

Keywords

Duckweed flower; Interspecific hybrids; Meiosis

NOTES

On the Way to Domesticate Duckweeds: Hidden Sexuality, Hybridization and Polyploid Evolution

Claire Smith; Thomas Welch; Rahul Bhosale; Anthony Bishopp

Plant and Crop Sciences, School of Biosciences, University of Nottingham

Corresponding author: claire.smith2@nottingham.ac.uk

Evolutionary studies mainly focus on the acquisition of new traits in highly diversified plant lineages, with comparatively few studies showing how these traits have undergone secondary loss. Duckweeds provide a tractable system to address this within angiosperms, as they have gradually lost the ability to grow roots and vasculature. These lost traits are regulated by auxin in other angiosperms. The core auxin signalling network relies on three key protein families: AUXIN RESPONSE FACTORS (ARFs), AUXIN/INDOLE-3 ACETIC ACIDS (AUX/IAAs), and TRANSPORT INHIBITOR 1/AUXIN SIGNALLING F-BOX (TIR1/AFBs). During the evolution of land plants there have been expansions in the number of network components, creating a diverse network with high specificity of response. There have been many studies on how this network has evolved from algae to angiosperms, but no research how components have been lost secondarily. We found that there has been a reduction in the number of components across all duckweeds, however most subclades have been conserved, and that a wide panel of duckweeds respond to exogenous auxin in growth assays. We found that the rootless *Wolffia australiana* responds transcriptionally to auxin, although the genes that are induced are distinct from those induced in rooted duckweeds. This work shows a correlation between the loss of structural complexity and reduction of the auxin signalling network, which is the opposite of what is observed during the evolution of angiosperms. This positions duckweeds as a good model for research into secondary loss and provides a minimal system with low levels of genetic redundancy.

Keywords

Auxin; Evolution; Secondary loss; Transcriptomics

NOTES

Investigating the secondary loss of auxin signalling components in duckweed

Constructing designer duckweed holobionts for comprehensive analyses of plant-microbe interactions

#82

Eric Lam¹; Zili Pang¹; Kenneth Acosta¹; Kyohei Koseki²

¹ Department of Plant Biology, Rutgers the State University of New Jersey, New Brunswick, NJ 08901, USA

² Department of Food Science, Ishikawa Prefectural University, Nonoichi, Ishikawa, Japan

Corresponding author: eric.lam@rutgers.edu

Holobiont refers to a holistic view of the organism as a combination of an eukaryote and its associated microbiome, which is a dynamic assortment of microbes inhabiting various niches that the host presents. The bacterial microbiome of water lentils (aka duckweeds) has been characterized over the past decade and may enhance or inhibit the growth of these aquatic plants. Most of these studies involved presenting isolates of duckweed-associated bacteria (DABs) to gnotobiotic duckweeds and observing their effects on the plant's behavior after days or weeks. However, the mechanism of how DABs can modulate duckweed growth in different context is still lacking due to the complexity of the interacting partners as well as with the changing environmental context during the assays. Leveraging the clonal propagation lifestyle of duckweeds, we have constructed duckweed holobionts (DHBs) using characterized DABs and their combinations that stably colonize gnotobiotic *Lemna minor* 5576, a duckweed strain with a well characterized genome. These associations may be stably propagated for months or years under laboratory conditions, showing that assembled DABs could be transferred between generations of fronds. Preliminary studies show that these designer DHBs can provide a convenient platform to study how stable colonization by individual DABs and their mini-communities can modify the host's physiological properties, such as their relative growth rate and levels of vitamin B12, as well as the response of plant-microbe association to environmental factors. The DHB approach can thus help generate deep datasets on plant-microbe interactions while producing value-added strains as candidates for practical applications.

Keywords

Microbiome; Holobiont; Synthetic communities; VitaminB12

NOTES

Constructing designer duckweed holobionts for comprehensive analyses of plant-microbe interactions

Vitamin B₁₂ Accumulation in Duckweed Emerges from a Cooperative, Environmentally Responsive Microbiome

#37

Osnat Gillor¹; Avital Friedjung Yosef¹; Dalia Rishmawi¹; Francois Prudot d'Avogny^{1,2}; Lusine Ghazaryan¹; Miri Lapidot³; Rhishika Dutta¹; Romain Bernard²

¹ Ben Gurion University of the Negev; ² French National Institute for Agriculture, Food, and Environment; ³ Hinoman Ltd.

Corresponding author: gilloro@bgu.ac.il

Vitamin B₁₂ is synthesized exclusively by bacteria and archaea, yet duckweeds accumulate bioavailable B₁₂ despite lacking the capacity to produce or use it. We asked how this function is organized within the duckweed microbiome, and whether cultivation conditions could enhance it. Bioavailable B₁₂ was detected across rooted and rootless duckweed genera, and in additional aquatic plants. Among 16 B₁₂-containing duckweed haplotypes spanning seven species, endophyte composition varied markedly, but neither community structure nor predicted B₁₂-biosynthesis potential tracked host taxonomy; distinct microbiomes converged on the same function. In the rootless species *Wolffia globosa*, the endosphere formed a host-filtered community in which a subset of bacteria encoded complete or near-complete cobalamin pathways; most co-occurring taxa instead carried partial pathways, precursor-salvage genes, or cobamide-remodeling capacity. Network analysis linked putative producers to salvagers, and endophytic genomes carried fewer biosynthetic genes than related planktonic strains, suggesting adaptation to metabolic interdependence over autonomous production. Experimental manipulation confirmed that B₁₂ production is a community-level trait. Epiphytic *Rhizobium* and *Caulobacter* isolates produced B₁₂ in culture, but co-cultivation with duckweed did not increase plant B₁₂ and instead disrupted plant-microbiome performance. Cobalt supplementation, by contrast, increased bioavailable B₁₂ up to fivefold without cost to growth, and reshaped endophyte composition, diversity, and core-community membership in species-specific ways. Our results show that B₁₂ accumulation in duckweed is an emergent property of a cooperative, environmentally responsive microbiome, not the output of a single producer. This positions duckweed as a tractable model for cobamide-mediated microbial interactions and a promising platform for microbiome-guided B₁₂ biofortification.

Keywords

B₁₂ biosynthesis; Metabolic interdependence; Endophytes

References

Dutta R, Obayomi O, Freidjung Yosef A, Ghazaryan L, Chalifa-Caspi V, Lapidot M, Gillor O. 2026. A cooperative cobalamide biosynthesis guild in the endosphere of the edible aquatic plant *Wolffia globosa* Mankai. *Environmental Microbiome*. <https://doi.org/10.1186/s40793-026-00917-4> Friedjung Yosef A, Ghazaryan L, Klamann L, Kaufman KS, Baubin C, Poodiack B, Ran N, Gabay T, Didi-Cohen S, Bog M, Khozin-Goldberg I, Gillor O. 2022. Diversity and Differentiation of Duckweed Species from Israel. *Plants-Basel* 11 (23): 3326. <https://doi.org/10.3390/plants11233326>

NOTES

Vitamin B₁₂ Accumulation in Duckweed Emerges from a Cooperative, Environmentally Responsive Microbiome

Arinthip Thamchaipenet; Chakrit Bunyoo; To Nhu Le Thuy; Juthaporn Pholmakam;
Karan Lohmaneeratana

Kasetsart University, Bangkok

Corresponding author: arinthip.t@ku.th

Potential cobalamin (vitamin B12)-producing *Wolffia globosa*-associated bacteria were investigated through shotgun metagenomic sequencing analysis. Taxonomic classification assigned microbiomes consisting of 5 phyla, 8 classes, 36 families, and 68 genera. High-quality metagenome-assembled genomes (MAGs) were assigned to *Allorhizobium* sp., *Alsobacter* sp., *Bosea* sp., *Bradyrhizobium huaxiensis*, *Brevundimonas* sp., *Methylophilus* sp., *Microbacterium* sp., *Mycobacterium* sp., *Neoroseomonas marina*, *Nevskia* sp., *Novosphingobium* sp., *Phenylobacterium* sp., *Pseudonocardia carboxydovorans*, *Rhizobium* sp., *Rhizorhaddus* sp., *Roseomonas* sp., *Sphingomonas* sp., and *Xanthomonas sontii*. Functional annotation across all MAGs revealed potential pathways related to aromatic compound degradation, cofactor and vitamin metabolism, serine and threonine metabolism, nitrogen metabolism, and other carbohydrate metabolic processes. Several MAGs contained complete modules of cobalamin biosynthesis including *hem*, *cob*, *cbi* genes responsible for corrin ring synthesis, cobalt transport, lower-ligand formation, and final cobalamin assembly. Interestingly, the definitive molecular marker for active B12 production, *bluB*, was identified in several MAGs. Subsequently, eight bacterial genera were isolated using culture dependent methods and three novel species have been proposed. Co-cultivation experiments are underway to understand the associated B12 production of *W. globosa* holobionts.

Keywords

W. globosa microbiome; MAGs; Cobalamin; Novel species

References

Manuscript in preparation.

NOTES

Potential cobalamin-producing *Wolffia globosa*-associated microbiome

François Prudot D'Avigny^{1,2}; Osnat Gillor²; Romain Bernard¹

¹ INRAE - French National Institute for Agriculture, Food, and Environment

² Ben Gurion University of the Negev

Corresponding author: fprudot@gmail.com

Duckweeds are small, fast-growing aquatic plants that provide a useful model for studying plant-microbiome interactions in aquatic environments. Among them, *Spirodela polyrhiza* is particularly interesting because it can switch from active vegetative growth to dormant turion formation (starch-rich vegetative propagules), in a turion-plant-turion cycle that allows to examine plant-associated microbiome transmission across contrasting developmental stages within a clonal lineage. We investigated the transmission of epiphytic and endophytic microbiomes across the life cycle of *S. polyrhiza*. Specifically, we examined whether microbial communities associated with turions persist during germination and vegetative growth, and whether newly formed turions retain microbial signatures from the preceding plant stage. We also compared epiphytic and endophytic fractions to determine whether they display distinct patterns of continuity, turnover, and reassembly. Our results indicate that microbiome transmission across the growth cycle involves both persistence and community reassembly. Turion-associated communities partially overlapped with those of fronds and roots, showing that a subset of microorganisms was retained across developmental transitions, yet with substantial community turnover, and variation associated with plant genotype and the surrounding medium. Epiphytic and endophytic communities were not completely differentiated based on their overall composition. We hypothesize that endophytic communities show stronger persistence across developmental transitions, whereas epiphytic communities are more strongly reshaped by interactions with the surrounding aquatic environment. By resolving microbiome dynamics across the turion-plant-turion cycle, this study clarifies the role of dormant vegetative propagules in maintaining host-associated microbial communities and improves our understanding of microbiome transmission in clonal aquatic plants.

Keywords

Microbiome; Assembly/transmission, Turion

NOTES

Microbiome Assembly and Transmission Across the Turion–Vegetative Growth Cycle in *Spirodela polyrrhiza*

Sandor Szabo¹; Gábor Borics²; Mihály Braun³; Gergő Koleszár¹

¹ University of Nyiregyhaza

² Ecological Research Center

³ Institute for Nuclear Research

Corresponding author: szabo.sandor@nye.hu

In duckweed pond systems established either for biofiltration or biomass production, excessive proliferation of planktonic algae reduces ecosystem services. In this study we revealed those algal related mechanisms resulting duckweed growth inhibition. We isolated 26 algal species from duckweed covered mesocosms and measured their impact on axenic culture of *Lemna gibba*. At low duckweed coverage (<50%), 18 algal and cyanobacterial species significantly reduced the growth rate of duckweed. The strongest inhibitory effects were exerted by unicellular green algae. When the combined effects of dominant algal species were examined, all tested algae drastically inhibited duckweed growth. The elemental flux between the water, algal biomass and *Lemna* fronds was followed in time. Algal biomass increase was closely associated with the depletion of nutrients from the water column. In aquarium experiment, under low and moderate nitrogen concentrations together with the absence of algal shading, algae significantly inhibited the growth and chlorophyll content of *Lemna* by 60-80%. The algal inhibitory effect weakened with increasing nitrogen concentration and under conditions of stronger shading affecting the algae. Laboratory experiments demonstrated that duckweed growth was most strongly inhibited by algal-induced nitrogen deficiency, followed by elevated pH (10.2), phosphorus deficiency, and iron deficiency, resulting in growth inhibition of 72%, 52%, 51%, and 47%, respectively. By the final day of the experiment, both algal-induced nitrogen depletion and high pH completely reduced duckweed growth.

Keywords

algae inhibition competition pH

References

Szabó S, Roijackers R, Scheffer M, Borics G. 2005. The strength of limiting factors for duckweed during algal competition. *Archiv für Hydrobiologie* 164:127–140. Szabó S, Braun M, Borics G. 1999. Elemental flux between algae and duckweeds (*Lemna gibba*) during competition. *Archiv für Hydrobiologie* 146:355–367.

NOTES

Why Algal Blooms Suppress Duckweed Growth: Mechanisms Behind Growth Inhibition

Shuqing Xu

University of Mainz

Corresponding author: shuqing.xu@uni-mainz.de

Duckweeds combine ecological importance with remarkable evolutionary and morphological diversity. In this talk, I will first present our recent work using *Spirodela polyrhiza* to investigate evolution in complex ecological communities. I will then introduce our ongoing efforts to establish *Lemna aequinoctialis* as a model for studying the evolution and development of highly reduced flowers. Evolutionary ecology has often simplified the ecological conditions under which evolution occurs. In nature, however, organisms live and evolve within communities composed of complex networks of species interactions. Using *S. polyrhiza*, we have shown that direct herbivory by snails and indirect effects mediated through algal competition jointly shape duckweed population dynamics and evolution (Böttner et al. 2025, Schäfer et al. 2025). Because these interactions vary over time, they can generate strongly fluctuating selection and make short-term evolutionary outcomes difficult to predict. These findings demonstrate that duckweed is a power system for revealing evolutionary principles in nature. At a broader evolutionary scale, duckweeds display striking variation in body plans and reproductive structures. Our recent comparative analysis suggests that duckweed reproductive structures evolved through progressive reduction and compression of the ancestral aroid spadix-spathe system (Siadjeu et al. 2026). To investigate the mechanisms underlying this diversity, we are developing *Lemna aequinoctialis* as a functional evo-devo model. A single-nucleus transcriptomic atlas and stable genetic transformation now provide tools for identifying cell-type-specific regulatory programmes and experimentally testing their roles in floral development.

Keywords

Community ecology; Species interactions; Flora development; *Spirodela polyrhiza*; *Lemna aequinoctialis*

References

Böttner, L., Dudenhausen, F., Nouere, S., Malacrino, A., Schäfer, M., Koene, J. M., Huber, M. & Xu, S. (2025). Herbivory can increase plant fitness via reduced interspecific competition-evidence from models and mesocosms. *Proceedings of the Royal Society B*, 292, 20241149. Schäfer, M., Malacrino, A., Walcher, C., et al. (2025). Aphid herbivory on macrophytes drives adaptive evolution in an aquatic community via indirect effects. *Proceedings of the National Academy of Sciences USA*, 122, e2502742122. Siadjeu, C., Chakraborty, A. & Xu, S. (2026). Duckweeds as a model for minimal floral development. *Trends in Genetics*, 42, 555–564.

NOTES

Duckweeds as models for understanding evolution in ecological communities and floral diversity

Plasticity and Adaptation of Fatty Acid Profiles in Four Lemnaceae Species under Varying Thermal and Nutrient Regimes

#47

Manuela Bog; Phillip Dam Harry Bernhardt; Alexander Wacke

University Greifswald

Corresponding author: manuela.bog@uni-greifswald.de

The Lemnaceae family exhibits remarkable ecological plasticity, allowing these aquatic plants to thrive in diverse freshwater habitats. A key mechanism of this adaptation is the modulation of membrane lipids, which is crucial for maintaining biological functions under fluctuating environmental conditions. While their potential as a sustainable protein source is well-known, the ecological drivers shaping interspecific variations in fatty acid profiles remain under-explored. This study examines the physiological adaptations of four species (*Lemna gibba*, *L. minor*, *L. turionifera*, and *Spirodela polyrhiza*) from Mecklenburg-Western Pomerania, Germany. Through a common garden experiment, we analyzed the influence of temperature (18, 24, 30 °C) and nutrient availability (half, standard, and double concentrated N-medium) on fatty acid profiles. Results show generally comparable profiles across species, regardless of turion-forming abilities. However, *L. gibba* exhibited higher proportions of stearidonic and γ -linolenic acids, whereas *S. polyrhiza* showed only traces of these compounds. Temperature exerted a stronger influence than nutrients; higher temperatures increased saturated fatty acids while reducing polyunsaturated fatty acids, particularly n-3 types. Minor nutrient effects were observed in *L. gibba* and *S. polyrhiza*. These findings highlight thermal adaptation as a primary driver of lipid metabolism. Further research on nitrogen-phosphorus ratios and clones from warmer regions may further unveil specialized desaturase adaptations to higher temperatures.

Keywords

Fatty acid profiles; Plasticity, Adaptation

References

Bernhardt PDH (2024) Fatty acid profiles of regional Duckweed species. Master Thesis - University Greifswald, Faculty of Mathematics and Natural Sciences, Institute of Botany and Landscape Ecology

NOTES

Plasticity and Adaptation of Fatty Acid Profiles in Four Lemnaceae Species under Varying Thermal and Nutrient Regimes

Laura Morello; Yuri Lee; Luca Braglia; Maria Adelaide Iannelli; Cristian Perna; Silvia Gianì; Massimiliano Lauria; Iride Mascheretti

Istituto di Biologia e Biotecnologia Agraria (IBBA-CNR) 20133 Milano, Italy

Corresponding author: lauraemmamaria.morello@cnr.it

Duckweeds are powerful model plants thanks to their reduced anatomic structure and promising novel crops due to rapid production of valuable biomass. However, transforming this small family into an agricultural crop requires understanding its unexpected complexity and exploring its significant genotypic and phenotypic diversity. Beyond his extensive research, Elias Landolt (1926–2013) left an invaluable legacy: hundreds of living clonal accessions from worldwide and a comprehensive herbarium with over 2,000 specimens, recently transferred to the Italian Central Herbarium. This material serves as a fundamental resource for taxonomy, phylogenomics, basic and applied research. To unlock the potential within these germplasm collections, a collaborative effort is ongoing to genetically characterize available accessions, clarify taxonomy, and identify cryptic species, soon integrating living clones with herbarium specimens. This work establishes a robust framework for genomic studies and phenotypic screening, aiding the selection of key target traits for sustainable technological applications. To fully exploit these resources, physical collections must be paired with standardized digital metadata, ensuring long-term preservation and alignment with FAIR (Findable, Accessible, Interoperable, Reusable) principles. Metadata, integrated with sequence data, genome size values, images, references, are being digitized and hosted in the Biomemory repository, the network of germplasm collections of the National Research Council (CNR). Ultimately, these data are directly accessible via GBIF and integrated in the nascent Distributed System of Scientific Collections (DiSSCo) a European Research Infrastructure, also aimed at providing services through its community. We believe this work represents the due tribute to E. Landolt, in the centenary of his birth.

Keywords

Germplasm collections; Genetic diversity, Taxonomy

NOTES

Landolt's collection 2.0 – Valorization and Digitization of duckweed germplasm resources

Sara Nouere¹; Martin Schäfer¹; Guangqiong Li¹; Martin Lohr²; Dieter Ebert³; Shuqing Xu¹

¹ Institute of Organismic and Molecular Evolution (iomE), Johannes Gutenberg University Mainz, Mainz 55128, Germany

² Institute for Molecular Physiology, Johannes Gutenberg University Mainz, Mainz 55128, Germany

³ Department of Environmental Sciences, Zoology, University of Basel, Basel 4051, Switzerland

Corresponding author: snouere@uni-mainz.de

Future climate change may reshape ecological communities not only by increasing mean temperature, but also by altering the consequences of increasingly frequent heatwaves. Predicting these effects requires understanding how background warming interacts with short heatwaves in natural communities, where responses can arise through direct thermal stress and species interactions. We tested this using 32 outdoor freshwater mesocosms exposed to sustained near-future warming while capturing a documented natural heatwave. Warming raised temperature maxima that exceeded the thermal threshold of the pond snail, a main grazer in the community. Warmed communities showed lower grazer abundance, increased macrophyte and insect herbivore abundance, reduced phytoplankton biomass, and lower zooplankton density. Complementary assays showed that heatwave-level temperatures promoted macrophyte growth and reduced grazer survival, whereas reduced zooplankton performance mainly reflected indirect warming effects via food-web cascades. Thus, near-future warming can amplify natural heatwave impacts by exceeding consumer thermal thresholds and propagating through species interactions.

Keywords

Climate warming; Heatwave; Freshwater community

NOTES

Near-future warming amplifies natural heatwave impacts and reorganizes freshwater communities

Martin Turcotte; Tia-Lynn Ashman

University of Pittsburgh

Corresponding author: turcotte@pitt.edu

Whole genome duplication, leading to polyploidy, is a common macromutation throughout the tree of life and especially in plants. It can have strong impacts across biological scales, from gene expression to cellular processes, to morphology and ecological dynamics. This presentation will summarize knowledge gained in our labs on these impacts using *Spirodela polyrhiza* as a model study system. We have generated replicated neotetraploid genetic lineages and report both shared and divergent changes across genetic backgrounds. Furthermore, we leverage the unique advantages of this aquatic plant to test population level hypotheses advancing insights beyond most other polyploid plant study systems. For instance, we find that polyploids grow more slowly in abundance and reach lower carrying capacities than their diploid progenitors. In addition, our experimental work shows that neotetraploid populations are generally more resistant to urban pollutants, but the extent depends on the pollutant type and the genetic background. Finally, population-level competition experiments applying methods from The Modern Coexistence Theory have revealed how stress alters competitive outcomes and sets the stage for more comparative work in other systems.

Keywords

Stress; Polyploidy; Competition; Variation; Ecology

NOTES

The immediate impacts of whole genome duplication (neopolyploidy) in Spirodela polyrhiza on its functional traits and competitive outcomes.

*Leone Ermes Romano*¹; *Marco Saltini*²; *Jack J. W. A. van Loon*³; *Gabor Milassin*³; *Giovanna Aronne*¹

¹ Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

² Mathematical and Statistical Methods, Plant Sciences Group, Wageningen University, The Netherlands

³ European Space Agency (ESA) Technology Centre (ESTEC), Noordwijk, The Netherlands

Corresponding author: leoneermesromano@gmail.com

The transition from land to water in Lemnaceae represents an extreme case of reductive evolution, culminating in the minute, rootless genus *Wolffia*. Aerenchyma explains buoyancy, but not how microscopic fronds remain upright, avoid rolling, and aggregate into cohesive colonies without any biological anchor. We addressed this in *Wolffia globosa* combining microscopy, surface metrology, histochemistry and mathematical modelling. High-magnification and laser imaging revealed that the waterline around each frond is not flat: reflective points along the perimeter mark localised deformations of the air–water interface, while ESEM excluded any anatomical protrusion. Laser scanning profilometry showed alternating upward and downward menisci, with a bimodal distribution of contact angles ($\theta_{up} = 78.0 \pm 3.0^\circ$, $\theta_{down} = 96.1 \pm 2.2^\circ$; $n = 68$). Neutral Red histochemistry mapped a matching wettability pattern: an undulated contact line between the unstained hydrophobic dorsal epidermis and the stained hydrophilic ventral one. Removing surface tension with a surfactant abolished the menisci and submerged the plants, showing that aerenchyma provides buoyancy while surface tension provides anchoring and orientation. A finite-element model parameterised on these values reproduces the observed multipolar capillary field and predicts a short-range (<0.4 mm) attractive force between neighbouring fronds — the “Cheerios effect” — sufficient to drive passive self-assembly into stable rafts. We propose that biologically patterned epidermal wettability functionally replaces the root system lost in derived Lemnaceae, with implications for colony dynamics, mat behaviour in cultivation systems, and bio-inspired interfacial engineering.

Keywords

Wolffia; Surface tension; Cheerios effect

References

- Acosta, K., Appenroth, K. J., Borisjuk, L., Edelman, M., Heinig, U., Jansen, M. A. K., Oyama, T., Pasaribu, B., Schubert, I., Sorrels, S., Sree, K. S., Xu, S., Michael, T. P., & Lam, E. (2021). Return of the Lemnaceae: Duckweed as a model plant system in the genomics and postgenomics era. *The Plant Cell*, 33(10), 3207–3234. <https://doi.org/10.1093/plcell/koab189>
- Vella, D., & Mahadevan, L. (2005). The “Cheerios effect.” *American Journal of Physics*, 73(9), 817–825. <https://doi.org/10.1119/1.1898523>
- White, S. L., & Wise, R. R. (1998). Anatomy and ultrastructure of *Wolffia columbiana* and *Wolffia borealis*, two nonvascular aquatic angiosperms. *International Journal of Plant Sciences*, 159(2), 297–304. <https://doi.org/10.1086/297550>

NOTES

The Wolffia effect: from biological to physical anchoring — meniscus engineering in a rootless duckweed



NOTES

Circular Feed Innovations for Sustainable Aquaculture and Blue Transformation

#84

Jogeir Toppe

Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO)

Corresponding author: jogeir.toppe@fao.org

As global demand for aquatic foods continues to grow, transforming aquatic food systems requires innovative, cost-effective and environmentally sustainable feed solutions. This presentation examines how circular bioeconomy approaches and locally produced alternative feed ingredients can enhance the resilience and sustainability of aquaculture while advancing the Food and Agriculture Organization of the United Nations (FAO) vision for Blue Transformation. The presentation will highlight the valorization of underutilized biomass and organic side streams, including the conversion of agricultural by-products and other organic materials into high-value feed resources such as duckweed, black soldier fly larvae and fish silage for aquaculture and livestock production. Particular emphasis will be placed on the potential of these resources to reduce environmental impacts and production costs, decrease reliance on imported feed commodities, and thereby strengthen local food security and rural livelihoods. Framed within the FAO Strategic Framework and its Four Betters (better production, better nutrition, a better environment and a better life), the presentation connects science, innovation and policy. It identifies actionable pathways through which research institutions, entrepreneurs, industry stakeholders and international organizations can collaborate to scale sustainable feed solutions across diverse production contexts.

Keywords

Circular bioeconomy; Aquaculture; Alternative feed

NOTES

Circular Feed Innovations for Sustainable Aquaculture and Blue Transformation

High quality and quantity duckweed biomass production & protein, starch biosynthesis

#20

Hai Zhao

Chengdu Institute of Biology, Chinese Academy of Sciences

Corresponding author: zhaohai@cib.ac.cn

Duckweeds are the smallest and fastest-growing flowering plants and represent a promising source of high-value aquatic biomass. Here, we summarize recent progress from our group in duckweed research. More than 10 years studies in a 0.33-ha production system in Yunnan demonstrated that duckweed can generate abundant biomass under practical cultivation conditions, with annual biomass productivity reaching 39.2–55.0 t ha⁻¹. Beyond high yield, duckweed also showed clear quality advantages over water hyacinth, characterized by higher nutritional value and lower lignocellulosic components. These features support its potential as a digestible and nutrient-rich feed ingredient. Consistently, pig feeding trials showed that duckweed supplementation improved growth performance compared with the control diet, with a 46.15% increase in average daily gain and a 43.71% reduction in the feed-to-gain ratio. Improvements in meat quality further supported the practical value of duckweed for animal production. Further mechanistic and genetic studies revealed that cultivation optimization and physiological induction can promote starch accumulation above 75% of dry weight, associated with altered carbon allocation and reduced lignocellulosic components. In parallel, high-protein duckweed strains with protein contents exceeding 50% were selected, and several genes associated with enhanced nitrogen assimilation and protein accumulation were cloned and functionally characterized, providing targets for improving protein production. Efficient genetic transformation systems have also expanded the application of duckweed as a plant bioreactor for recombinant protein production, including edible vaccines. Together, these studies establish duckweed as a high-quality biomass resource and a versatile synthetic biology platform for future food, feed, and plant-based biomanufacturing.

Keywords

Duckweed biomass; Protein; Starch; Biomanufacturing

References

1. Ling Guo†, Yang Fang†, Songhu Wang, Yao Xiao, Yanqiang Ding, Yanling Jin, Xueping Tian, Anping Du, Zhihua Liao, Kaize He, Shuang Chen, Yonggui Zhao, Li Tan, Zhuolin Yi, Yuqing Che, Lanchai Chen, Jinmeng Li, Leyi Zhao, Peng Zhang, Zhengbiao Gu, Fanguan Zhang, Yan Hong, Qing Zhang, Hai Zhao. Duckweed: a starch-hyperaccumulating plant under cultivation with a combination of nutrient limitation and elevated CO₂. *Frontiers in plant science*, 2025, 16:1531849.
2. Hu Zhubin, Fang Yang, Yi Zhuolin, Tian Xueping, Li Jinmeng, Jin Yanling, He Kaize, Liu Penghui, Du Anping, Huang Yuhong, Zhao Hai. Determining the nutritional value and antioxidant capacity of duckweed (*Wolffia arrhiza*) under artificial conditions. *LWT-Food Science and Technology*, 2022, 53: 112477.
3. Ling Guo #, Yonggui Zhao #, Yao Xiao, Xueping Tian, Shu Bao, Xinhui Wang, Kaize He, Yanling Jin, Yang Fang, Hai Zhao*. Pilot-scale duckweed-CO₂ wastewater bioremediation system: a synergistic approach for nutrient removal, carbon capture, and biomass production. *Bioresource Technology*, 2025, 434:132785

NOTES

High quality and quantity duckweed biomass production & protein, starch biosynthesis

Ingrid van der Meer¹; Mark van Hoogdalem ²; Jurriaan Mes ³

¹ Wageningen University and Research

² Wageningen University and Research, Greenhouse Horticulture Research

³ Wageningen Food and Biobased Research; Food, Health and Consumer Research

Corresponding author: ingrid.vandermeer@wur.nl

Now EFSA approval has been obtained for the introduction of *Lemna minor* and *Lemna gibba* as vegetable for human consumption we concentrated research activities on methods to obtain stable plant biomass production, adhering to the EC legislation. As we foresee an indoor (vertical) farming system as most successful and controllable, we tested artificial lighting strategies to reach lowest energy input as possible. To determine required light intensity and spectra we studied different light intensities resulting in good plant growth. High light intensity showed more plant stress and anthocyanin accumulation which was slightly modulated by fertilization. Next, we analysed different photoperiods resulting in a linear correlation of cumulative production and photoperiod without clear growth saturation at longer photoperiods. Fertilization had a small effect on plant biomass and root growth, but a large effect on cyanobacteria and microalgae contamination which will be a major risk determinant to increase yield and high-quality fresh products. As artificial light will be the main cost determinant for year-round plant production in vertical farming system, we tested the effect of intermitted light regimes, showing that commercial cultivation can make use of flexible hours with a low energy rate. Dynamic light strategies with far red did not show any effect on plant biomass. An objective for the future is to product organic certified *Lemna* plant products, currently based on Regulation (EU) 2018/848. We tested several currently available fertilizers for their potential use in *Lemna* cultivation. Next steps include analysis of plant composition and adherence to EU set specifications.

Keywords

Waterlentils; Lighting, Vertical farming, Organic

References

COMMISSION IMPLEMENTING REGULATION (EU) 2025/153 of 29 January 2025 authorising the placing on the market of *Lemna minor* and *Lemna gibba* plants as novel food and amending Implementing Regulation (EU) 2017/2470 (<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32025R0153>)

NOTES

Optimising *Lemna* cultivation for food applications

Sara Demaria; Alessandro Pistillo; Giuseppina Pennisi; Giorgio Gianquinto; Francesco Orsini

DISTAL, Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, Italy

Corresponding author: sara.demaria3@unibo.it

Non-conventional crops with high nutritional value and efficient resource use may improve food security while reducing environmental pressures. Duckweeds are fast-growing aquatic plants with high potential as alternative protein sources. However, their ability to assimilate several compounds may impose food-safety concerns. Controlled-environment agriculture (CEA) could reduce microbiological risks and accumulation of undesirable compounds, but knowledge of indoor cultivation strategies remains fragmented. This systematic review synthesized how growing conditions and management affect duckweed growth and biochemical composition in indoor CEA. Four databases were searched for studies reporting growth and/or biochemical outcomes. Responses varied among genera, species, clones, and outcomes, preventing definition of common cultivation ranges. Most studies examined *Lemna*, particularly *Lemna minor*, in small-scale batch systems; semi-continuous, recirculating, photobioreactor, and multilayer systems were uncommon. In *Lemna*, nitrogen availability and source affected biomass and protein production, while nutrient limitation and environmental stress promoted starch or soluble sugar accumulation, often reducing growth. Light intensity influenced biomass more consistently than composition. In *Spirodela*, red light and carbon availability promoted starch accumulation, while blue light increased amino acids and photosynthetic pigments. *Landoltia* showed consistent starch accumulation under nutrient limitation, especially when combined with extended photoperiods. In *Wolffia*, nutrient availability influenced protein, starch, biomass nitrogen, and nitrate accumulation, while evidence for *Wolffiella* was limited. Minerals, undesirable compounds, and microbiological safety were occasionally assessed. Evidence supports food-oriented production and shows that cultivation conditions can direct biomass towards protein- or starch-oriented uses. Scaling up cultivation alongside comprehensive nutritional and safety assessments is needed to develop standardized production strategies.

Keywords

CEA; Productivity, Quality; Systematic review

NOTES

Growing duckweed indoors: how cultivation conditions and management strategies modulate productivity and nutritional quality - A systematic review

Viktor Oláh¹; Bach Nguyen Phuong¹; Raja Amri^{1,2}; Pham Thi Hong Xuan¹; Muhammad Irfan³; Tamás Garda¹; Ilona Mészáros¹; Szilárd Szabó⁴

¹ Department of Botany, Faculty of Science and Technology, University of Debrecen

² Hungarian University of Agriculture and Life Sciences, Doctoral School of Biological Sciences

³ School of Biological, Earth and Environmental Sciences, University College Cork

⁴ Department of Physical Geography and Geoinformatics, Faculty of Science and Technology, University of Debrecen

Corresponding author: olahviktor@unideb.hu

Commercial duckweed cultivation may require regular monitoring of biomass quality, but chemical analyses can be laborious and costly. Optical signatures –that is selective light absorbance of fronds would offer a promising alternative, yet reports on testing such proxies on duckweeds are still scarcely available. To address this knowledge gap, we tested light transmittance- and reflectance-derived indices of duckweed fronds as quick tools in estimating biomass quality. We analyzed biochemical and optical traits in a total of 9 duckweed clones belonging to 3 widely spread species with economic potential: *Lemna gibba*, *Lemna minor* and *Spirodela polyrrhiza*. The plants were cultivated in a growth chamber under 2 different light intensities, and the studied parameters included relative growth rate, dry matter content, protein and photosynthetic pigment contents, as well as various indices derived from hyperspectral reflectance spectra and frond transmittance. Our results indicated strongly interrelated frond traits. Reflectance indices also correlated well with protein and photosynthetic pigment concentrations in the biomass. Similarly, frond transmittance assessed by a handheld chlorophyll meter proved to be reliable in estimating protein content of the biomass. Optical signatures, therefore, can be applicable in routine monitoring of biomass quality and physiological status of duckweed cultures in larger-scale applications too.

This research was funded by the NKFIH OTKA grant No. FK 134296. Viktor Oláh was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by the University of Debrecen Program for Scientific Publication.

Keywords

Biomass; Protein; Hyperspectral reflectance; Transmittance

NOTES

Non-invasive estimation of duckweed biomass quality can be achievable by spectral signatures

Weijuan Huang¹; Ling Deng¹; Xiang Zhou²; Zhenbiao Yang¹

Shenzhen University of Advanced Technology
Shenzhen Institute of Advanced Technology, CAS

Corresponding author: huangweijuan@suat-sz.edu.cn

Duckweed is a promising plant-based protein and biomass resource, but efficient breeding and large-scale cultivation require rapid, high-throughput, and non-destructive evaluation of key traits. Conventional methods for determining crude protein and chlorophyll contents are labor-intensive, chemically demanding, and destructive, limiting their application in large-scale germplasm screening and real-time cultivation management. In this study, we developed an integrated high-throughput phenotyping platform combining hyperspectral imaging (HSI) and deep learning for simultaneous non-destructive prediction of crude protein content, leaf area-based relative growth rate (RGR), and chlorophyll content in duckweed. More than 200 duckweed samples cultivated in standardized 12-well microplates were scanned using an HSI system covering 400–2000 nm with a spectral resolution of 3 nm. A DeepLab v3+ semantic segmentation model was used to delineate duckweed fronds and quantify leaf area, enabling automated extraction of growth-related phenotypes. A Transformer-based spectral-spatial feature fusion model was further developed to integrate RGB-derived spatial features with hyperspectral signatures for biochemical and growth trait prediction. The platform achieved strong predictive performance for crude protein content ($R^2 = 0.938$, RMSE = 1.030, prediction accuracy = 95.8%), chlorophyll content ($R^2 = 0.893$, RMSE = 1.514, prediction accuracy = 94.7%), and leaf area-based RGR (prediction accuracy = 98.5%). With a throughput of up to 120 samples per hour, this HSI- and deep learning-based platform provides an efficient tool for duckweed germplasm evaluation, high-protein strain selection, and precision cultivation management.

Keywords

Protein; Hyperspectral imaging; Deep learning

References

- [1] Gao, R., Li, Z., Ma, Z., et al. (2021). "Research on detection of crude protein content in pasture based on hyperspectral imaging." *Spectroscopy and Spectral Analysis* 41(10): 3158-3163.
- [2] Aulia, R., Kim, Y., Amanah, H.Z., et al. (2022). "Non-destructive prediction of protein contents of soybean seeds using near-infrared hyperspectral imaging." *Infrared Physics & Technology* 127: 104365.
- [3] Xuan, G., Jia, H., Shao, Y., & Shi, C. (2024). "Protein content prediction of rice grains based on hyperspectral imaging." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 320: 124589.
- [4] Wang, P., et al. (2022). "An improved DeepLab v3+ deep learning network applied to the segmentation of grape leaf black rot spots." *Frontiers in Plant Science* 13: 795410.

NOTES

High-Throughput Non-Destructive Prediction of Duckweed Protein Content Using Hyperspectral Imaging and Deep Learning

Felipe Alves^{1,2}; Carlos Seit¹; Rui Abreu¹; Manuel Feliciano¹; Márcio Carochio¹; Miguel Lage²

¹CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; ² Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Instituto de Agroecología e Alimentación (IAA)—CITEXVI, Universidade de Vigo, 36310 Vigo, Spain

Corresponding author: felipemacedo.alves20@gmail.com

The protein tyrosine phosphatase 1B (PTP1B; EC 3.1.3.48), encoded by the PTPN1 gene, dephosphorylates the activated insulin receptor and IRS-1, acting as a negative regulator of insulin and leptin. PTP1B knockout mice, which are more insulin sensitive and resistant to diet-induced obesity, genetically validate PTP1B as a dual target for type 2 diabetes and obesity. However, no inhibitor has made it to clinical trials. We applied an integrated and phased flow, with prior validation, to the 53 phenolic compounds of *Lemna minor*, found in literature and hosted in the LMW Database against PTP1B (PDB 2QBS): (1) validation by redocking of the co-crystallized inhibitor; (2) molecular docking (AutoDock Vina) [4]; (3) consensus by second scoring function (Vinardo) and by machine learning (RF-Score); (4) AI co-folding with confidence metric (Boltz-2); and (5) internal control with cross-validation in the literature. *Lemna minor* was rich in PTP1B ligands (about two-thirds of the phenolics showed a strong predicted interaction). Flavone luteolin stood out as a lead in all criteria (docking -9.14 kcal/mol; best flavonoid by affinity; ipTM 0.90), corroborated by literature: luteolin derivatives inhibit PTP1B (luteolin-7-diglucuronide, IC₅₀ = $2.10 \mu\text{M}$); quercetin is a reference inhibitor of aldose reductase (IC₅₀ = $6.6 \mu\text{M}$); and apigenin inhibits α -glucosidase (IC₅₀ = $10.5 \mu\text{M}$). *Lemna minor* extracts emerge as multi-component, multitarget antidiabetic candidates, with a solid basis for subsequent in vitro experimental confirmation.

Keywords

Extract; PTP1B; Luteolin; Flavonoids; Diabetes; Docking

References

- [1] Elchebly, M. et al. (1999). Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science*, 283(5407), 1544–1548.
- [2] Klamann, L. D. et al. (2000). Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Molecular and Cellular Biology*, 20(15), 5479–5489.
- [3] Combs, A. P. (2010). Recent advances in the discovery of competitive protein tyrosine phosphatase 1B inhibitors for the treatment of diabetes, obesity, and cancer. *Journal of Medicinal Chemistry*, 53(6), 2333–2344.
- [4] Trott, O., Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461.
- [5] Wohlwend, J. et al. (2024). Boltz-1: Democratizing biomolecular interaction modeling. *bioRxiv*.
- [6] Passaro, S. et al. (2025). Boltz-2: Towards accurate and efficient binding affinity prediction. *bioRxiv*.

NOTES

AI-Driven Virtual Screening of *Lemna minor* Phenolic Compounds in the Search for Novel Glycemic Control Agents

From Traditional Food to Emerging Industry: The Rise of *Wolffia* in Thailand

#88

Metha Meetam

Corresponding author: metha.mee@mahidol.ac.th

Wolffia globosa has been consumed in Thailand and neighboring countries for generations. However, its consumption declined substantially, partly due to limited awareness of its nutritional properties and the unreliable availability of biomass. Prior to 2020, *W. globosa* was primarily harvested from natural ponds, resulting in low yields, seasonal variability, and food safety concerns. The taxonomic and genetic diversity of *Wolffia* and other duckweed genera in the region was also poorly understood. Over the past decade, growing demand for plant-based proteins and wellness foods, together with increasing recognition of duckweed as an alternative protein source, has renewed interest in this tiny duckweed among Thai consumers. Driven largely by Thai startups developing large-scale cultivation technologies, related food products ranging from fresh ingredients to innovative health foods are now available in more than 300 shops and restaurants nationwide. The Thai government has also established Good Agricultural Practice (GAP) standards for *W. globosa* production and promoted public awareness of its nutritional and health benefits. Currently, 150 farms are GAP-certified, producing an estimated 500 tonnes of fresh biomass annually, with production projected to reach 3,000 tonnes in 2027. The expansion of the industry has been accompanied by rapid growth in scientific research. Duckweed-related publications increased from an average of 1.8 articles per year during 2016–2020 to 9.25 during 2021–2024, followed by 39 publications in 2025 and 46 in 2026. These developments demonstrate the rapid emergence of *Wolffia* spp. as a high-value crop and highlight Thailand's growing role in advancing duckweed research and commercialization.

Keywords

Wolffia; Cultivation; Applications; Translational research

NOTES

From Traditional Food to Emerging Industry: The Rise of Wolffia in Thailand

Ivan Loaiza^{1,2}; Marcel A.K. Jansen^{1,2}

¹ School of Biological, Earth and Environmental Sciences, University College Cork, Ireland;

² Environmental Research Institute, University College Cork, Ireland

Corresponding author: m.jansen@ucc.ie

Innovative cultivation techniques are required for new circular economy applications that require cultivation of large surface areas of duckweed under indoor conditions. To increase duckweed cultivation area relative to floor space, multilayered cultivation systems have been developed. In these stacked systems, duckweed is cultivated on liquid medium, an approach inspired by the natural, floating habit of these aquatic plants. LEDs are sandwiched between each cultivation layer to provide homogenous light conditions. Stacked systems can successfully generate large amounts of duckweed biomass, but their construction involves an engineering challenge due to the sheer weight of the water column and associated mechanical support structures. As a result, most stacked systems comprise less than 10 cultivation layers. As an alternative, growth of duckweed in a mist of nutrient-supplemented fine droplets, i.e. fog-o-ponics, was pioneered. The advantage of fog-o-ponic cultivation of duckweed is that trays with water are omitted and replaced by a thin layer of fabric on which the duckweed is positioned. Fog droplets pass through the hydrophobic fabric, to be returned to the mister, creating a circular flow-thru system. The use of fabrics substantially decreases the weight and thickness of each layer of duckweed in a stacked fog-o-ponic cultivation system, and as a consequence enables increases in the number of duckweed layers per unit of floor space. *Lemna minor* was grown in a fog-o-ponic system comprised of 10 cultivation layers in an incubator of just 40 cm height, demonstrating good growth and an attractive metabolic profile, as well as upscaling potential.

Keywords

Fog-o-ponic; *Lemna minor*; Biomass; Circular-economy

NOTES

Fog-o-ponics; a novel duckweed cultivation method

Patricia Maag; Cornelia Rauh; Özlem Özmutlu Karslioglu

Hochschule Weihenstephan-Triesdorf University of Applied Sciences, TU Berlin

Corresponding author: patricia.maag@hswt.de

Efficient extraction methods are required to maximize protein recovery from duckweed (*L. minor*) while preserving nutritional quality and functionality. The Institute of Food Technology at Weihenstephan-Triesdorf University of Applied Sciences compared ultrasound-assisted extraction (UAE) and pulsed electric field-assisted extraction (PEF) with conventional alkaline-acidic extraction. Fresh greenhouse-grown *L. minor* was processed immediately after harvest. UAE parameters were optimized, while PEF conditions were adopted from previous work. Protein concentrates were characterized for protein content and yield, chlorophyll, antioxidant capacity, and techno-functional properties. UAE achieved the highest protein extraction efficiency at 100% amplitude and 2 min sonication, whereas the investigated PEF treatment (5 min homogenization prior to 5kV/cm) comparatively enhanced cell permeabilization. UAE and PEF yielded protein concentrates containing 60.81% and 65.24% protein, with protein yields of 55.02% and 34.70%, respectively. Conventional extraction produced the lowest protein yield (30.78%) but the highest protein content (66.19%), which did not differ significantly from PEF. Extraction technology also influenced pigment recovery, antioxidant capacity, and techno-functional properties. UAE achieved the highest chlorophyll extraction by ~64% and preferentially recovered hydrophilic antioxidants, consistent with previous findings [1], whereas PEF favored hydrophobic antioxidants. The PEF-derived protein concentrate showed the highest protein solubility and superior foaming and emulsifying capacities, while UAE improved foam and emulsion stability. These findings demonstrate that UAE and PEF are promising alternatives to conventional extraction, enabling the production of duckweed protein concentrates with tailored compositional and techno-functional properties for diverse food applications.

Keywords

Non-thermal technologies; Protein recovery; Functionality

References

Maag, P.; Cutroneo, S.; Tedeschi, T.; Grüner-Lempar, S.; Rauh, C.; Karslioglu, Ö.Ö. Optimization of Protein Extraction from Duckweed Using Different Extraction Processes. *Food Bioprocess Technol* 2025, 18, 5510–5531, doi:10.1007/s11947-025-03777-x.

NOTES

Comparison of Ultrasound- and Pulsed Electric Field-Assisted Protein Extraction from fresh *Lemna minor*: Process Evaluation and Functional Characterization

Anim Ujong^{1,2}; Sarah Kearney¹; Brijesh Tiwari¹; Shay Hannon¹; Uma Tiwari²

¹ Technological University Dublin

² Teagasc Food Research Centre, Ashtown

Corresponding author: d23129048@mytudublin.ie

Duckweeds have gained increasing attention as sustainable sources of bioactive compounds with promising applications in functional foods and nutraceuticals. This study comprehensively characterized the phenolic profiles of three duckweed species (*Lemna minor*, *Lemna gibba*, and *Spirodela polyrrhiza*) using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The bioactive potential of the extracts was evaluated through in vitro antioxidant, enzyme inhibitory, anti-inflammatory and ACE-inhibitory assays to assess their functional properties. Targeted UHPLC-MS/MS analysis revealed distinct species-specific phenolic profiles, with quinic acid identified as the predominant compound in all species, particularly in *L. minor* (3.99 µg/mg extract DM), followed by *L. gibba* (2.31 µg/mg extract DM) and *S. polyrrhiza* (1.64 µg/mg extract DM). *S. polyrrhiza* exhibited a characteristic flavonoid-rich profile, with markedly higher concentrations of luteolin-7-O-glucoside (2.11 µg/mg extract DM) and vitexin (2.13 µg/mg extract DM) compared with *L. minor* (0.47 and 0.20 µg/mg extract DM, respectively) and *L. gibba* (0.12 and 0.10 µg/mg extract DM, respectively). Apigenin was detected exclusively in *S. polyrrhiza*, whereas caffeic acid was most abundant in *L. gibba* (0.49 µg/mg extract DM). Among the three species, *L. minor* demonstrated the highest total phenolic content (23.54 mg GAE/g DW), ferric reducing antioxidant power (FRAP; 43.55 mmol Fe²⁺/g DW). Principal component analysis revealed strong associations between specific phenolic constituents and measured biological activities, suggesting that species-specific phenolic profiles contribute to the functional properties of duckweed extracts. These findings highlight duckweed as a promising sustainable source of phenolic compounds with potential applications as functional food ingredients and health-promoting nutraceuticals.

Keywords

Duckweed; Bioactive compounds; Phenolic compounds

NOTES

UHPLC-MS/MS profiling and biological activities of phenolic compounds from three duckweed species for the identification of potential health-promoting biomolecules

Shi Er Ng¹; Derek Juinn Chieh Chan¹; Ezaki Satoshi²; Masao Tsutsui²; Tatsu Takimoto³; Eishi Morita³

¹ Universiti Sains Malaysia

² CS Innovation Co. Ltd

³ TSUKASA PETCO SINGAPORE PTE. LTD

Corresponding author: chderekchan@usm.my

Duckweeds are gaining attention as sustainable nutrient-rich biomass due to their exceptional capacity to accumulate proteins and carbohydrates. However, maximizing biomass production alone does not guarantee the preservation of nutritional quality after harvest. This study investigated both cultivation and post-harvest processing conditions for three duckweed species (*Lemna minor*, *Spirodela polyrhiza*, and *Wolffia globosa*) using a two-phase approach. During cultivation, single and sequential nutrient media with different nitrogen-to-phosphorus (N:P) ratios were evaluated over 12–28 days. Distinct species-specific responses were observed. *L. minor* achieved the highest relative growth rate (0.138 day^{-1}) and protein content (50.2% dry weight) under a sequential shift from a high to low N:P ratio (18:1 to 6:1). *S. polyrhiza* produced the highest protein concentration overall (51.7% dry weight) and performed best under prolonged cultivation at a low N:P ratio (6:1). In contrast, *W. globosa* exhibited slower growth (0.071 day^{-1}) but accumulated the greatest lipid content (6.2%) and carbohydrate content (52.4% dry weight) during extended cultivation at a high N:P ratio (18:1). Drying temperature significantly affected biochemical composition. Lower temperatures (40–50°C) better preserved protein integrity, maintaining protein yields of approximately 300–340 mg g⁻¹ while minimizing lipid oxidation. Higher temperatures (50–60°C) promoted degradation of non-carbohydrate components, increasing apparent carbohydrate concentrations (>400 mg g⁻¹) and enhancing anthocyanin extractability from approximately 12 to over 43 mg g⁻¹. These results demonstrate that species-specific nutrient management combined with low-temperature drying (40–50°C) is essential for preserving duckweed biomass quality and supporting large-scale, sustainable production systems

Keywords

Nutrient management; Protein preservation

NOTES

Synergistic Nutrient Formulations and Thermal Drying Strategies for Enhanced Duckweed Nutrition

Tripartite Synergy in a Baffled Duckweed Reactor for 2-Chloro-4-Nitroaniline Remediation: Metabolic Pathways, Microbial Dynamics, and Biochar Valorization

#4

Ahmed Tawfik

Kuwait university, College of Life Sciences

Corresponding author: ahmed.elmitwalli@ku.edu.kw

This study presents a novel, integrated treatment approach for petrochemical wastewater laden with 2-chloro-4-nitroaniline (2-Cl-4-NA) and heavy metals using a baffled duckweed reactor (BDR). Unlike traditional systems, this BDR leverages a unique tripartite synergy between *Lemna gibba*, a specialized symbiotic bacterial community, and in-situ photocatalytic oxidation. At an optimized hydraulic retention time (HRT) of 12 days, the system achieved a superior 2-Cl-4-NA removal efficiency of 84.3%. For the first time, the metabolic pathway in this integrated system was mapped, identifying 4-amino-3-chlorophenol and 2-amino-3-chloro-5-hydroxyphenol as key intermediates. The mechanical novelty lies in the dual-role of *L. gibba*: it facilitates (H₂O₂)-mediated photocatalytic degradation and provides essential oxygen to the rhizosphere, stimulating the production of bacterial monooxygenase and dioxygenase enzymes for ring cleavage. Concurrently, the bacteria break down the toxic parent compound into metabolites that the duckweed subsequently assimilates as a carbon source. High-throughput sequencing confirmed a specialized microbial consortium, dominated by Proteobacteria and Bacteroidetes, with functional genera such as *Defluviimonas* and *Thioclava* driving the degradation. These findings establish the BDR as a highly efficient, sustainable, and multi-pathway bioremediation technology for complex industrial pollutants. The harvested biomass was used for biochar productivity for further application (0.77 g biochar/gbiomass).

Keywords

Duckweed Rhizosphere; Synergy; Microbial

NOTES

Tripartite Synergy in a Baffled Duckweed Reactor for 2-Chloro-4-Nitroaniline Remediation: Metabolic Pathways, Microbial Dynamics, and Biochar Valorization

Alexandra Katsara; Olivia K. Lordan; Neil E. Coughlan; Marcel A.K. Jansen

School of Biological, Earth and Environmental Sciences, University College Cork

Corresponding author: alexandra.katsara@ucc.ie

Seafood processing industries generate significant volumes of highly polluted wastewater that poses serious environmental challenges. The characteristics of seafood processing wastewaters were identified, focussing on the different processing stages. Fish filleting wastewater, generated from food-grade raw materials, contains plant nutrients, oils, proteins, and other organic compounds that are costly to remove yet represent underutilized resources. Within the Horizon Europe IMPRESS project, duckweed cultivation was explored as a strategy to both remediate these wastewaters and convert their nutrients into high-value biomass. Duckweed species (*Landoltia punctata*, *Lemna minor*, *Lemna minuta*) displayed good growth on fish processing wastewater, measured as a relative growth rate (RGR). Different dilutions of wastewater from the fish deheading stage gave excellent growth of *Le. minor* and plant health was good as ascertained using chlorophyll fluorometry. Different adjustments were applied to the wastewater to optimize *Le. minor* biomass growth, including pH adjustment, addition of micro-nutrients and of potassium, calcium and magnesium. Dilutions of 25% and 50% of the “raw” fish wastewater were found to be optimal for duckweed cultivation. A small-scale duckweed treatment system revealed that *Le. minor* cultivation in fish deheading wastewater results in rapid nitrogen removal from the wastewater and considerable levels of total soluble protein in the biomass. This approach highlights duckweed’s potential as a biological agent, capable of capturing valuable compounds from food-industry wastewaters, reducing waste, and contributing to circular bioeconomy strategies for future food systems. A challenging and multifaceted question is whether duckweed grown on food-grade waste can be considered fit for human food consumption.

Keywords

duckweed · wastewater treatment · circular economy

References

DOI: 10.1016/j.envpol.2025.126761

NOTES

Exploring Duckweed for Remediation of, and Resource Recovery from Industrial Fish Filleting WasteWater

Jingrou Chen; Emily Mulvey; Marcel A.K. Jansen; Neil E. Coughlan

¹ School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland

Corresponding author: jingrou.chen@ucc.ie

Small, fast-accumulating, floating, aquatic macrophytes such as Lemnaceae (duckweeds) and *Azolla* have gained attention as an eco-friendly and cost-effective solution to be used for wastewater remediation due to their ability to remove nutrients and their potential for valorisation. Polycultures in agricultural systems have the potential to outperform monocultures through complementary resource use, niche partitioning, and enhanced stability, although their performance can be dependent on species identity, relative abundance, and environmental conditions. This study investigated the phytoremediation potential and growth performance of polyculture combinations of *Lemna minor*, *Lemna minuta*, and *Azolla filiculoides* (*Azolla*), grown on local meat processing wastewater, assessing how species composition and surface covers influenced biomass yield and nutrient removal efficiency. Results indicated that species interactions significantly impacted growth, with *L. minuta* generally facilitating higher *Azolla* growth rates than *L. minor* at laboratory scales. Owing to its greater tolerance of nutrient-rich wastewater, 20% surface cover of *L. minuta* combined with 40% surface cover of *A. filiculoides* emerged as an optimal configuration (community RGR: 0.065 ± 0.065 day⁻¹), achieving high growth performance with $38.13 \pm 0.93\%$ removal of total phosphorus. The transition to upscaled conditions showed less pronounced growth effects, highlighting the challenges of translating controlled laboratory findings to larger-scale applications and the need for further optimisation of system design and operational parameters. These findings demonstrate the potential of macrophyte polycultures for agri-food wastewater treatment, offering a sustainable approach to protecting aquatic ecosystems and creating additional value streams through biomass valorisation.

Keywords

Duckweed; *Azolla*; Polyculture; Plant growth

References

1. Muradov, N., Taha, M., Miranda, A.F., Kadali, K., Gujar, A., Rochfort, S., Stevenson, T., Ball, A.S. and Mouradov, A. (2014). Dual application of duckweed and azolla plants for wastewater treatment and renewable fuels and petrochemicals production. *Biotechnology for Biofuels*, 7(1). doi:10.1186/1754-6834-7-30.
2. Picasso VD, Brummer EC, Liebman M, Dixon PM, Wilsey BJ. (2011). Diverse perennial crop mixtures sustain higher productivity over time based on ecological complementarity. *Renewable Agriculture and Food Systems*. 26(4):317-327. doi:10.1017/S1742170511000135

NOTES

Group therapy: Macrophyte polyculture of Lemna species and Azolla filiculoides for the optimized remediation of meat processing wastewater

Gergő Koleszár¹; Prynces Lacdang¹; Viktor Oláh ²; Mihály Braun³; Sándor Szabó¹

¹ University of Nyiregyhaza

² Department of Botany, Faculty of Science and Technology, University of Debrecen

³ Hun-REN, Institute for Nuclear Research, Debrecen

Corresponding author: koleszar.gergo@nye.hu

Rare earth elements (REEs) are increasingly used in modern technologies, raising concerns about their release into aquatic environments and subsequent transfer through food webs. This study investigated the response of the floating macrophyte *Lemna gibba* to three ionic REEs (Ce, Nd, and Sm), focusing on phytotoxicity, uptake, and bioaccumulation. Plants were exposed to contaminated water for eight days, during which REE concentrations in the growth medium and plant biomass were monitored to establish a complete mass balance. The highest non-toxic concentrations were 0.8 mg L⁻¹ for Ce, 1.8 mg L⁻¹ for Nd, and 7.7 mg L⁻¹ for Sm, whereas 50% inhibition of relative growth rate occurred at 2.0, 2.4, and 13.3 mg L⁻¹, respectively. Elevated phosphate concentration markedly reduced the toxicity of all investigated REEs. Among the six physiological parameters evaluated, total chlorophyll content proved to be the most sensitive indicator of REE-induced stress. The removal efficiency of dissolved REEs reached 94–96%. However, only 30–55% of the initial REE load accumulated in plant tissues, while the remainder was attributed to precipitation. Bioconcentration factors were 460 for Ce, 774 for Nd, and 419 for Sm. Neodymium showed the highest accumulation (3579 mg kg⁻¹ dry biomass). These results demonstrate that *Lemna gibba* efficiently removes ionic REEs from contaminated water. Nevertheless, the considerable accumulation of REEs in plant tissues suggests a potential route for their transfer into aquatic food webs, highlighting the ecological risks associated with increasing environmental REE contamination.

Keywords

Bioaccumulation; Toxicity; Phytoremediation; Duckweed

References

Szabó, S., Zavanyi, G., Koleszár, G., Del Castillo, D., Oláh, V., Braun, M., 2023. Phytoremediation, recovery and toxic effects of ionic gadolinium using the free-floating plant *Lemna gibba*. *Journal of Hazardous Materials*, 458, 131930. <https://doi.org/10.1016/j.jhazmat.2023.131930>

NOTES

BIOACCUMULATION AND PHYTOTOXICITY OF IONIC RARE EARTH ELEMENTS (CERIUM, NEODYMIUM, SAMARIUM) IN *LEMNA GIBBA*

Umit Baris Kutman; Kubra Tutkun Akdeniz; Rifat Buğra Bildiri; Bahar Yildiz Kutman

Institute of Biotechnology, Gebze Technical University, Turkey

Corresponding author: bkutman@gtu.edu.tr

Agricultural drainage waters contain recoverable plant nutrients but may contribute to eutrophication when discharged untreated. This study evaluated *Lemna minor* for nutrient recovery from agricultural drainage water and, more importantly, investigated the unexplored potential of its dried biomass to bind boron (B) in soil and mitigate B toxicity in a crop plant. Duckweed was cultivated in drainage water from a soilless tomato greenhouse and a vertical farm, with nutrient solution as a reference. Nutrient removal and mineral accumulation were determined. The resulting dried biomass was assessed (i) in water, (ii) during a 90-day soil incubation, and (iii) in a cucumber seedling experiment under increasing B supply. *Lemna minor* grew successfully in both drainage waters and substantially depleted nitrate and phosphorus while accumulating macro- and micronutrients, including B. Incorporation of dried duckweed into soil at 0.5% (w/w) markedly reduced hot-water-extractable B, and this effect persisted throughout incubation. At soil-relevant pH, the biomass released B into B-free water but bound more than half of the B in B-enriched water, indicating bidirectional buffering capacity. Duckweed amendment increased cucumber shoot biomass across all B levels and prevented visible B-toxicity symptoms at the highest B treatment. Although the high B-accumulation capacity of living *Lemna* and the involvement of cell-wall apiogalacturonan are known, this is, to our knowledge, the first demonstration that mineral-rich, dried *Lemna* biomass retains B-binding functionality in soil and can mitigate B toxicity during crop growth. Thus, agricultural wastewater treatment and nutrient recovery generate a multifunctional soil amendment within a circular agricultural system.

Keywords

Boron; Cucumber: Organic amendment: Toxicity

NOTES

Dried Duckweed Biomass as a Boron-Binding Soil Amendment Produced through Nutrient Recovery from Agricultural Drainage Water

Ngosa Bwalya¹; Samuel Appelbaum²; Guy Ofer³; Nicola Trivellin⁴; Gideon Oron⁵

¹ Genius Watter Srl, Carmignano di Brenta, Padova, Italy

² French Associates Institute, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Kiryat Sde-Boker, Israel

³ Ramat Negev Desert Agro-Research Center (RN-DARC), Ramat Negev Works Ltd., D.N. Halutza, Israel

⁴ Department of Industrial Engineering, University of Padua, Padova, Italy

⁵ Zuckerberg Water Research Institute, Jacob Blaustein Institutes for Dessert Research, Ben-Gurion University of the Negev, Kiryat Sde-Boker, Israel

Corresponding author: gidi@bgu.ac.il

Desalination brine, typically considered a disposal challenge, is evaluated through a resource recovery model that converts the concentrate into a valuable input for food production. A sequential system consisting of duckweed cultivation, fish production, and final irrigation of salt-tolerant halophytes is developed. Duckweed (*Lemna gibba*) serves as a key component by assimilating nutrients from fish influents and enhancing biomass production. It can achieve yields of approximately 10 g/m²/day (dry weight), with edible protein content of about 35%. The produced duckweed is primarily utilized as feed for herbivorous fish (grass carp), while contributing to the overall food production chain. The remaining saline brine is applied for cultivating halophyte crops (*Atriplex lentiformis*; *Salicornia bigelovii*). The model integrates the serial relationship between duckweed, fish, and halophyte, like agricultural practices implemented in Ramat Negev Regional Council, where groundwater salinity is approximately 4.4 dS/m. This similarity demonstrates the feasibility of utilizing saline resources for productive applications. For a small reverse osmosis desalination facility producing 50 m³/day of desalinated water and 27 m³/day of concentrate with an electrical conductivity of 20.31 mS/cm, approximately 0.36 hectares are required for brine utilization. The system can generate an estimated 10.3 tons of forage annually, supporting approximately 37 goats or sheep, 11 camels, or 5 cattle. The approach is scalable according to brine availability and land capacity. Although the direct economic value of the crops is relatively low (approximately €467/ha/year), the primary benefit is the conversion of desalination by-products into livestock feed resources while reducing risks.

Keywords

Duckweed; Fish; Halophyte; Desalination; Food

NOTES

Integrating Duckweed into a Chain of Brine Reuse Enhancing the Value of Desalination for Food Production: The Case of Somalia

K. Sowjanya Sree

School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi-221005, India

Corresponding author: kssree9@bhu.ac.in

Duckweeds (Water lentils) are progressing towards their incorporation into the Planetary Health Diet. Several studies have focused on analyzing the quality and quantity of the wide spectrum of nutritional components in these tiny flowering plants. Based on these investigations, duckweeds exhibit excellent nutritional profile suitable for human nutrition. However, the bioavailability of these nutrients is dependent on several factors, one of them being the content of antinutritive compounds like Phytate and Oxalate.

Phytate, inositol hexakisphosphate, is the main storage form of Phosphorus in plants. When consumed by humans, they can lower the absorption of some of the minerals like Zinc, Magnesium, Calcium and so on, because of their strong affinity towards these mineral ions. Oxalates play diverse roles in plants, such as in calcium homeostasis, detoxification of heavy metals, pH regulation and so on. They can be found in plants as soluble or insoluble salts. When consumed by humans through plant-rich diet, they can interfere with the bioavailability of minerals and may also show potential nephrotoxicity.

Although consumed in sufficient amounts, the extent of bioavailability of the minerals is key to assessing mineral malnutrition. Here, the role of antinutritive compounds can be crucial. Our current understanding and the quantities of oxalate, phytate and the minerals in different duckweed species will be discussed.

Acknowledgements: KSS acknowledges the Anusandhan National Research Foundation (ANRF), India, for the SERB POWER grant (grant no. SPG/2022/002036; dated 31 October 2023) and Banaras Hindu University, India for the IoE-SEED Grant (grant no. R/Dev/D/IoE/SEED GRANT (Phase-VI)/2024-25/83259; dated 21 January 2025).

Keywords

Phytates; Antinutritives; Oxalate

NOTES

Antinutritives in Duckweeds

Olena Kishchenko¹; Anton Peterson¹; Mikolaj Adamek²; Verena Jung Schroers²; Veronika Piackova³; Tomas Korytar⁴; Anatoli Giritch⁵; Ingo Schubert⁶; Manuela Nagel⁶

¹ Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Germany & Institute of Cell Biology and Genetic Engineering, National Academy of Science of Ukraine; ² Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Germany; ³ University of South Bohemia in Ceske Budejovice Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic; ⁴ Laboratory of Fish Immunology, Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic; ⁵ Nomad Bioscience GmbH, Germany; ⁶ Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Germany

Corresponding author: kishchenko@ipk-gatersleben.de

Vaccination is a cornerstone of modern health management. It's particularly important in intensive animal production systems, such as aquaculture, where high stocking densities facilitate the rapid spread of pathogens. Oral vaccines offer a stress-free, scalable alternative to traditional fish injections. Our study explores duckweed as a platform for vaccine production and as a vehicle for oral administration. Using a deconstructed potato virus X-based transient expression system [1], recombinant proteins analogous of antigens of koi herpesvirus (KHV) and carp edema virus (CEV) were expressed in duckweed. Freeze-drying was shown to preserve the recombinant antigens in the duckweed biomass, enabling long-term storage at room temperature. Obtained freeze-drying duckweed biomass expressing KHV antigens was used for carp immunization via intraperitoneal injection and intubation [2]. Both methods of administration induced release of the specific KHV-neutralising antibodies. Such data provide a strong support for the efficacy of duckweed as a producer of oral vaccines. Transient expression of recombinant proteins in duckweed can facilitate and accelerate the development of oral vaccines for animals.

Keywords

Transient transgene expression; Oral vaccines

References

- [1] Peterson, A., Kishchenko, O., Zhou, Y., Vasylenko, M., Giritch, A., Sun, J., et al. (2021). Robust Agrobacterium-mediated transient expression in two duckweed species (Lemnaceae) directed by non-replicating, replicating, and cell-to-cell spreading vectors. *Front. Bioeng. Biotechnol.* 9, 761073.
- [2] Kishchenko, O., Peterson, A., Adamek, M., Schroers, V. J., Piačková, V., Korytář, T., et al. (2026). Fish immunization by duckweed biomass accumulating recombinant cyprinid herpesvirus 3 antigens induces specific immune response. *New Biotechnol.* 94, 272–282.

NOTES

Duckweed as a platform for oral vaccines in sustainable fish aquaculture

Ron Vunsh¹; Maja Cohen²; Manpreet Kaur¹; Aviva Samach¹; Avraham Levy¹; Marvin Edelman¹

¹ Weizmann Institute of Sciences

² Averma Therapeutics

Corresponding author: lpron@weizmann.ac.il

Newcastle virus disease (ND) in poultry is highly contagious. Weak strains cause loss in egg production, virulent strains kill the birds¹. NDV virions possess an envelop and contain a single molecule of linear, negative sense RNA coding for at least six proteins. The important proteins for subunit vaccines are two glycoproteins: the hemagglutinin-neuraminidase (HN), and the fusion protein (F), which are used as antigens for immunization. Immunization is currently by injection, nasal spray, ocular or drinking water that contain the HN antigen. Duckweeds are small, edible monocot plants that float on the surface of fresh water and need only light, air and some common minerals for rapid growth. Plants of these genera are preferred food as indicated by their name. Transgenic edible plants can deliver vaccines to different animals². We introduced HN and the F protein genes into a duckweed species to deliver these antigens for induction of protective immune response in chickens. We get high expression of both antigens in the transgenic plants, which grow normally with no apparent phenotypic change.

Keywords

NDV; Duckweed; Vaccine; Chicken

References

1 Moller, L. et al. (2018) Preventive Veterinary Medicine, 152, 41-48. 2 Shaaltiel, Y. Et al. (2007) Plant Biotechnol. J. 5, 579-590

NOTES

Towards a subunit oral vaccine to Newcastle disease in transgenic duckweeds

Julia Eva Fortmueller; Naama Zioni; Ron Vunsh; Priel Siman-Tov; Asaph Aharoni

Weizmann Institute of Science

Corresponding author: julia-eva.titz@weizmann.ac.il

Silk sericin, a glycoprotein produced by *Bombyx mori*, naturally coats and binds silk fibroin fibers during cocoon formation. Beyond this role, sericin's biocompatibility, low immunogenicity, and bioactive properties, including antioxidant and adhesive effects, have made it attractive for a range of biomedical and biomaterial applications. However, conventional degumming methods are harsh, reduce protein purity, and are constrained by low yield and poor reproducibility between batches. To overcome these limitations, we explored duckweed (*Lemna japonica*), a fast-growing, readily transformable aquatic plant, as an alternative production platform. We generated stable transgenic duckweed lines expressing silk sericin, representing one of the first efforts to establish duckweed as a platform for recombinant silk-derived protein expression, and characterized expression levels and tissue-specific accumulation. Soluble sericin was recovered from plant extracts via heat-treatment method. We then evaluated the functional bioactivity of duckweed-derived sericin using an established mammalian cell model, benchmarking its performance against commercially available sericin under standardized conditions. Quantitative and morphological assessments confirmed that duckweed-derived sericin significantly enhanced the assessed functional endpoints, outperforming commercial sericin. This work establishes proof-of-concept for duckweed as a scalable, plant-based platform for producing functional recombinant sericin via a mild extraction route, offering a sustainable, cost-effective alternative to conventional methods with broader implications for recombinant protein production in plants and downstream biomaterial applications.

Keywords

Sericin; Lemna; Recombinant expression; Biomaterial

References

Silk sericin — properties and biomedical relevance Lamboni, L., et al. Silk Sericin: A Promising Sustainable Biomaterial for Biomedical and Pharmaceutical Applications. PMC (2022). Covers sericin's biocompatibility, low immunogenicity, antioxidant/anti-inflammatory activity, and conventional degumming-based extraction limitations. <https://pmc.ncbi.nlm.nih.gov/articles/PMC9699483/>

NOTES

Recombinant Expression of Silk Sericin in Duckweed (*Lemna japonica*) as a Sustainable Platform for Functional Biomaterial Production

Anne Lincoln; Anthony Bishopp

Plant and Crop Sciences, School of Biosciences, University of Nottingham

Corresponding author: anne.lincoln@nottingham.ac.uk

This poster centres around exploring the flowering response potential of *Lemna minor*, a species not known to be able to flower in laboratory conditions. We use phylogenetic assays to identify potential regulators of flowering in *L. minor* to better understand the potential mechanism behind flowering in this species, and use modern molecular biology to identify the regulation of these elements in conditions designed to induce flowering. We also explore the function of orthologous components in *Lemna aequinoctialis*, a species of duckweed known for its capacity to flower to better understand the mechanism present, and potentially relate its functionality to other *Lemna* species.

Keywords

Flowering; FT; *Lemna minor*; *L. aequinoctialis*

References

Yoshida A, Taoka K, Hosaka A, Tanaka K, Kobayashi H, Muranaka T, Toyooka K, Oyama T and Tsuji H (2021) Characterization of Frond and Flower Development and Identification of FT and FD Genes From Duckweed *Lemna aequinoctialis* Nd. *Front. Plant Sci.* 12:697206. doi: 10.3389/fpls.2021.697206 Kumiko Miwa, Masayuki Serikawa, Sayaka Suzuki, Takao Kondo, Tokitaka Oyama, Conserved Expression Profiles of Circadian Clock-related Genes in Two *Lemna* Species Showing Long-day and Short-day Photoperiodic Flowering Responses, *Plant and Cell Physiology*, Volume 47, Issue 5, May 2006, Pages 601–612, <https://doi.org/10.1093/pcp/pcj027>,

Jingrou Chen¹; Matthew Holland²; Marcel A.K. Jansen²; Neil E. Coughlan²

¹ University College Cork, Ireland

² School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland; Sustainable Institute, University College Cork, Cork, Ireland

Corresponding author: jingrou.chen@ucc.ie

Lemnaceae (duckweeds) and *Azolla*, are floating aquatic plants, that exhibit rapid growth in nutrient-rich waters and have considerable potential for biomass production using wastewater derived nutrients. This study evaluated *Lemna* species and *Azolla filiculoides* (*Azolla*) growth performance on industrial meat processing effluent under controlled laboratory conditions. Growth effects of agitations (0, 30, 60, 90, 120 rpm) and surface covers (10, 20, 40, 80%) were investigated over 7 days (Exp.1), followed by continuous cultivation of *Azolla* under different hydraulic retention times (HRT; 3, 5, 7 days) at 10% surface cover for 15 days (Exp.2). *Lemna minor* and *Lemna minuta* exhibited great growth at 60 rpm, achieving RGRs of $0.290 \pm 11.34 \text{ day}^{-1}$ and $0.293 \pm 2.23 \text{ day}^{-1}$, respectively. *Azolla* cultivated at lower-moderate surface covers at 60 rpm achieved the highest RGR (20%: $0.10 \pm 0.006 \text{ day}^{-1}$). For Exp.2, 3-day HRT supported greater growth (RGR: $0.067 \pm 0.002 \text{ day}^{-1}$) than 5- and 7-day HRTs for 9 days. Findings provide insights into the development and optimisation of floating macrophyte-based phytoremediation systems and plant biomass generation.

Keywords

Agitation; Hydraulic retention; Plant growth

References

1. Redmond, C., O'Mahoney, R., Blanchard, M., and Coughlan, N. E. (2025). Varied Growth Media Necessitate Different Light Regimes for Indoor Duckweed Cultivation. *Plants*, 14(3), 397. <https://doi.org/10.3390/plants14030397>.
2. Chen, J., Ring, L.P., Jansen, M.A.K., and Coughlan, N.E. (2026). Thick and thin: high plant abundance but low surface cover enhances growth and remediation by floating *Azolla filiculoides* following wastewater acidification. *Water Resources and Industry*, 35, 100355. <https://doi.org/10.1016/j.wri.2026.100355>.

Alexej Sonnenfeld; Erwin Berchtold

University of Rostock

Corresponding author: alexej.sonnenfeld@uni-rostock.de

The present study evaluated the suitability of recirculating aquaculture system (RAS) process water from African catfish (*Clarias gariepinus*) for cultivating *Lemna minor* (9440) in a greenhouse system. An indoor vertical farming (IVF) system for decoupled aquaponics was developed and implemented. Cultivation in process water had no significant effect on frond area or frond weight, while root length increased significantly. Nutrient and amino acid composition remained within ranges reported in the literature, with protein contents exceeding 32% in all treatments. Biomass productivity reached 3.0 g DW/m²/d in the control and 2.6 g DW/m²/d in both process water treatments. These results indicate that RAS process water is a suitable cultivation medium for *L. minor* (9440) without compromising biomass quality.

Keywords

Aquaponics; IVF; RAS; *Lemna*; catfish

References

Petersen, F., Demann, J., von Salzen, J., Olfs, H.-W., Westendarp, H., Wolf, P., Appenroth, K.-J., & Ulbrich, A. (2022a). Re-circulating indoor vertical farm: Technicalities of an automated duckweed biomass production system and protein feed product quality evaluation. *Journal of Cleaner Production*, 380. <https://doi.org/10.1016/j.jclepro.2022.134894> Palm, H. W., Seidemann, R., Wehofsky, S., & Knaus, U (2014a). Significant factors affecting the economic sustainability of closed aquaponic systems. Part I: System design, chemo-physical parameters and general aspects. *Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux Society*, 7(1), 20–32. Molnár, P., Bereczki, C., Bényi, B. C., Bársony, P., & Fehér, M. (2025). The use of Common duckweed (*Lemna minor*) as a biological filter in the rearing of hybrid African catfish (*Clarias gariepinus* x *Heterobranchus longifilis*). *Acta Agraria Debreceniensis*(1), 107–112. <https://doi.org/10.34101/actaagrar/1/15457>

Felipe Alves¹; Miguel A. Prieto²; Manuel Feliciano¹; Marcio Carocho¹

¹ Bragança Polytechnic University

² University of Vigo

Corresponding author: mcarocho@ipb.pt

The growing demand for sustainable food has driven the development of alternative protein sources with a lower environmental impact. *Lemna minor* emerges as a promising alternative due to its high protein content, essential amino acid rich profile, rapid growth, and high adaptability to adverse environmental conditions. The goal of this project is to explore this species as a sustainable source of protein for the food industry. To this end, protocols will be established to characterize the nutritional, chemical, and bioactive profile of the produced biomass; compare and optimize the growth of *L. minor* in two culture media (Hoagland and Steinberg); assessing the effect of factors such as temperature, light intensity, and photoperiod on biomass production, protein content, and antioxidant activity; developing sustainable methods for protein extraction and isolation; and incorporating the obtained proteins into plant-based food products. The proposal aims to contribute to the development of innovative, environmentally sustainable protein ingredients by promoting the utilization of high-productivity, low-impact aquatic resources. The proposed project involves multidisciplinary research groups (IPB/PT, UVIGO/ES, MORE/PT) with diverse expertise in the food and environmental sectors. The objectives described are in line with the EU Strategy for the 2030 Agenda for Sustainable Development and aim to address food security issues and promote circular food systems in the region.

Acknowledgements to FCT/MCTES(PIDDAC): CIMO, UIDB/00690/2020 (DOI:10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI:10.54499/LA/P/0007/2020). Also to FCT for institutional scientific employment program-contracts of S. A. Heleno and M. Carocho. F. Macedo also thanks FCT for his PhD grant (2023.04962.BD).

Keywords

Vegetable protein; Food science

Maria Adelaide Iannelli¹; Marco Fabriani¹; Iori Valentina¹; Giovanni Sciarretta¹; Laura Morello²; Luca Braglia²; Yuri Lee²; Cristian Perna²; Giovanna Frugis¹

¹ Istituto di Biologia e Biotecnologia Agraria (IBBA-CNR) - Montelibretti

² Istituto di Biologia e Biotecnologia Agraria (IBBA-CNR) - Milano

Corresponding author: mariaadelaide.iannelli@cnr.it

Duckweeds (Lemnaceae) are the smallest flowering plants and reproduce predominantly through vegetative propagation, while flowering occurs only rarely under natural and laboratory conditions, limiting breeding and germplasm conservation. Although salicylic acid (SA) efficiently induces flowering in several duckweed species, the molecular mechanisms regulating floral transition remain largely unknown. Following the identification of the Mediterranean hybrid *Lemna × mediterranea* (*L. minor* × *L. gibba*; Braglia et al., 2024), we investigated SA-induced flowering competence in parental (*L. minor* and *L. gibba*) and hybrid *L. × mediterranea* clones. Plants were grown in natural mineral water supplemented with 20 µM SA, and flowering frequency, vegetative growth, and developmental traits were quantified. To identify molecular determinants associated with flowering competence, the expression of key components of the florigen activation complex such as FLOWERING LOCUS T (FT) protein and transcription factor FD protein, was compared among the different genotypes. To further dissect the molecular basis of floral induction, *L. minor* strain 5500 was selected for transcriptomic analysis. RNA sequencing of fronds collected at key developmental stages following SA treatment was used to characterize temporal transcriptional responses. This integrated phenotypic and transcriptomic approach provides new insights into genotype-dependent flowering competence and SA-mediated floral transition in duckweeds, advancing our understanding of reproductive development in *L. minor* and *L. × mediterranea* and providing a foundation for improved flowering induction, breeding, and germplasm conservation.

Keywords

Lemna; Flowering; Salicylic-acid; Phenotypic analysis

References

Braglia et al., 2024. Characterization of the cryptic interspecific hybrid *Lemna×mediterranea* by an integrated approach provides new insights into duckweed diversity, *Journal of Experimental Botany* <https://doi.org/10.1093/jxb/erae059>

Dalia Rishmawi Avraham Levy¹; François Prudot D'Avigny^{1,2}; Lusine Ghazaryan¹; Osnat Gillor¹; Rhishika Dutta; Romain Bernard²

¹ Ben Gurion University of the Negev

² French National Institute for Agriculture, Food, and Environment (INRAe)

Corresponding author: daliarishmawi@gmail.com

Vitamin B₁₂ (cobalamin) is essential for animal and human metabolism, while plants neither synthesize nor require it. Yet, aquatic plants such as duckweeds can accumulate bioavailable B₁₂ synthesized by their endophytic microbiome. We hypothesized that cobalt, the central metal ion in cobalamin would enhance B₁₂ accumulation by altering endophytic community structure and function. To test our hypothesis, we cultivated the rooted duckweed species *Lemna minor* and *Spirodela polyrrhiza* and the rootless species *Wolffia globosa* across a cobalt gradient, ranging from 0 to 1 μ M (just below the reported toxicity threshold). We quantified growth and bioavailable B₁₂, profiled the corresponding endophytic bacterial communities and examined B₁₂ partitioning between roots and fronds. Responses to cobalt were species-specific. In *L. minor*, B₁₂ increased by up to fivefold without affecting growth rate. Cobalt explained 54% of the variation in bacterial community composition and significantly altered the abundance of *Novosphingobium* and *Roseomonas*. In *S. polyrrhiza*, B₁₂ increased by up to threefold, while cobalt explaining 38% of the variation in community composition and significantly influencing alpha diversity and overall community structure. In *W. globosa*, B₁₂ increased significantly at the highest cobalt concentration without detectable changes in community composition, suggesting regulation through microbial activity rather than taxonomic replacement. B₁₂ localization was also species-specific, with significantly greater accumulation in the roots of *S. polyrrhiza* than in those of *L. minor*. These findings demonstrate that cobalt enhances microbiome derived B₁₂ accumulation in duckweed and provides a practical strategy for improving the nutritional value of duckweed biomass.

Keywords

Cobalamin; Endophytic microbiome; Cobalt supplementation

Laura Diezma-Navas; Filipp Krasnovid; Verónica Barragán-Borrero; Christian Parteli; Rana Elias; Arturo Marí-Ordóñez

Gregor Mendel Institute

Corresponding author: laura.diezma@gmi.oeaw.ac.at

In eukaryotes, post-transcriptional gene silencing (PTGS) involves the production of small interfering RNAs (siRNAs) and their incorporation into ARGONAUTE (AGO) proteins. The resulting siRNA-AGO complex mediates mRNA cleavage and/or translational repression, leading to degradation via RNA decay pathways and ultimately resulting in reduced protein expression. In plants, mRNA cleavage is believed to be the main mode of action of siRNAAGO complexes, and although translational repression has been shown, it is not yet well understood. Interestingly, in duckweeds, a group of small aquatic flowering plants, siRNA-mediated transgene silencing causes strong loss of protein synthesis, while mRNA levels remain largely unchanged. On the contrary, loss of siRNA results in resoration of protein levels. This observation suggests that translational repression, rather than mRNA degradation, may be a predominant mechanism of gene regulation in this group of plants. To investigate this hypothesis, we are exploiting transgene silencing in duckweeds to analyze the fate and turnover of mRNAs, targeted or not by siRNAs, using a combination of polysome profiling, SLAM-seq, and ribosome footprinting. Understanding silencing mechanisms in duckweeds will expand the knowledge of silencing mechanisms, mostly investigated in a handful of plant models, and potentially provide further insights into their translational repression mechanisms.

Keywords

Silencing; PTGS; TGS

References

[bioRxiv:2026.02.27.708460](https://doi.org/10.1101/2026.02.27.708460). [bioRxiv:2025.08.28.672806](https://doi.org/10.1101/2025.08.28.672806). [bioRxiv: 2024.04.03.587901](https://doi.org/10.1101/2024.04.03.587901).

Samuel Appelbaum¹; Ofel Guy; Ngosa Bwalya²; Nicola Trivellin³; Gideon Oron⁴

¹ French Associates Institute, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Kiryat Sde-Boker, Israel

² Genius Watter Srl, Carmignano di Brenta, Padova, 35010, Italy

³ Department of Industrial Engineering, University of Padua, Padova, Italy

⁴ Zuckerberg Water Research Institute, Jacob Blaustein Institutes for Dessert Research, Ben-Gurion University of the Negev, Kiryat Sde-Boker, Israel; The Department of Industrial Engineering and Management, Ben-Gurion University of the Negev, Beer-Sheva 8410500, Israel

Corresponding author: gidi@bgu.ac.il

Desalination brine is increasingly recognized as a potential resource rather than a waste stream requiring disposal. This study presents a modelling strategy for sequentially reusing desalination concentrate for food production, fish culture (food) and irrigation of salt-tolerant halophytes crops, including *Atriplex lentiformis* and *Salicornia bigelovii*. The proposed system follows a serial structure in which brine is progressively utilized through duckweed cultivation, fish production, and halophyte irrigation. The halophyte component is particularly relevant to the Ramat Negev region, where groundwater salinity is approximately 4.4 dS/m, demonstrating the feasibility of saline-water agriculture under local conditions. *S. bigelovii* plant is emphasized because of its high productivity, nutritional value, livestock acceptability, and broad salinity tolerance. For a small reverse-osmosis plant processing $50 \text{ m}^3 \text{ d}^{-1}$ and generating $27 \text{ m}^3 \text{ d}^{-1}$ of concentrate with an electrical conductivity of 20.3 mS/cm, the model estimates that 0.36 ha of cultivated land would be required to utilize the entire concentrate stream. The system could produce approximately 10.3 metric tons of forage annually, potentially supporting 37 goats or sheep, 11 camels, or 5 cattle. Although the estimated crop value is modest (approximately €467/(ha*yr), the principal benefit is resource recovery: converting desalination brine into livestock feed while reducing concentrate disposal. The approach is scalable according to brine availability, land resources, and livestock demand.

Keywords

Duckweed; Fish; Desalinated-brine; Reuse; Food

Madison McGregor; Caroline Henry; Nicole Burkett; Kevin Cox

Washington University in St Louis, Donald Danforth Plant Science Center

Corresponding author: chenry@danforthcenter.org

In the face of climate change and the demand for more sustainable alternatives to industrial operations, duckweed plants are utilized across a diverse set of applications – from wastewater treatment, to feedstock production, to bioenergy and bioproduct generation. The *Wolffia* genus is the smallest and fastest-growing known angiosperm, making it of particular interest for duckweed research, as well as plant biology more broadly. With these plants becoming more relevant under an increasingly volatile climate, it is crucial to understand stress susceptibility of these plants. Here we examine early salt stress response in *Wolffia australiana* plants over time and under different degrees of salt stress. We examined the phenotypic effects of different salt concentrations (0mM, 50mM, 100mM, 150mM) on growth rate and chlorophyll content. All concentrations of salt stress resulted in decreased growth rate compared to the 0mM control, as well as a persistent increase in chlorophyll content. Given that 100mM produced the clearest stress phenotype without being lethal to the plants, we assessed transcriptomic response at this concentration via bulk RNA-sequencing over a 48-hour timecourse, which identified specific genes, gene families, and functional pathways involved in response to salt stress. Genes upregulated under stress tended to be enriched in metabolic processes and stress response, whereas downregulated genes were enriched in reproductive and biosynthetic processes. This study will provide important insights into the mechanisms of salt stress response in *Wolffia*, and how they differ from other plants.

Keywords

Abiotic stress; Salt; Transcriptomics; Chlorophyll

Karnjana Ruenpham¹; Mori Kazuhiro²; Tadashi Toyama²

¹ Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi

² Graduate Faculty of Interdisciplinary Research, University of Yamanashi, Japan

Corresponding author: k.ruenpham@gmail.com

Duckweeds (Lemnaceae) are rapidly growing aquatic plants with potential as sustainable feedstocks for human nutrition, animal feed, biofuel production, and wastewater bioremediation. In natural environments, duckweed coexists with diverse microbial communities, forming a complex host-microbiome assemblage known as a holobiont. Within this assemblage, duckweed-associated plant growth-promoting bacteria (PGPB) play a critical role in enhancing duckweed growth through direct and indirect mechanisms. However, the specific mechanisms driving strain-specific growth promotion remain largely uncharacterized. Therefore, this study aimed to elucidate these mechanisms by comparing the effects of two PGPB with distinct colonization strategies—*Terrimicrobium* sp. PS02 and *Aeromicrobium* sp. PS05—on the growth, biochemical composition, and transcriptomic profile of the giant duckweed, *Spirodela polyrhiza*. Under SEM observation, strain PS02 attached to the duckweed surface through the aggregation of 1–3 cells, while strain PS05 formed filamentous extracellular polymeric substance (EPS)-mediated microcolonies. These distinct colonization strategies aligned with bacterial cell density, where PS05 established approximately one order of magnitude higher bacterial populations than PS02. Both PGPB strains enhanced duckweed biomass, yielding 1.2- and 1.4-fold increases, respectively. Markedly, both strains enhanced starch accumulation and turion formation without reducing protein or photosynthetic pigment content. Transcriptomic profiles demonstrated that PS05 regulated carbohydrate biosynthesis and carbon allocation. This study provides integrated evidence linking bacterial colonization strategy, biomass production, and host transcriptomic regulation, allowing us to better understand the mechanisms required for engineering high-performance duckweed–microbiome systems for sustainable biomass production.

Keywords

Plant-growth promoting bacteria; *Spirodela polyrhiza*

Clair Margot; Marcel Jansen

University College Cork, Ireland

Corresponding author: cmargot@ucc.ie

Integrated Multi-Trophic Aquaculture is an aquaculture system in which extractive species (algae, aquatic plants, bivalves, etc.) recycle the waste produced by fed species (fish, shrimp, etc.). By integrating organisms from different trophic levels, IMTA reduces the potential environmental impacts of aquaculture and improves the sustainability and economic value of production systems. The CIRCULAR-IMTA-DEMO pilot site at Mount Lucas, County Offaly, Ireland, comprises an IMTA farm producing 30 tonnes of rainbow trout in four split-ponds. Fish effluents are directed into 16 channels (12,800 m²) where duckweed is cultivated. Duckweed removes nutrients, improving water quality for recirculation while producing valuable biomass. The main duckweed species are *Lemna minor* “Blarney” and *Lemna gibba* “Keywater”, together with a spontaneous hybrid. Studies conducted in 2019 and 2020 estimated a production potential of 35–48 t yr⁻¹ of dry duckweed biomass (Paolacci et al., 2022). However, productivity remains dependent on environmental conditions, including fish density and feeding regime, which determine waste production. Following six years of inactivity, the site has restarted and has undergone structural changes. Vegetation has expanded within and around the channels, providing shelter and shade. However, the invasive aquatic plant *Elodea nuttallii* remains a problem, competing for nutrients. A diverse fauna, including swans, herons, amphibians and insects, has become established. Swans consume large amounts of duckweed, prompting the construction of exclusion zones. In addition, heatwave events in July 2026 caused changes in water physicochemical properties, affecting trout and duckweed production. Our work aims to identify management strategies to maintain duckweed growth under varying conditions

Keywords

IMTA; Duckweed; Nutrient recycling; Circular economy

References

Paolacci, S., Stejskal, V., Toner, D., & Jansen, M. A. (2022). Wastewater valorisation in an integrated multitrophic aquaculture system; assessing nutrient removal and biomass production by duckweed species. *Environmental Pollution*, 302, 119059.

Abhisek Chakraborty¹; Todd P. Michael²; Shuqing Xu¹

¹ Johannes Gutenberg University of Mainz, Germany

² Salk Institute for Biological Studies, 10010 N Torrey Pines Rd, La Jolla, CA 92037

Corresponding author: achakrab@uni-mainz.de

Evolution does not always lead to increased complexity, and in plants, the loss of floral organs is a recurring phenomenon across different lineages. However, the mechanisms behind this simplification remain poorly understood. To investigate the evolutionary processes leading to floral organ loss, duckweeds, especially *Wolffia microscopica*, provide an ideal system because of the smallest flowers with the simplest morphology – with a single stamen and a unicarpellate gynoecium, which are even simpler than the model species *Arabidopsis thaliana*, yet with essential genetic makeups. Therefore, by integrating single-nucleus multi-omics with comparative genomics, we ask the specific questions: i) which floral cell-types remain in this smallest flowering plant? and ii) which cell-types and genes are showing evolutionary signatures? We constructed a haplotype-resolved genome assembly of *W. microscopica* with a total size of 666.5 Mb, consisting of 20 chromosomes and 12,623 protein-coding genes. We performed single-nucleus RNA-Seq and single-nucleus ATAC-Seq (10,720 nuclei) to identify all cell-types, including the male and female floral cell-types. We identified the marker genes and the transcription factor-binding motifs enriched in the floral cell-types, which could be responsible for establishing and maintaining the floral cell-type identities. Further, we performed natural selection and gene family evolution analyses to understand the evolutionary mechanisms underlying the development of the world's simplest flower. The detailed single-nucleus multi-omics and evolutionary insights aid in uncovering the fundamental genetic mechanisms and regulatory networks responsible for the development of the simplest flowers, which likely represent the minimal controls of sexual reproduction in plants.

Keywords

Genome; Single-nucleus sequencing; Simplest flower

References

1. Siadjeu, C., Chakraborty, A., & Xu, S. Duckweeds as a model for minimal floral development. *Trends in Genetics* 42, 555-564 (2026). 2. Sree, K. S., Maheshwari, S. C., Boka, K., Khurana, J. P., Keresztes, Á., & Appenroth, K. J. The duckweed *Wolffia microscopica*: A unique aquatic monocot. *Flora* 210, 31-39 (2015).

Martina Lombardi¹; Jacopo Troisi¹; Andrea Viggiano¹; Antonino Testa²; Antonietta Santoro¹

¹ University of Salerno, Dept. Medicine, Surgery and Dentistry; "Scuola Medica Salernitana"

² University of Campania "Luigi Vanvitelli", Dept Environmental, Biological and Pharmaceutical Sciences and Technologies

Corresponding author: marlombardi@unisa.it, aviggiano@unisa.it

Polylactic acid (PLA) has gained considerable industrial relevance, often considered an environmentally preferable material, but it can actually undergo fragmentation, generating micro- and nano-plastics (MPs/NPs), whose biological consequences are still insufficiently characterized. In the present study, we used an untargeted metabolomics approach to investigate the in vivo metabolic alterations associated with chronic oral exposure to PLA-NPs and to determine whether supplementation with *Lemna minor* (LM) could modulate the metabolic response to PLA-NPs. Wistar rats were randomly allocated to four experimental groups: untreated controls (CTR), oral PLA-NPs exposure (0.05 mg/ml in drinking water), dietary LM supplementation (20g/day), and combined PLA-NP and LM treatment. Fecal samples were collected at baseline (T0) and after 14 (T1) and 21 days (T2) of treatment and analyzed by liquid chromatography-mass spectrometry (LC-MS) using a high-resolution Orbitrap mass spectrometer. PLA-NPs exposure induced a broad metabolic rearrangement, mainly involving pathways related to bile acid homeostasis, energy regulation and microbial metabolism. Co-supplementation with LM partially counteracted the metabolic alterations induced by PLA-NPs. At T1, LM shifted bile acids, branched-chain fatty acids, and acylcarnitines toward CTR levels, suggesting modulation of bile acid homeostasis, microbial fermentation, and lipid utilization. At T2, partial normalization extended to metabolites involved in carbohydrate, lipid, and nucleotide metabolism. Although residual differences from untreated CTR persisted, these data support a partial rescue of the PLA-NP-associated metabolic phenotype. Collectively, our findings demonstrate that PLA-NPs can induce profound disturbances in metabolic homeostasis and provide a strong rationale for further investigating LM as a potential nutritional countermeasure.

Keywords

Polylactic acid; Nanoplastics; Dietary supplements

Anna Kolehmainen¹; Shuqing Xu ²

¹ Institute of Organismic and Molecular Evolution (iomE), Johannes Gutenberg University Mainz

² University of Mainz

Corresponding author: anna.kolehmainen@uni-mainz.de

Duckweeds possess some of the most highly reduced flowers among angiosperms; however, their sexual reproduction is poorly understood. Although flowering can be induced in multiple duckweed species under controlled conditions, successful seed set remains limited. A better understanding of pollination is required to establish controlled crossing systems, while reliable seed production is essential for the broader application of duckweeds in genetic studies. Here, we characterize the progression of flowering, pollination, and seed development in *L. aequinoctialis* (genotype KSS014), a duckweed species in which we can reliably produce seeds. We define distinct developmental stages from floral initiation to mature seed formation and establish their temporal progression. Under an 8-hour photoperiod, flowering is initiated 7–10 days after induction, after which an individual flower completes development within 2–3 days. Flower maturation proceeds through the simultaneous emergence of the pistil and first anther, followed by asynchronous emergence of the second anther, formation of a stigmatic liquid droplet associated with fertilization, and the onset of seed development. Seed maturation is completed approximately 10 days after fertilization. Based on these developmental stages, we propose the optimal timeframe for crossing in *L. aequinoctialis*. Together, these findings provide a practical framework for controlled fertilization and seed production, facilitating the use of duckweeds in genetic engineering and breeding applications.

Keywords

Flowering; pollination; Seed development; Crossing

*Martin Schäfer*¹; *Sara Nouere*¹; *Antonino Malacrinò*²; *Martin Lohr*³; *Christoph Vorburger*⁴; *Shuqing Xu*¹

¹ Institute of Organismic and Molecular Evolution (iomE), Johannes Gutenberg University Mainz

² Department of Biological Sciences, Clemson University, USA

³ Institute for Molecular Physiology, Johannes Gutenberg University Mainz

⁴ Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology (EAWAG)

Corresponding author: mschaefer@uni-mainz.de

Predicting the outcome of species interactions in complex communities requires separating the direct effects that organisms have on each other from those that function indirectly through their influence on other community members and the environment. We performed a two-year outdoor experiment with 14 mesocosms containing the giant duckweed, *Spirodela polyrhiza*, in the presence or absence of its aquatic snail herbivore, *Lymnaea stagnalis*. We analyzed the snail effects on the aquatic community and the evolutionary consequences for the duckweed. The direct and indirect effects of snails turned out to be often antagonistic. In the first year, snail herbivory reduced the duckweed abundance by direct grazing but also promoted duckweed growth indirectly by reducing algae competition and increasing phosphate availability, thereby shifting plant traits and genotype frequencies. In the second year, the ponds experienced an algae bloom and strong phosphate depletion which abolished and reversed various effects and trait-fitness associations observed in the previous year. Over the two years, the direct effects were observed to be stronger but fluctuating, while the indirect effects were weaker but more consistent. The results highlight the need for multiple season experiments with complex communities to understand the forces in species interactions that drive evolution.

Keywords

Herbivory; *Spirodela polyrhiza*; *Lymnaea stagnalis*

References

Sara Nouere, Martin Schäfer, Antonino Malacrinò, Martin Lohr, Christoph Vorburger, Shuqing Xu (2025) Contrasting and fluctuating selection from direct and indirect herbivory drives transient plant evolution. Authorea (Preprint) DOI: 10.22541/au.176219502.28259701/v1

Sandra Vitko; Tina Milišić; Jelena Braut; Biljana Balen; Mirta Tkalec

University of Zagreb, Faculty of Science, Department of Biology, Croatia

Corresponding author: sandra.vitko@biol.pmf.unizg.hr

Silver nanoparticles (AgNPs) and microplastics (MPs) are among the most prevalent emerging contaminants in freshwater ecosystems, yet the role of nanoparticle surface coating in modulating phytotoxicity, particularly under co-exposure conditions, remains poorly understood. To address this, we examined the individual and combined effects of two surface-coated AgNPs, cetyltrimethylammonium bromide (AgCTAB) and polyvinylpyrrolidone (AgPVP), and two MPs, polystyrene (PS) and polymethylmethacrylate (PMMA), on the duckweed *Lemna minor* over a 7-day exposure period. Control plants were grown without the addition of AgNPs or MPs. Relative growth rate, chlorophyll a fluorescence (JIP-test), pigment content, and expression of photosynthesis-related genes were assessed on days 1, 3, and 7. AgPVP-based combinations with MPs (AgPVP+PS, AgPVP+PMMA) induced the most pronounced physiological stress from the earliest timepoint, while AgCTAB and AgPVP alone caused moderate effects on day 1. By day 3, stress in AgPVP-treated plants intensified, whereas AgCTAB-exposed plants showed signs of recovery towards control values. On day 7, AgPVP+PS remained the most severely affected treatment, while plants treated with AgPVP and Ag-PVP+PMMA displayed partial recovery, though with persistently reduced growth suggesting lasting physiological consequences. The observed physiological responses in the most affected treatments indicate a decline in photosynthetic efficiency, ultimately resulting in reduced plant growth. These results highlight the coating-dependent nature of AgNP phytotoxicity and suggest that microplastic co-exposure may modulate, but not eliminate, the adverse effects of AgNPs on aquatic macrophytes.

Keywords

Duckweed; AgNPs; MPs; Co-exposure toxicity

Amare Fassil Abebaw¹; Raja Amri ¹; Viktor Oláh ²; Ilona Mészáros ²

¹ Doctoral School of Natural Sciences, Szent István Campus, Hungarian University of Agriculture and Life Sciences

² Department of Botany, Faculty of Science and Technology, University of Debrecen

Corresponding author: amarefassil@gmail.com

Abstract This study investigated the growth and physiological responses of four *Lemna gibba* clones (UD0101, UD0103, UD0106, and UD0114) following 7-day exposure to Mn (0.05–160 mg L⁻¹) under controlled conditions (Steinberg's medium containing 0.05 mg Mn L⁻¹). Growth was assessed using frond area relative growth rate (RGRFA) and frond number relative growth rate (RGRFN). Photosynthetic performance was evaluated using Fv/Fm, Y(II), Y(NPQ), and Y(NO). All clones were affected at Mn concentrations ≥20 mg L⁻¹, whereas UD0103 showed growth inhibition from 5 mg L⁻¹. Under control conditions, RGRFA ranged from 0.287 day⁻¹ (UD0106) to 0.334 day⁻¹ (UD0101), while RGRFN ranged from 0.269 day⁻¹ (UD0106) to 0.294 day⁻¹ (UD0101); UD0103 and UD0114 showed intermediate growth rates. At 160 mg Mn L⁻¹, RGRFA relative to the control indicated Mn tolerance in the order UD0101 (50%) > UD0114 (40%) > UD0103 (27%) > UD0106 (25%). Despite growth inhibition, Fv/Fm remained at or slightly above control levels (99–108%) in UD0101 and UD0106, whereas UD0103 and UD0114 showed reduced Fv/Fm at ≥20 mg Mn L⁻¹, reaching minima of 94.9% and 95.2% of the control, respectively. Similarly, increasing Mn concentrations reduced Y(II) in all clones. At 40 mg L⁻¹, Y(NPQ) increased significantly, peaking at approximately 1.8-fold and 1.7-fold above the control in UD0106 and UD0114, respectively. In contrast, UD0101 maintained stable Y(II), Y(NPQ), and Y(NO), whereas UD0103 showed the greatest increase in Y(NO) and a reduced Y(II). These findings suggest that UD0103 could serve as a bioindicator of Mn pollution, whereas UD0101 shows potential for phytoremediation.

Keywords

Duckweeds clone; Chlorophyll fluorescence; Manganese

References

Irfan M, Mészáros I, Szabó S, Oláh V. Comparative Phytotoxicity of Metallic Elements on Duckweed *Lemna gibba* L. Using Growth- and Chlorophyll Fluorescence Induction-Based Endpoints. *Plants* 2024;13. <https://doi.org/10.3390/plants13020215>.

Hayley Sussman; Kevin Cox

Corresponding author: h.sussman@wustl.edu

Wolffia australiana offers a unique opportunity to study spatial variation in stress responses given a small portion of its tissue is exposed to air while the remainder is submerged in water, creating distinct above- and below-water regions within a physically condensed space (<1 mm in diameter). As a result, in a high saline environment, the below-water region has direct exposure to sodium chloride, while the above-water tissue does not. Previous research has shown which gene regulatory networks are induced during salinity stress across the whole tissue of a variety of duckweed species. However, the spatial organization of these transcriptomic responses has not been elucidated. As a first step towards addressing this question, we sought to identify where sodium ions accumulate spatially and the corresponding physiological impacts. To visualize the cellular-level physiological impact of salinity stress on *W. australiana*, this project uses fluorescent stains and indicators under confocal microscopy. CoroNa Green, a cell-permeant compound that fluoresces upon binding sodium ions, is used to spatially observe sodium ion accumulation. In tandem, we spatially observe and quantify both chlorophyll autofluorescence and reactive oxygen species production as proxies for photosynthetic productivity and oxidative stress, respectively. Together, these measurements reveal cellular-level differences in stress exposure, suggesting compartmentalized stress responses between the above- and below-water tissues.

Keywords

abiotic stress · microscopy · physiology

Filipp Krasnovid¹; Veronica Barragan-Borrero²; Arturo Ponce²; Laura Diezma-Navas²; Daniel Buendia²; Arturo; Marí-Ordóñez²

¹ Gregor Mendel institute, Vienna Biocenter PhD Programm

² Gregor Mendel Institute

Corresponding author: filipp.krasnovid@gmi.oeaw.ac.at

Small RNA silencing pathways in plants play crucial roles in development, antiviral resistance, and transposon control. There are two types of silencing: transcriptional gene silencing (TGS) and posttranscriptional (PTGS). TGS acts via the RNA-directed DNA methylation pathway and 24nt-long small interfering RNAs (siRNAs). PTGS functions at the mRNA level, where viral or transgene RNA transcripts trigger the formation of 21nt- and 22nt-long siRNAs that target AGO proteins toward these transcripts leading to their cleavage and degradation. Duckweeds have simplified their silencing pathways and challenge the current model of PTGS in several ways. Dicer-like protein 2 (DCL2), the protein responsible for the production of 22nt siRNA, conserved among plants, is lost in duckweeds, yet we observe 22nt siRNA in all three species studied in our lab. Transgenic silencing in *Lemna minor* is characterised by the equal presence of both 21nt- and 22nt-long siRNAs; transient expression of an inverted repeat (IR) in *Spirodela polyrhiza* and in *Wolffia brasiliensis* results in the production of both 21nt and 22nt siRNA derived from this IR. These 22nt siRNAs, in turn, do not seem to trigger transitivity, the silencing amplification process, despite the presence of all the necessary elements that we know of. The goal of our project is to understand the biogenesis of 22nt-long siRNA and find the mechanisms responsible for the difference in PTGS functioning in duckweeds, including transitivity — a phenomenon that is not well understood even in conventional models such as *Arabidopsis*.

Keywords

small RNA; PTGS; Silencing

References

Barragan-Borrero V, de Santana Lopes A, Rodrigues-Batista ED, et al. (2026) Strain, procedures, and tools for reproducible genetic transformation and genome editing of the emerging plant model *Spirodela polyrhiza* *New Phytol* 250(2):735-56 preprint bioRxiv:2025.08.28.672806 Dombey R, Buendía-Ávila D, ..., Marí-Ordóñez A (2025) Atypical epigenetic and small RNA control of degenerated transposons and their fragments in clonally reproducing *Spirodela polyrhiza*. *Genome Res* 35(3):522-44 preprint bioRxiv:2024.04.03.587901 Buendia-Avila D, Barragan-Borrero V, ..., Mari-Ordóñez A (2026) Transposon expansion is associated with reorganization of small RNA and DNA methylation landscapes in the morphologically minimal angiosperm *Wolffia brasiliensis*. bioRxiv:2026.02.27.708460

Natasha Manyenga¹; Meritxell Cuevas Abril ¹; Laura Guerra Diaz¹; Lorenzo Proia¹; Yasmina Chourak¹; Aubanell Quim Zaldo ²

¹ BETA Technological Centre-University of Vic-Central University of Catalunya (BETA-UVIC- UCC)

² Forest Science and Technology Centre of Catalonia (CTFC)

Corresponding author: natasha.manyenga@uvic.cat

Intensive swine farming generates nutrient-rich wastewater that, if mismanaged, poses environmental risks but also offers opportunities for resource recovery. Duckweed, noted for its rapid growth and high nutrient-uptake capacity, offers a promising solution for wastewater treatment and holds untapped potential for agricultural valorisation. This study investigated duckweed cultivated on both swine wastewater and mineral fertilizer to produce a nutrient-rich biomass for application as an organic amendment, thereby enhancing sustainable food production. Agronomic evaluations were conducted to assess the potential of duckweed as an organic amendment in comparison to mineral fertilizer and raw manure. Duckweed biomass, both dried and fresh, was tested in a 52-day pot experiment with spinach (*Spinacia oleracea*) and lettuce (*Lactuca sativa*). Additionally, wheat (*Triticum aestivum*) was grown for 168 days under laboratory conditions. The findings indicate that duckweed-based amendments, when combined with raw manure, enhanced the growth and biomass accumulation of spinach, lettuce and wheat, outperforming the mineral fertilizer treatment. The fertilizing potential of the produced biomass, in both dried and fresh forms, was demonstrated to be comparable to the use of raw manure and other inorganic fertilizers. The integrated use of duckweed with raw manure further enhances soil nutrient cycling through synergistic effects on mineralization, reinforcing the approach of combining organic nutrient sources to optimize crop performance and soil health. Duckweed-based amendments reduce nutrient losses, slowly releasing nutrients, and enabling wastewater bioremediation and production of nutrient-rich biomass that supports sustainable crop production and soil regeneration.

Keywords

Duckweed biomass; Organic-amendment; Nutrient-cycling; Sustainable agriculture

Anim Ujong¹; Monjurol Hogue²; Brijesh Tiwari²; Shay Hannon²; Uma Tiwari³

¹ Technological University Dublin and Teagasc Food Research Centre

² Teagasc Food Research Centre, Ashtown

³ Technological University Dublin

Corresponding author: d23129048@mytudublin.ie

Duckweed is a sustainable aquatic biomass with potential for biorefinery applications owing to its rapid growth and rich proteins, starch composition, and bioactive compounds. However, conventional processing methods often fail to efficiently fractionate its components, limiting biomass valorization. This study investigated aqueous fractionation assisted by pulsed electric field (PEF) and high-pressure processing (HPP) as non-thermal pretreatments to enhance the recovery of functional duckweed ingredients. PEF was applied at 12 and 24 kV (9 μ s; 5 or 10 treatment cycles) or HPP at 200, 400, and 600 MPa for 5 min, followed by centrifugation and fractionation into (i) an aqueous extract rich in phenolic compounds, minerals, and soluble proteins and (ii) a solid fraction enriched in starch, dietary fiber, and residual proteins. Protein solubilization increased with HPP pressure (27.91–28.44%) and PEF electric field strength (26.10–27.80%), while additional PEF cycles had minimal effects. Starch enrichment in the pellet increased with higher treatment intensity (4.50–5.32%), whereas phenolic recovery was enhanced by PEF, reaching 13.8 μ mol/g at 24 kV–10 cycles, and by HPP, with the highest recovery at 200 MPa (18.52 μ mol/g). Functional characterization showed superior foaming capacity in aqueous extracts, reaching 236% after HPP (400 MPa) and 345% after PEF (12 kV–10 cycles), while pellet fractions exhibited greater water-holding and fat-binding capacities. Thermal analysis and FTIR indicated structural modifications and molecular rearrangement without significant chemical degradation. Overall, HPP- and PEF-assisted aqueous fractionation represents a promising approach for duckweed valorization and the sustainable production of multifunctional food and biobased ingredients.

Keywords

Duckweed; Pulse-electric field; High pressure

References

Ujong, A., Naibaho, J., Ghalamara, S., Tiwari, B. K., Hannon, S., & Tiwari, U. (2024). Duckweed: Exploring its farm-to-fork potential for food production and biorefineries. *Sustainable Food Technology*, 3(1), 54–80.

Rebecca Fairburn

The University of Nottingham

Corresponding author: rebecca.fairburn@nottingham.ac.uk

Changes in morphology have occurred frequently over the course of plant evolution, and in some cases involve the loss of entire organs or structures, yet the mechanisms that underpin organ loss are not well understood. We are using duckweed as a model to understand organ loss. As duckweed evolved, they experienced a dramatic reduction in anatomical complexity. Their roots losing the ability to take up nutrients, branch, and produce root hairs. In some species roots are lost entirely. This project aims to provide knowledge on how structures and traits and the networks which encode them are lost or reduced over time. Using a combination of bioinformatics, molecular genetics, and tissue culture techniques, we aim to identify genes regulating root hairs that are missing in duckweed and then reintroduce homologs from related species using transgenic approaches. This project will give insights the complex genetic pathways that determine plant organ loss and evolution. Additionally, reintroducing genes that complete a hypothesised ancestral genetic pathway, will precede further synthetic biology projects, which aim to transfer complex genetic networks between organisms and unlock the benefits of ancestral genomes. This, all while firmly cementing duckweed as a plant of the future.

Keywords

Root hairs; Organ loss; Vestigiality

Enrico Diniz Rodrigues Batista; Charlotte Köhle²; Meret Huber

Institute of Organismic and Molecular Evolution, University of Mainz

Corresponding author: edinizro@uni-mainz.de

Transgenerational plasticity is considered a flexible and rapid adaptive mechanism that enables organisms to respond to environmental change over relatively short evolutionary timescales. However, how long these effects persist and the extent to which they influence organismal fitness remain largely unknown. Here, we investigated these questions using the giant duckweed (*Spirodela polyrhiza*) and its native herbivore, the waterlily aphid (*Rhopalosiphum nymphaeae*). We exposed clonal duckweed lineages to aphid herbivory for ten consecutive generations and subsequently allowed them to recover under herbivore-free conditions for 1, 3, 5, 7, or 9 generations. We then grew the descendants in the presence and absence of aphids to assess their transgenerational responses. Duckweed exhibited a remarkably persistent stress memory: the ancestral herbivory environment fundamentally altered the growth of descendant fronds across multiple subsequent generations. Ancestral aphid feeding enhanced plant growth in the absence, but not the presence, of renewed herbivory. This pattern suggests that previously stressed plants overcompensate by accelerating growth once herbivore pressure is removed, rather than by increasing resistance to recurring attack. Remarkably, ancestral aphid herbivory also enhanced aphid fitness, even after nine generations without herbivore exposure. Together, these findings demonstrate that duckweed possesses a long-lasting, multigenerational stress memory that herbivores may exploit to their own advantage.

Keywords

Spirodela polyrhiza; Aphids; Transgenerational plasticity

References

Huber et al., 2021; Proc. R. Soc.

Susi Mulyanti Lubis¹; Wilhelmus Terang Arga Sanjaya²; Dwi Guntoro³; Stefano Mazzoleni⁴; Anja Meryandini⁵; Anak Agung Istri Kesumadewi⁶; Yemima Gresia Sagala⁷; Dwi Andreas Santos²

¹Graduate Program in Biotechnology, Graduate School, IPB University; ²Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University; ³Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University; ⁴Department of Agricultural Sciences, School of Agriculture and Veterinary Medicine; ⁵Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University; ⁶Agroecotechnology Study Program, Faculty of Agriculture, Udayana University; ⁷Biotech Center, IPB University

Corresponding author: wilhelmus_arga@apps.ipb.ac.id

Extracellular self-DNA has emerged as a species-specific regulator of plant growth, yet its effects and physiological responses in aquatic plants remain poorly understood. This study investigated whether extracellular self-DNA preferentially inhibits growth in a two-species duckweed community and whether this response is associated with oxidative stress. *Lemna perpusilla* and *Spirodela polyrhiza* were exposed to self-DNA, non-self DNA, and mixed DNA. A dose-response assay using genomic DNA solutions ranging from 0 to 2,000 ng μL^{-1} identified the most inhibitory treatment. Growth was evaluated using frond number, root number, root length, fresh biomass, and dry biomass. Species specificity was examined by co-cultivating both species and exposing the culture separately to DNA from each species. Physiological responses to self-DNA at 1,000 ng μL^{-1} were assessed through reactive oxygen species (ROS), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA). Self-DNA caused stronger inhibition than non-self DNA and mixed DNA. In the two-species community, *L. perpusilla* DNA reduced *L. perpusilla* fresh biomass by 76.5% but *S. polyrhiza* by only 3.2%. Conversely, *S. polyrhiza* DNA reduced *S. polyrhiza* fresh biomass by 93.3% but *L. perpusilla* by only 14.5%, demonstrating reciprocal species-preferential inhibition. Self-DNA also increased ROS accumulation by 39% in *L. perpusilla* and 54% in *S. polyrhiza*, accompanied by enhanced SOD, POD, and CAT activities and elevated MDA levels. These findings show that extracellular self-DNA preferentially inhibits its source species within a mixed duckweed community and that this response is associated with oxidative stress, supporting investigation of self-DNA recognition and species-targeted aquatic plant management.

Keywords

Autotoxicity; eDNA; *Lemna*; stress; *Spirodela*

References

Carteni F, Bonanomi G, Giannino F, Incerti G, Vincenot CE, Chiusano ML, Mazzoleni S. 2016. Self-dna inhibitory effects: Underlying mechanisms and ecological implications. *Plant Signal. Behav.* 11(4):1– 4.. Mazzoleni S, Carteni F, Bonanomi G, Senatore M, Termino P, Giannino F, Incerti G, Rietkerk M, Lanzotti V, Chiusano ML. 2015. Inhibitory effects of extracellular self-DNA: A general biological process? *New Phytol.* 206(1):127–132. Bog M, Appenroth KJ, Schneider P, Sree S.K. 2022. Intraspecific Diversity in Aquatic Ecosystems: Comparison between *Spirodela polyrhiza* and *Lemna minor* in Natural Populations of Duckweed. *Plants.* 11(7).

Jesis Silvano; Dwi Andreas Santosa

IPB University

Corresponding author: jessissilvano22@gmail.com

Duckweed (*Lemna* sp.) is a promising alternative ruminant feed due to its rapid growth and high protein content. However, extracellular self-DNA released from plant tissues may exert species-specific inhibitory effects on microbial activity, a phenomenon remaining unexplored in rumen systems. This study evaluated the effects of *Lemna* supplementation and *Lemna*-derived self-DNA on in vitro rumen fermentation characteristics. Five dietary treatments were evaluated: P0 (basal diet: 60% forage, 40% concentrate); P1 (60% forage + 36% concentrate + 4% *Lemna* self-DNA); P2 (60% forage + 36% concentrate + 4% *Lemna* control); P3 (50% forage + 30% concentrate + 20% *Lemna* self-DNA); and P4 (50% forage + 30% concentrate + 20% *Lemna* control). Rumen pH, NH₃-N, total volatile fatty acids (VFA), and VFA profiles were determined post-in vitro incubation. Rumen pH and NH₃-N concentrations were not significantly affected by treatments ($P > 0.05$). Conversely, total VFA production and composition were significantly altered ($P < 0.05$). The highest total VFA concentration was observed in P3, followed by P2 and P1, indicating enhanced fermentation with higher *Lemna* inclusion. Notably, treatments containing self-DNA tended to produce lower fermentation responses than their respective controls, suggesting a potential inhibitory effect on microbial activity. Additionally, acetate proportion decreased while propionate and butyrate increased, shifting toward more efficient fermentation pathways. In conclusion, while *Lemna* nutrients stimulate rumen fermentation, its self-DNA partially modulates microbial activity through inhibitory mechanisms, highlighting the importance of considering both nutritional and molecular factors in plant biomass evaluation.

Keywords

Extracellular self-DNA; Oxidative stress

References

- Apel, K., & Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55, 373–399. <https://doi.org/10.1146/annurev.arplant.55.031903.141701>
- Mazzoleni, S., Carteni, F., Bonanomi, G., Senatore, M., Termolino, P., Giannino, F., Incerti, G., Rietkerk, M., Lanzotti, V., & Chiusano, M. L. (2015). Inhibitory effects of extracellular self-DNA: A general biological process? *New Phytologist*, 206(1), 127–132. <https://doi.org/10.1111/nph.13306>
- Zhou, X., Gao, H., Zhang, X., Khashi u Rahman, M., Mazzoleni, S., Du, M., & Wu, F. (2023). Plant extracellular self-DNA inhibits growth and induces immunity via the jasmonate signaling pathway. *Plant Physiology*, 192(3), 2475–2491. <https://doi.org/10.1093/plphys/kiad195>

Shi Er Ng¹; Derek Juinn Chieh Chan¹; Ezaki Satoshi²; Masao Tsutsui²; Takimoto Tatsu³; Eishi Morita³

¹ Universiti Sains Malaysia

² CS Innovation Co. Ltd

³ TSUKASA PETCO SINGAPORE PTE. LTD.

Corresponding author: chderekchan@usm.my

Duckweed is a sustainable biomass resource capable of high-value protein production, carbon sequestration, nutrient recovery, and water purification. This study evaluated the feasibility of commercial-scale duckweed production in Malaysia through pilot-scale cultivation, sequential nutrient optimization, drying process development, and life cycle assessment (LCA). A two-stage sequential media strategy was employed to optimize biomass growth and nutrient accumulation independently. Pilot-scale cultivation successfully reproduced laboratory-scale performance, demonstrating scalability while maintaining species-specific characteristics. *Lemna minor* achieved the highest relative growth rate (0.227 day^{-1}) with 36.8% protein (dry weight), whereas *Spirodela polyrhiza* accumulated 50.2% protein under sustained low-nitrogen conditions. *Wolffia globosa* exhibited unique metabolic traits, producing both high carbohydrate (50.7%) and protein (44.0%) contents under prolonged high-nitrogen cultivation. Drying conditions (40–55°C) significantly affected nutrient retention, pigment stability, powder properties, and downstream processing suitability, enabling biomass quality to be tailored for specific applications. LCA identified electricity use ($80.4 \text{ kg CO}_2\text{-eq kg}^{-1} \text{ dry biomass}$) and water production ($39.8 \text{ kg CO}_2\text{-eq kg}^{-1}$) as the major environmental hotspots. Nevertheless, cultivation of *L. minor* and *S. polyrhiza* achieved net carbon removal of $33.6 \text{ kg CO}_2\text{-eq kg}^{-1} \text{ dry biomass}$ through biogenic carbon sequestration. Furthermore, integrating photovoltaic electricity transformed *W. globosa* cultivation into a net carbon sink, sequestering $81.0 \text{ kg CO}_2\text{-eq kg}^{-1} \text{ dry biomass}$. These findings demonstrate that optimized cultivation and processing strategies can support application-specific duckweed production while enhancing environmental and economic sustainability in tropical regions.

Keywords

Commercial cultivation; Life Cycle Assessment

Alberto Giuliano¹; Giovanna Aronne¹; Vincent Jalink²; Ester Scotto di Perta¹; Stefania Pindoizzi¹; Leone Ermes; Romano¹

¹ Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy

² PhenoVation B.V., Agro Business Park 65a, 6708 PV Wageningen, The Netherlands

Corresponding author: alberto.giuliano@unina.it

Cultivating duckweed (Lemnaceae) on livestock wastewater represents a leading strategy for the circular bioeconomy, particularly relevant in Campania (Italy), where about 80% of the national buffalo herd is concentrated and where sustainable wastewater management is a priority challenge. Several image analysis techniques are now available to calculate the relative growth rate (RGR) of these macrophytes, ranging from classic RGB imaging to more sophisticated chlorophyll fluorimetry-based approaches, yet a systematic comparison of their reliability is still lacking, particularly under physiological stress. In this work, *Lemna minuta* (clone LER035) was grown on ozonated buffalo wastewater at different total ammoniacal nitrogen (TAN) concentrations, and RGR was calculated using three imaging methods — RGB camera, light-adapted PAM fluorimetry, and dark-adapted PAM fluorimetry — to compare their agreement. Among the treatments showing a positive RGR, the treatment based on centrifuged digestate at low nitrogen concentration (50 mg L⁻¹ TAN, DC_low) was the only one to outperform the control (0.343 vs. 0.307 day⁻¹). Overall, light-adapted PAM and RGB showed a high, robust correlation ($\rho = 0.988$), confirmed when separately analyzing the positive-RGR ($\rho = 0.980$) and negative-RGR ($\rho = 0.916$) subgroups. Under stress, however, the two methods were not numerically interchangeable, with divergence ranging from negligible in healthy treatments to marked (+0.141 on average) in stressed ones. The RGB method therefore remains valid for RGR determination; however, PAM technology detects stress well ahead of RGB, identifying growth decline approximately 4 days earlier in 81% of stressed replicates and capturing impaired photosynthetic efficiency before visible tissue disintegration.

Keywords

Lemnaceae; Image analysis; PAM; RGR

Dóra Szilágyi¹; István Gyulai²; Bácsi István²; Attila Kiss³; Ilona Mészáros¹; Viktor Oláh¹

¹ Department of Botany, Faculty of Science and Technology, University of Debrecen

² Department of Hydrobiology, Faculty of Science and Technology, University of Debrecen

³ Department of Organic Chemistry, Faculty of Science and Technology, University of Debrecen

Corresponding author: szilagydorika2001@gmail.com, olahviktor@unideb.hu

High phenotypic plasticity of duckweeds affects their biomass productivity. Reliable estimation of biomass quantity and quality, hence, are important when culturing duckweed outdoors. Spectral proxies, that is, indices derived from light reflectance and transmittance, became fundamental in crop production, but their application on duckweeds has only been reported scarcely. We aimed at sampling naturally occurring duckweed populations and analyzing their biomass with analytical (dry matter and protein contents) and optical (hyperspectral reflectance and transmittance) approaches due to test applicability of the latter proxies. A total of $n=20$ duckweed and water samples were analyzed from 10 sites in the Trans-Tisza region (E-Hungary) during two sampling campaigns in June and September of 2025. The sampled waters showed wide gradients of N- and P-concentrations, but biomass dry matter and protein contents showed no significant correlation with the nutrient availability. Similarly, neither seasonality, nor species-specific patterns were found in biomass traits. Cell phone imaging proved to be reliable in estimating plant biomass (Pearson's correlation: $p<0.001$) under field conditions. We could not establish link, however, between the reflectance- (e.g. NDVI, PBI) and transmittance-based (CCI) indices, and the biomass composition, respectively. Our results highlight, that duckweed biomass traits cannot be predicted mechanistically from spectral indices, but customized approach is needed for particular applications.

This research was funded by the NKFIH OTKA grant No. FK 134296, and was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, by the University of Debrecen Program for Scientific Publication, and by the Tempus Public Foundation.

Keywords

Light reflectance; Transmittance; Biomass composition

Raja Amri; Amare Fassil Abebaw; Viktor Oláh; Ilona Mészáros

Turions, the vegetative dormant organs, are vital for some duckweed species, enabling their survival under harsh conditions. We studied the turion germination and then sprouting of four *Spirodela polyrhiza* clones. Turions were collected from 7-day old growth cultures treated by 0.5 μM abscisic acid (ABA) in Steinberg medium. Germination tests were performed under continuous light for 7 days, immediately after harvesting the turions or after cold storage in darkness. The growth and photosynthetic activity of turion-derived fronds was compared with those developed in non-ABA treatment, culture-derived fronds. Germination capacity of turions was clone-specific. Immediately after harvesting, turions of UD0402 and UD0408 germinated readily at 81.2% and 73.3%, whereas those of UD0401 and UD0407 germinated poorly at 8.7% and 0.0%, respectively. The cold storage influenced differently the turion germination: increased largely in UD0401, moderately in UD0407 while decreased in UD0402 and UD0408. Turion-derived fronds of UD0401, UD0402, and UD0407 showed greater frond area, and lower LMA and LDMC than the culture-derived fronds, suggesting reserve-supported area expansion in the former ones, rather than increasing dry-matter accumulation. However, there were no differences in these traits between the two frond types of UD0408. Independently of turion storage duration, the turion-derived fronds showed mostly comparable physiological traits to culture-derived fronds. Consistently, the chlorophyll fluorescence results showed that, turion-derived fronds reached similar levels of photosynthetic activity. Overall, the results show that exogenous 0.5 μM ABA was able to trigger turions formation of all studied clones, but the germination success depended on the clone and the storage duration.

Keywords

S. polyrhiza; ABA; Turion-storage; Germination; Post-dormancy.

References

Ley et al. 1997; Oláh et al. 2021; Appenroth and Ziegler, 2008

*Yelizaveta Prokhorova*¹; *Verónica Barragán-Borrero*²; *Elias Rana*²; *Arturo Marí-Ordóñez*²; *Richard Strasse*³; *Piers A. Hemsley*^{1,4}

¹ Division of Plant Sciences, School of Life Sciences, University of Dundee

² Gregor Mendel Institute of Molecular Plant Biology (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC)

³ Institute of Applied Genetics and Cell Biology, University of Natural Resources and Applied Life Sciences, Vienna

⁴ Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

Corresponding author: yprokhorova001@dundee.ac.uk

Duckweeds are a group of aquatic plants that represent an attractive platform for molecular farming due to their rapid vegetative propagation, genetic stability, and low cultivation costs. In this study, we investigated the production of recombinant human interferon alpha-2b (HsIFN2b), a cytokine widely used for antiviral and anti-proliferative therapies, in duckweed, with potential to reduce costs associated with downstream processing, cold-chain storage, and parenteral administration. Respiratory viral infections remain a major global health burden, causing significant morbidity, mortality, and economic losses, while access to prevention and treatment remains limited in many low-income countries. Oral delivery of HsIFN2b has been shown to produce a systemic immune response at doses up to 104-fold lower than required for injections, potentially reducing its adverse effects. Moreover, in edible duckweeds, the plant cell wall may provide bioencapsulation, protecting recombinant protein from digestion via gastrointestinal enzymes. To improve the protein stability and biological activity of HsIFN2b, four artificial N-glycosylation sites were introduced by site-directed amino acid substitution. Transgenic lines of *Spirodela polyrhiza* 162 and *Lemna minor* 8623 expressing 4N-HsIFN2b were generated via *Agrobacterium*-mediated transformation and indirect organogenesis. LC-MS glycan analysis of plant 4N-HsIFN2b identified complex plant-type glycans, indicating Golgi-mediated processing and targeting to the secretory pathway. Biological activity was assessed using human A375 melanoma cells carrying an ISRE-mCherry reporter. Plant-produced 4N-HsIFN2b induced activation of the IFNAR signalling pathway, resulting in an approximately threefold increase in reporter activity at 70 ng ml⁻¹ compared with the buffer control, demonstrating functional activity in human cells.

Keywords

Spirodela polyrhiza; *Lemna minor*; Highly-glycosylated human-interferon-alpha-2b; Molecularfarming; Biopharmaceuticals

Cristian Perna; Maria Adelaide Iannelli; Luca Braglia; Chiara Forti; Paolo Cozzi; Paola Cremonesi; Marco Fabriani; Silvia Giani; Barbara Menin; Laura Morello

¹ Institute of Agricultural Biology and Biotechnology - National Research Council (IBBA-CNR)

Corresponding author: cristian.perna@cnr.it

Effluents generated by the dairy sector constitute a nutrient-rich matrix with a significant environmental impact. Conventional treatments are generally energy-intensive and based on biological oxidation, leading to nutrient dissipation and secondary waste products. Phytoremediation emerge as sustainable strategy for nutrient recovery and biomass production within a circular economy framework. Duckweeds are particularly attractive for these applications, and their nutritional properties open perspectives as feed supplements. Their remarkable biodiversity, characterized by extensive genetic variability, polyploidization and hybridization, translates into adaptability to heterogeneous aquatic environments. Such diversity likely corresponds to a wide spectrum of functional traits suitable to phytoremediation systems. This work explores the diversity of autochthonous (EU) duckweed clonal accessions sourced from the IBBA collection, representative of multiple taxonomic levels, including species from different genera, genotypes within the same species, allopolyploids and their parental species. Tested accessions were evaluated for their growth performance on dairy wastewater. Microbial community dynamics were also investigated in order to explore the contribution of cooperative plant-microbe interactions. Specific harsh growth conditions favoured certain species and clones over others, revealing large inter- and intraspecific variability. Two accessions emerged as the best-performing: *Lemna minor* (#5500) and its natural allotriploid *Lemna × mediterranea* (#LER0021). Further characterization of their physiological and biochemical traits, e.g. pigment and protein content, oxidative stress markers as well as nutrient depletion efficiency, revealed two distinctive eco-physiological strategies.

Keywords

Phytoremediation; Biodiversity; Dairy-wastewater; Duckweed

Zoé Faes^{1,2}, Bruno De Meulenaer², Emmy Dhooghe¹

¹ Department of Plants and Crops, Ghent university, Belgium

² Department of Food Technology, Safety and Health, Ghent university, Belgium

Corresponding author: zoe.faes@ugent.be

The rising demand for sustainable food sources has brought attention to unconventional and innovative alternatives like duckweed, a small aquatic plant recognized for its rapid growth rate and high nutritional value. To initiate large-scale cultivation, sufficient sterile biomass must first be produced from axenic in vitro stock cultures. Therefore, this study evaluated the effect of recipient gas exchange capacity on relative growth rate (RGR) and nutrient medium pH for two *W. arrhiza* clones at 7 and 14 days of in vitro cultivation. Plants were grown axenically in liquid N-medium with an adjusted NO₃⁻/NH₄⁺ ratio of 90/10, under controlled environmental conditions (25.2 ± 0.8 °C, 150 µmol m⁻² s⁻¹, 50% RH, 16:8 h light:dark), with five biological replicates per treatment. Four recipient types were used: sterile parafilm-sealed jars (no gas exchange) and Microbox Eco2 containers with increasing gas exchange capacity (L, XL, XXL). One clone generally showed higher RGR than the other. Recipient effects on RGR showed no uniform significant differences among gas-exchange treatments. Medium pH decreased over time in all treatments, consistent with preferential NH₄⁺ uptake and associated proton release into the medium. These results demonstrate that gas exchange influences in vitro *Wolffia* cultivation, mainly through changes in medium pH, while clone identity remains a dominant driver of growth performance

Keywords

Phytoremediation; Biodiversity: Dairy-wastewater; Duckweed

Evan Ernst¹, Cristian Mateo-Elizalde¹, Robert A. Martienssen¹

¹ Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA

Corresponding author: martiens@cshl.edu

Contemporary *de novo* assemblers often produce near-perfect telomere-to-telomere assemblies of chromosomes from long-read data, alongside hundreds of shorter accessory contigs with diverse origins: under-collapsed heterozygous sequence, organellar genome isoforms, rDNA, and cobionts. We developed a software tool called dnanis (*de novo* assembly disambiguator) to automate common analyses following assembly and produce machine-readable tabular reports, rich HTML summaries, and classified sequence files to classify contigs, diagnose assembly errors, produce useful biological insights, and prepare for downstream annotation.

Beginning with whole genome alignment to one or more reference genomes, dnanis orchestrates chromosome-length contig assignment, naming, reorientation, scaffolding. When genomic read sets are available, dnanis automates coverage analysis to surface aneuploidy and distinguish rearrangements from misassemblies. Hybrid and polyploid chromosome sets are disentangled with an implementation of Gaussian mixture model segmentation and/or simultaneous mapping to multiple reference genomes. Classification modules using community-standard external tools and novel algorithms perform organelle identification, rDNA array annotation, cobiont and contaminant screening, and quality assessment. If several closely related query assemblies are provided, dnanis produces additional comparative reports and plots which can be especially helpful in identifying conserved changes to chromosome architecture within a clade.

dnadis is open-source software and supports local and distributed execution for scalable use with dozens of assemblies.

Keywords

Comparative genomics, Curation, Validation, Visualization

INDEX OF FIRST AUTHORS

Presenting and first authors, in alphabetical order, with the page of their contribution.

A

Abebaw, Amare Fassil	131
Alves, Felipe	83, 118
Amri, Raja	141
Appelbaum, Samuel	122
Appenroth, Klaus J.	3

B

Batista, Enrico Diniz Rodrigues	137
Bishopp, Anthony	5
Bog, Manuela	59
Bwalya, Ngosa	105

C

Chakraborty, Abhisek	126
Chen, Jingrou	99, 116
Cox, Kevin	37
Crombez, Ewout	39

D

Demaria, Sara	77
Diezma-Navas, Laura	121

E

Ernst, Evan	147
--------------------------	-----

F

Faes, Zoé	146
Fairburn, Rebecca	136
Fortmueller, Julia Eva	115

G

Gabry, Halina	11
Gillor, Osnat	49

Giuliano, Alberto	141
--------------------------------	-----

H

Hou, Hongwei	7
Huang, Weijuan	81
Huber, Meret	27

I

Iannelli, Maria Adelaide	119
---------------------------------------	-----

J

Je, Sujeong	25
--------------------------	----

K

Katsara, Alexandra	97
Kishchenko, Olena	109
Kolehmainen, Anna	128
Koleszár, Gergő	101
Krasnovid, Filipp	133
Kutman, Umit Baris	103

L

Lam, Eric	47
Lauritsen, Markus Dahl	13
Lee, Yuri	43
Lincoln, Anne	115
Loaiza, Ivan	87
Lombardi, Martina	127
Lubis, Susi Mulyanti	138

M

Maag, Patricia	89
Manyenga, Natasha	134
Margot, Clair	125
Marí-Ordóñez, Arturo	31
Martienssen, Robert	69
Mateo-Elizalde, Cristian	35
McGregor, Madison	123
Meetam, Metha	85

Monselise, Edna Ben-Izhak	9
Morello, Laura	61
N	
Ng, Shi Er	93, 140
Nouere, Sara	63
O	
Oláh, Viktor	79
Oyama, Tokitaka	19
P	
Perna, Cristian	145
Peršić, Vesna	17
Polikovskiy, Mark	29
Prokhorova, Yelizaveta	144
Prudot d’Avigny, François	53
R	
Rishmawi, Dalia	120
Romano, Leone Ermes	67
Ruenpham, Karnjana	124
S	
Schäfer, Martin	129
Schubert, Ingo	33
Silvano, Jesis	139
Smith, Claire	45
Sonnenfeld, Alexej	117
Sree, Sowjanya Kandregula	107
Stepanenko, Anton	41
Sussman, Hayley	132
Szabo, Sandor	55
Szilágyi, Dóra	142
T	
Tawfik, Ahmed	95
Thamchaipenet, Arinthip	51
Toppe, Jogeir	71

Turcotte, Martin..... 65

U

Ujong, Anim..... 91, 135

V

van der Meer, Ingrid 75

Vitko, Sandra 130

Vunsh, Ron 111

W

Ware, Alexander..... 15

X

Xu, Shuqing..... 57

Y

Yamaoka, Yasuyo..... 23

Z

Zhang, Mi..... 21

Zhao, Hai..... 73