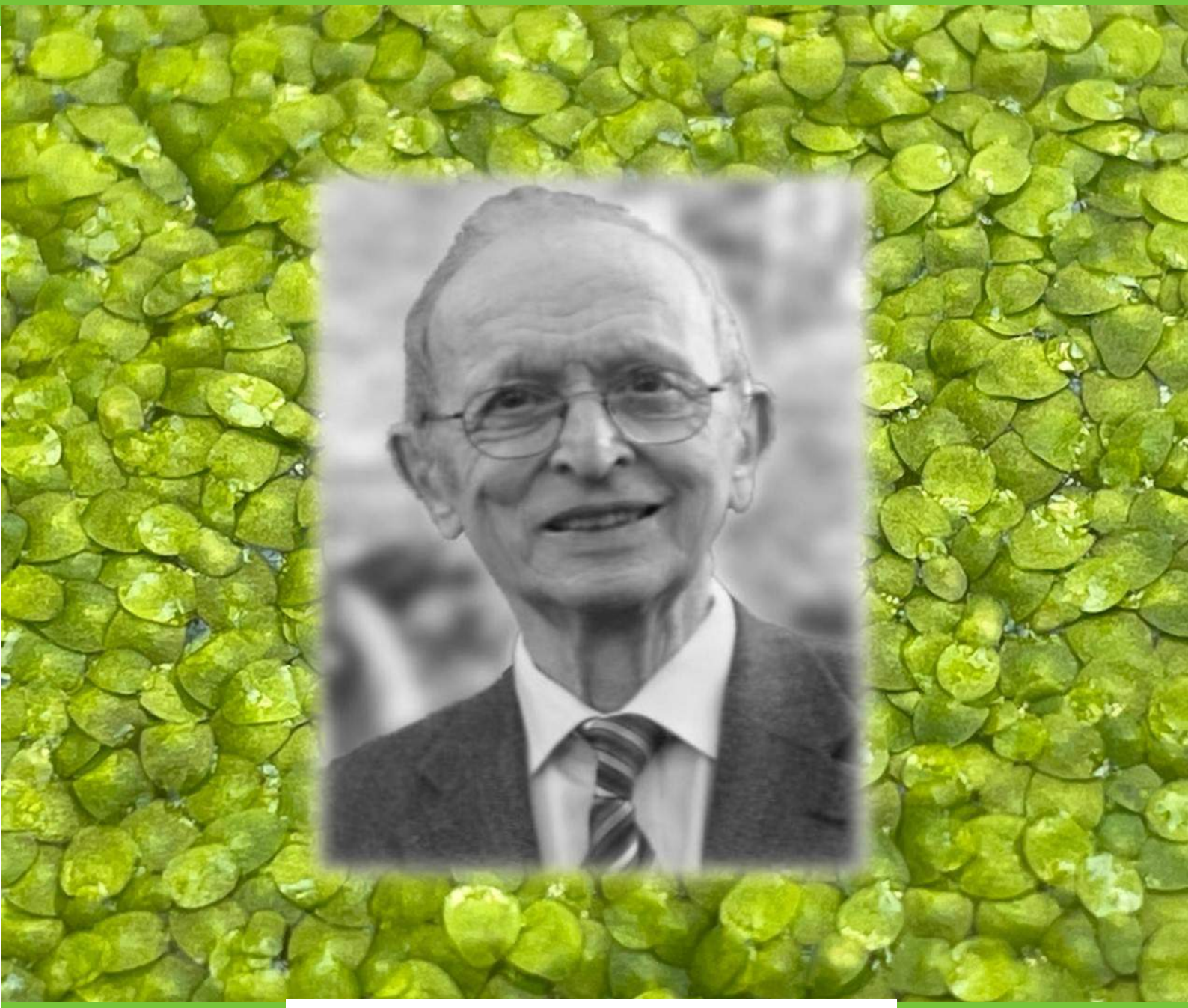
Newsletter for the Community of Duckweed Research and Applications, edited by the ISCDRA

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 11 (2), issue 41, pages 46 - 97 (2023)



Professor Elias Landolt
(1926 - 2013)

Cover page

Late Prof. Elias Landolt (1926 – 2013). This issue of Duckweed Forum is dedicated to Late Prof. Elias Landolt on the 10th anniversary of his passing (Photo credit and cover design: Prof. Eric Lam, Rutgers University, USA).

In this issue

The 5th International Steering Committee on Duckweed Research and Applications Members

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

Letter from the Editor:

April 29th, 2023

Dear readers,

Greetings from New Jersey and welcome to another issue of our community newsletter, the *Duckweed Forum* (DF). It is springtime in the northern hemisphere and a lot of plants are flowering now in New Jersey, while I wake up to calling of birds outside my window in the morning. The earth seems to be reawakening from being dormant in the winter and the longer days keeping us more energized.

In this spirit of renewal, it is perhaps fitting to share my feeling of gratification upon going through the numerous articles that are being curated for the Database section in this issue of the DF. In addition to a steady increase in the number of duckweed-related publications in peer-reviewed journals, I believe there is also continual improvement in the quality of many published studies as well. In addition to the descriptive and observational, increasing number of published work using duckweeds are now addressing problems at the mechanistic level and novel discoveries that could help shape our understanding of plant biology are being made. The renaissance of duckweed research as a model plant (Acosta *et al.*, *Plant Cell* 2021) has indeed arrived.

As examples, several recent reports in the Highlights of the Database section made exciting use of the duckweed system to advance diverse areas of plant biology research. In a paper from the lab of Tony Bishopp (Nottingham, the U.K.), a combination of cytological and transcriptomic approaches was used to examine the evolutionary path of structural minimalization in the duckweed family (Ware *et al.*, in *Current Biology*) to inform on how one organ, the root, became vestigialized as the plant adapt to a new habitat. On a very different subject, the lab of Hongwei Hou (Wuhan, China) published two papers (Sun *et al.*, in *New Phytologist* and in *Sustainable Energy and Fuels*) describing the physiological, metabolic and transcriptomic changes of duckweed grown under different types of trophic conditions to identify optimal regimes for biomass production. In one of the studies, they reported that xylose (a component of hemicellulose) could be metabolized effectively by duckweed under mixotrophic conditions that pointed to the potential use of duckweed for more efficient conversion and recycling of biomass. In addition to these highlighted reports, there are other interesting findings that are also very promising to yield exciting discoveries and applications. They include several that reported novel uses of duckweed in fuel cells and manufacturing of nanomaterials, while the Oyama lab (Kyoto, Japan) reported their finding that the commonly used plant viral promoter (CaMV 35S) appears to be under the control of a non-cell-autonomous circadian rhythm that is distinct from the known cellular oscillators such as the AtCCA1 protein. There are additional publications of interest, but I will let our readers to discover them on their own.

Aside from also pointing out to our readers a nice contribution by Dylan Jones (Nottingham, the U.K.) in our Student Spotlight and the announcement by the organizers of the 7th ICDRA in Thailand, I like to close out this Editorial Letter by acknowledging the immense contributions of Prof. Elias Landolt to duckweed research, on the occasion for the 10th anniversary of his passing. As our community's success continues to build on the foundational resources that Prof. Landolt's dedicated work has provided us, I hope we would also remember and emulate his spirit of mutual support to ensure our community's renaissance will develop fully and for the benefit of all. I am quite sure that would be his true legacy and best tribute from all of us.

For all the ISCDRA members, my warmest regards to you.

Sincerely,

Eric Lam

Chair, ISCDRA

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

Klaus-J. Appenroth[1], K. Sowjanya Sree[2], Eric Lam[3]

[1] Matthias Schleiden Institute – Plant Physiology, University of Jena, 07743 Jena. Email: Klaus.Appenroth@uni-jena.de

[2] Department of Environmental Science, Central University of Kerala, Periye- 671320, India. Email: ksowsree@gmail.com; ksowsree9@cukerala.ac.in

[3] The Rutgers Duckweed Stock Cooperative and Department of Plant Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA. Email: ericl89@hotmail.com

The late Elias Landolt was famous as the unquestionable top scientific expert on the plant family of Lemnaceae, commonly called duckweeds. He was an honorary member of the Swiss Botanical Society, as well as honorary citizen of the city Zurich, Switzerland. He was a Professor at the Swiss Federal Institute of Technology Zurich ("Eidgenoessisch Technische Hochschule", ETH) and director of the Geobotanical Institute until his retirement in 1993. Interestingly, Prof. Landolt 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 duckweeds and more than 150 papers about other topics, mainly related to the flora of Switzerland and of Zurich in specific. This year marks a decade since he passed away on the 13th of April, 2013.

Elias Landolt collecting duckweed in Zurich, Switzerland, *Lemna minor* 9606

As an undergraduate student, Prof. Landolt was interested in microbiology as evident from his diploma thesis entitled "Synergismen im Bereich von Mikroorganismen" (Synergisms in the area of microorganisms). Later he investigated *Ranunculus montanus* and *R. breyninus* (crowfoot or buttercup), two species from the family Ranunculaceae for his Ph.D., the results of which were published in "Berichte der Schweizerischen Botanischen Gesellschaft" (Landolt, 1954). Thereafter, he succeeded for a postdoc stay as a Carnegie Fellow at Stanford University (CA, USA) and was also associated with the California Institute of Technology, the Earhardt Laboratory (1953-1955) in USA (Laemmler and Bogner 2014). The foundation for his passion for duckweeds was laid during this visit to the U.S., where a collection of diverse plant species already existed and curated samples numbered with a four-digit code from 0001 to approximately 6500. Prof. Landolt contributed his duckweed samples to this existing collection and continued using this code number system. Therefore, the lowest code number we found for duckweed listed in his clone collection was 6566, a *Lemna gibba* from USA which unfortunately was lost over time. Showcasing his keen interest and efforts to building a global collection of duckweeds, the highest number in his clone collection is 9928, a clone of *Lemna aequinoctialis* from Dhaka, Bangladesh, is still existing to date. Over the past seven decades, keeping living duckweeds in stock collections has turned out to be quite challenging due to the necessary human and financial resources. Early attempts in Prof. Landolt's group to store and secure duckweeds by cryopreservation were not fruitful. Only recently a few research groups have started experimenting in this direction and are beginning to produce positive results (Olah et al., 2023). Nevertheless, Prof. Landolt recognized very early on that the duckweed clones can have large intraspecific variabilities concerning their physiological properties

and this led to his efforts towards collecting a large number of clones belonging to each duckweed species from various geographical locations. Interestingly, his first paper on duckweed (Landolt, 1955) described heterotrophic growth of duckweed species in which he measured relative growth rates for several clones of the species *Lemna minor*, *L. valdiviana*, *L. perpusilla* and *Spirodela polyrhiza* under different light conditions and in darkness. However, the manuscript does not give many details and was written in German language as it was common at that time by authors from Switzerland, Germany and Austria. Two years later, in 1957, Prof. Landolt published the detailed results of his habilitation (Dr. sc. nat., Doctor of science) under the title "Physiologische und ökologische Untersuchungen an Lemnaceen" (Physiological and ecological investigations of Lemnaceae) in "Berichte der Schweizerischen Botanische Gesellschaft", also in German (Landolt, 1957). This publication, having 139 printed pages, is like an overture to a classical opera presenting all themes that will be later unfolded. As such, this paper (Landolt, 1957) described duckweed-related topics that were to be investigated by the field over the next 6 decades. Having an interest in ecology, he carried out physiological experiments under defined laboratory conditions that simulated natural conditions, addressing the concepts of evolutionary adaptation of plants to the environment. He investigated as many as 80 clones belonging to 13 duckweed species under axenic ("sterile") conditions. This publication (Landolt, 1957) is a rich source of data concerning the influence of nutrient, light, and temperature on growth, flowering and turion formation, and supplemented with morphological descriptions and ecological observations on duckweeds.

Elias Landolt inoculating duckweeds

During the investigations, it was unavoidable for him to realize the underdeveloped taxonomic description of duckweeds, which he investigated and resolved to a great extent over the remaining time of his scientific career. He especially focused on resolving complexity in the section *Lemna* (for present definition cf. Bog et al., 2020), meaning the "group" *L. minor*. As a first step, he divided *L. minor* s.l. (= sensu lato, in the broad sense) into two species: *L. minor* I (forms turions, flowers under certain conditions and forms anthocyanin) and *L. minor* II (no turion formation, rarely flowering, rarely pigmented). Years later (Landolt,1975; 1980a), he returned to these problems and after investigated in more details, separated the species into *L. minor* s.s. (= sensu stricto, in the strict sense) and *L. turionifera* Landolt sp. nov., the turion forming (literally carrying turions) *Lemna*. It should be noted that Prof. Landolt based his classification on nothing more than morphological markers and physiological responses to uncover the similarity and dissimilarity between duckweed species, and from which to deduce taxonomic relationships. Unfortunately, the story of the *L. minor* "group" is even more complicated than was known at his time. A few clones that were investigated by Prof. Landolt are still available to date. As an example, one of the first collected clones was registered as *L. minor* 6580 and characterized as *L. minor* II (no turions). This clone was re-investigated by the group of Laura Morello, Milan, Italy, using the molecular method of tubulin-based polymorphism and revealed that it is a hybrid between *L. minor* and *L. turionifera*, i.e. belonging to the species *L. japonica* (Braglia et al., 2021). Without using the word "hybrid", Prof. Landolt wrote "The species combines characteristics of *L. minor* and *L. turionifera*" (Landolt, 1980b). He also mentioned that hybrids in the family Lemnaceae can easily survive because of dominant vegetative propagation strategy of duckweeds. Certainly, this would have been very challenging to prove using only morphological or physiological investigations. Still, at the present time, the *L. minor* "group" is not yet well understood. In a genome sequencing consortium led by Todd P. Michael's group (The Salk Institute, La Jolla, CA, USA), about 60 clones of this group have now been sequenced as well as investigated using several different molecular and

cytological approaches. The final evaluation of these data will hopefully shed light on the taxonomy of the *L. minor* "group", almost 70 years after Landolt became aware that the taxonomy of this group is highly complex.

A part of the Landolt Duckweed Collection in Zurich

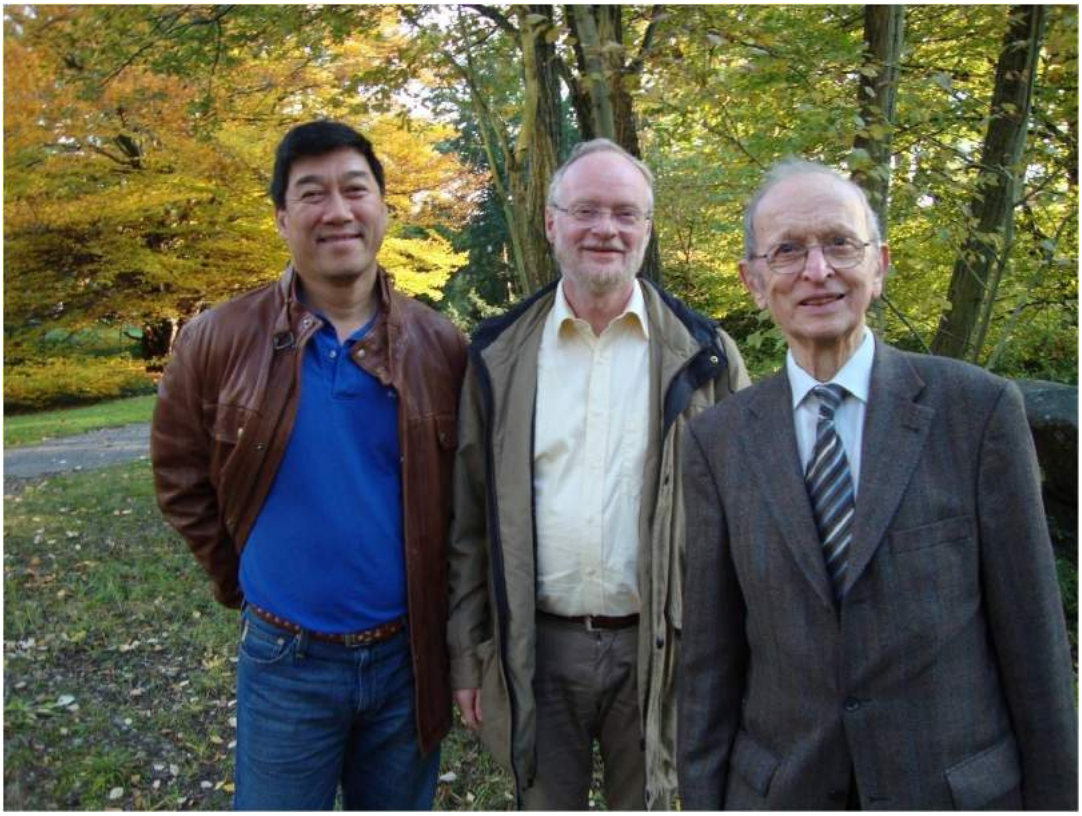
Prof. Landolt investigated the Lemnaceae family as a whole by visiting a large number of countries, especially after his retirement, and inspecting all available herbaria that he could get his hands on (e.g. Landolt, 1980a; Landolt and Lawalrée, 1988; Landolt, 1992). He described several new duckweed species (cf. Sree et al., 2016) and importantly, he never hesitated to revise his own earlier scientific conclusions. For example, when it became clear that the name *L. minuscula* has to be replaced by the older name *L. minuta* (Reveal 1990), or that the spelling of *S. polyrrhiza* was not in agreement with taxonomic rules and has to be replaced by *S. polyrhiza* (Appenroth et al. 1990), he did not hesitate for a moment. The same happened, when one of the authors (KJA) showed him the very first analysis of *L. yungensis* by AFLP, a species newly defined in 1998 (Landolt 1998d), indicating that there is no significant difference between this species and *L. valdiviana* (cf. Bog et al., 2020b, c). He commented that the original definition of the species was not based on very solid data but mainly on the unusual place of origin, i.e. wet rocks instead of a stagnant water body. In the very last weeks of his life, he considered separating the species *Spirodela biperforata* Koch from *Spirodela intermedia* (Koch, 1932, 1933) based on their morphology. Together with him we decided to test this hypothesis using molecular methods. Unfortunately, he passed away before we reached the conclusion by using molecular taxonomic analyses (three plastidic markers and AFLP), that this separation has no genetic basis (Bog et al., 2015).

In general, Prof. Landolt was very much interested in modern taxonomic methods to be applied on the Lemnaceae. He supported Daniel J. Crawford (University of Kansas, USA) and Donald H. Les (University of Connecticut, USA) and their co-workers to start analysing the chemical composition of flavonoids (Les et al., 1997) and isoforms of enzymes (allozymes) in duckweeds, an advanced form of chemotaxonomy at that time (Les et al., 1997; Crawford et al., 2001). These authors continued to investigate duckweed species and helped to start the modern period of molecular taxonomy of duckweeds using plastidic and nuclear barcoding (Les et al., 2002; Tippery et al., 2015). Further progress in this field was reported by several other research groups, among others (Borisjuk et al., 2015; Bog et al., 2019; and references therein). We are always impressed to find that Prof. Landolt's taxonomic conclusions based solely on morphological and physiological investigations are usually in agreement with the findings using molecular taxonomy by later studies.

In one point, however, Prof. Landolt was adamant: the definition of duckweed as a plant family of its own, i.e. Lemnaceae (Bog et al. 2013). This family was first described in 1820 by Martinov. The Angiosperm Phylogeny Group (APG) suggested to integrate duckweeds as subfamily in the superfamily Araceae, Lemnoideae, based on plastidic barcoding. According to Appenroth et al. (2015) "To circumvent this rather philosophical debate, one can separate the small group of Protoaraceae together with the group of Lemnaceae from the 'true Araceae' (as these two groups are the most basal elements in this group), which results in three monophyletic plant families, i.e. true Araceae, Lemnaceae and Protoaraceae. Arguments for keeping the taxonomic level of Lemnaceae instead of Lemnoideae were summarised by Appenroth et al. (2013) after thorough discussion with Elias Landolt". When we separate the small group of protoaraceae as Orontiaceae from the Araceae s.l., then Lemnaceae are not "nested" anymore as subfamily within the superfamily Araceae and can be kept as a distinct family (Tippery et al. 2021) – in accordance with the existing taxonomic rules and the opinion of Prof. Landolt.

As mentioned already above, Prof. Landolt started collecting duckweeds during his time as a postdoc in California, USA, and continued with his collection until the end of his life, compiling more than 1000 living duckweed clones over the years. Moreover, he collected tens of thousands of herbarium samples that are now mainly integrated into the Herbarium of the ETH Zurich. Parts of the live duckweed collection are now kept at the Rutgers University, NJ, USA (Eric Lam), at the University of Jena, Germany (Klaus-J. Appenroth), and the Landolt Duckweed Collection in Zurich was transferred in 2021 to the Institute of Agricultural Biology and Biotechnology, National Research Council, Milan, Italy (Laura Morello). A description of duckweed collections at various locations around the world is given in the newsletter "Duckweed Forum" "Registration and Collections of duckweed clones / strains", last update 2022, *10*, 21-22. Prof. Landolt not only collected duckweed clones but also identified them with his available methods, mostly on the basis of morphological characteristics. He had an "exceptional gift of assessing diagnostic characteristics of plants" (Laemmler and Bogner, 2014). As a result, the duckweed community inherited from him an invaluable resource for investigations in the present and future (Acosta et al., 2021).

In 1986, Prof. Landolt summarized his rich knowledge of the duckweed literature in the monograph "The family of Lemnaceae - A monographic study" (Vol. 1) and one year later the second volume, together with Riklef Kandeler (Appenroth, 2021; Landolt, 1986, Landolt and Kandeler, 1987). Although these two volumes were published almost four decades back, with approximately 3000 references they are indispensable to read as a rich source of detailed information for all professional duckweed researchers. They are indeed tangible legacies of Elias Landolt's lasting contribution to the modern era of duckweed research.



Eric Lam (Left) and Klaus Appenroth (Middle) together with Elias Landolt (Right) on a visit to Zurich

Duckweed biography of Elias Landolt

This biography was ordered chronologically

Landolt E. (1955) Über das Wachstum in der Dunkelheit bei einigen Lemnaceen. *Verhandlungen der Schweizerischen Naturforschenden Gesellschaft* 135, 135–136.

Landolt E. (1957a) Physiologische und ökologische Untersuchungen an Lemnaceen. *Berichte der Schweizerischen Botanischen Gesellschaft* 67, 271–410.

Landolt E. (1957b) Instruction for collecting and dispatch of living tropical Lemnaceae. *Flora Malesiana Bulletin* 13, 568 - 568. ISSN 0071-5778.

Landolt E. (1975) Morphological differentiation and geographical distribution of the *Lemna gibba–Lemna minor* group. *Aquatic Botany* 1, 345–363.

Landolt E., Wildi O. (1977) Ökologische Felduntersuchungen bei Wasserlinsen (Lemmnaceae) in den südwestlichen Staaten der USA. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **44**, 104–146.
Landolt E. (1979) *Lemna minuscula* Herter (= *L. minima* Phil.), eine in Europa neu eingebürgerte amerikanische Wasserpflanze. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **46**, 86–89.
Landolt E. (1980a) Key to the determination of taxa within the family of Lemnaceae – Biosystematic investigations in the family of duckweeds (Lemnaceae) vol. 1. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübe, Zürich* **70**, 13–21.
Landolt E. (1980b) Description of six new species of Lemnaceae. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübel, Zürich* **70**, 22–29.
Landolt E. (1980c) Bibliographie der Familie der Lemnaceae. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübel, Zürich* **70**, 142–204.
Landolt E., Urbanska-Worytkiewicz K. (1980d) List of the studied Lemnaceae samples: origin and chromosome numbers. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübel, Zürich* **70**, 205–247.
Landolt E. (1981) Distribution pattern of the family Lemnaceae in North Carolina. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübel, Zürich* **77**, 112–148.
Landolt E. (1982a) Distribution pattern and ecophysical characteristics of the European species of the Lemnaceae. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **49**, 127–145.
Landolt E. (1982b) Distribution patterns within the family Lemnaceae. In: *Studies on Aquatic Vascular Plants* (Symoens J. J., Hooper S. S. & Compère P., eds). Royal Botanical Society of Belgium, Brussels, Belgium: 313.
Landolt E., Dann W. (1983) Vergleich von zehn Klonen von *Lemna gibba* bei verschiedenen Stickstoffkonzentrationen. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel*, **50** 86–96.
Landolt E. (1984a) Flowers and fruits in the genus *Wolffiella* (Lemnaceae). *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **51**, 164–172.
Landolt E. (1984b) Verbreitungsmuster in der Familie der Lemnaceae und ihre ökologische Deutung. *Verhandlungen der Gesellschaft für Ökologie* **12**, 241–253.
Uotila P., Baytop A., Landolt E. (1984) Duckweeds (Lemnaceae) in Turkey. *Webbia* **38**, 839–844.
Landolt E. (1985) Höhendifferenzierung einiger Artengruppen von Blütenpflanzen in Fettwiesen des Davoser Gebietes (Graubünden, Schweiz) (vorläufige Mitteilung). *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **52**, 117–139.
Landolt E. (1986) The family of Lemnaceae – a monographic study, vol. 1. Biosystematic investigations in the family of duckweeds (Lemnaceae) vol. 2. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübel, Zürich* **71**, 566.
Landolt E. (1987) Eco-geographical differentiation in some aquatic plants: The Lemnaceae. In: *Differentiation Patterns in Higher Plants* (Urbanska K.M., ed). Academic Press, London, UK: 201-215.
Landolt E., Kandeler R. (1987) The family of Lemnaceae – a monographic study, vol. 2. Biosystematic investigations in the family of duckweeds (Lemnaceae) vol. 4. *Veröffentlichungen des Geobotanischen Instituts der Eidg. Tech. Hochschule, Stiftung Rübel, Zürich* **95**, 1–638.
Landolt E. (1988) Wasserlinsen, die kleinsten Blütenpflanzen der Erde. *Mitteilungen der Naturforschenden Gesellschaft Bern* **45**, 188–189.
Landolt E., Lawalrée A. (1988) Lemnaceae. In: *Flore d'Afrique centrale (Zaire - Rwanda - Burundi) Spermatophytes* (Bamps P., ed). Jardin botanique national de Belgique, Meise, Belgium: 1–12.
Landolt E. (1989) Geographisch-ökologisches Vorkommen der Lemnaceae-Arten in der Schweiz im Rahmen ihrer Gesamtverbreitung. *Flora* **182**, 87–98.
Landolt E. (1990) Über zwei seit kurzer Zeit in Europa neu beobachtete *Lemna*-Arten. *Razprave* **31**, 127–135.
Crawford D.J., Landolt E. (1992) Allozyme diversity within and divergence among species of *Spirodela* (Lemnaceae). *American Journal of Botany* **79** (6, suppl.), 139
Landolt E. (1992a) The flowers of *Wolffia australiana* (Lemnaceae). *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel*, **58**, 132–137.
Landolt E. (1992b) *Lemna tenera* Kurz, a little known species of Lemnaceae. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **58**, 124–131.
Landolt E. (1992c) Lemnaceae duckweed family. *Journal of the Arizona-Nevada Academy of Science* **26**, 10–14.
Landolt E. (1992d) *Wolffiella caudata*, a new Lemnaceae species from the Bolivian Amazon region. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel* **58**, 121–123.
Crawford D.J., Landolt E. (1993) Allozyme Studies in *Spirodela* (Lemnaceae): Variation among conspecific clones and divergence among the species. *Systematic Botany* **18**, 389–394.
Landolt E. (1993) Species names in current use in the Lemnaceae (Monocotyledones). In: *NCU-2: Names in Current Use in the Families Trichocomaceae, Cladoniaceae, Pinaceae and Lemnaceae* (Greuter W., ed). Koeltz Scientific Books, Königstein, Germany: 147–150.
Crawford D.J., Landolt E. (1994) Allozyme diversity within and divergence among species of *Wolffia* (Lemnaceae). *American Journal of Botany*, **81**(6, suppl.), 150.
Landolt E. (1994a) The Lemnaceae of Zimbabwe and Botswana. *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel*, **60**, 110–136.
Landolt E. (1994b) Taxonomy and Ecology of the Section *Wolffia* of the Genus *Wolffia* (Lemnaceae). *Berichte des Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel*, **60**, 137–151.
Landolt E., Zarzycki K. (1994) Ecological field investigations of duckweed (Lemnaceae) in Argentina. *Berichte des*

Geobotanischen Institutes der Eidg. Techn. Hochschule, Stiftung Rübel, **60**, 62–109.
Les D.H., Landolt E., Crawford D.J. (1994) Molecular systematics of the Lemnaceae. *American Journal of Botany*, **81**(6, suppl.), 168–169.
Wolff P., Landolt E. (1994) Spread of *Lemna turionifera* (Lemnaceae), the red duckweed, in Poland. *Fragmenta Floristica et Geobotanica*, **39**, 439–451.
Crawford D.J., Landolt E. (1995) Allozyme divergence among species of *Wolffia* (Lemnaceae). *Plant Systematics and Evolution*, **197**, 59–69.
Crawford D.J., Landolt E., Les D., Tepe E. (1995) Allozyme divergence among species of *Wolffiella* (Lemnaceae). *American Journal of Botany*, **82**(6, suppl.), 122–123.
Crawford D.J., Landolt E., Les D.H. (1996) An allozyme study of two sibling species of *Lemna* (Lemnaceae) with comments on their morphology, ecology and distribution. *Bulletin of the Torrey Botanical Club*, **123**, 1–6.
Landolt E. (1996a) Duckweeds (Lemnaceae): Morphological and Ecological Characteristics and their Potential for Recycling of Nutrients. *Environmental Research Forum*, **5-6**, 289–296.
Landolt E. (1996b) Lemnaceae S. F. Gray. In: *Flora Fanerogámica Argentina* (Gray S. F., ed). Córdoba, Argentina: 1–8.
Crawford D.J., Landolt E., Les D.H., Tepe E. (1997) Allozyme variation and the taxonomy of *Wolffiella*. *Aquatic Botany*, **58**, 43–54.
Landolt E. (1997a) How do Lemnaceae (duckweed family) survive dry conditions? *Bulletin of the Geobotanical Institute ETH*, **63**, 25–31.
Landolt E. (1997b) Lemnaceae. In: *Flora of Ethiopia and Eritrea* (Edwards S., Tadesse M. & Hedberg I., eds). Empda, Addis Ababa, Ethiopia: 51–54.
Les D.H., Crawford D.J., Landolt E., Aakjar R., Tepe E. (1997a) Systematics of Lemnaceae revisited. *American Journal of Botany*, **84**(6, suppl.), 211.
Les D.H., Landolt E., Crawford D.J. (1997b) Systematics of the Lemnaceae (duckweeds): inferences from micromolecular and morphological data. *Plant Systematics and Evolution*, **204**, 161–177.
Landolt E. (1998a) Anatomy of the Lemnaceae (duckweeds). In: *Handbuch der Planzenanatomie - Extreme Adaptations in Angiosperms Hydrophytes* (Carlquist S., Cutler D.F., Fink P., Ozenda P., Roth I., Ziegler H., eds). Gebrüder Borntraeger, Berlin, Germany: 1–127.
Landolt E. (1998b) *Lemna yungensis*, a new duckweed species from rocks of the Andean Yungas in Bolivia. *Bulletin of the Geobotanical Institute ETH*, **64**, 15–21.
Landolt E. (1998c) *Lemna*, *Spirodela*, *Wolffia*. In: *Standardliste der Farn- und Blütenpflanzen Deutschlands* (Wisskirchen R., Haeupler H., eds). Ulmer, Stuttgart, Germany: 287–288, 489, 549.
Landolt E. (1998d) Lemnaceae. In: *The Families and Genera of Vascular Plants* (Kubitzki K., ed). Springer, Berlin, Germany: 264–270.
Landolt E. (1999) Pleustonic communities with Lemnaceae in South America. *Applied Vegetation Science*, **2**, 7–16.
Landolt E. (2000a) Contribution on the Lemnaceae of Ecuador. *Fragmenta Floristica et Geobotanica*, **45**, 221–237.
Landolt E. (2000b) Flora North America Editorial Committee. Lemnaceae Gray. Duckweed Family. *Flora of North America-North of Mexico* **22**, 143-153.
Crawford D.J., Landolt E., Les D.H., Kimball R.T. (2001) Allozyme studies in Lemnaceae: variation and relationships in *Lemna* sections Alatae and Biformes. *Taxon*, **50**, 987–999.
Landolt E. (2001) Lemnaceae. In: *Flora of Thailand* (Santisuk T. & Larsen K., eds). Bangkok, Thailand: 394–399.
Les D.H., Crawford D.J., Landolt E., Gabel J.D., Kimball R.T. (2002) Phylogeny and systematics of Lemnaceae, the duckweed family. *Systematic Botany*, **27**, 221–240.
Kimball R.T., Crawford D.J., Les D.H., Landolt E. (2003) Out of Africa: molecular phylogenetics and biogeography of *Wolffiella* (Lemnaceae). *Biological Journal of the Linnean Society*, **79**, 565–576.
Les D.H., Crawford D.J., Kimball R.T., Moody M.L., Landolt E. (2003) Biogeography of discontinuosly distributed hydrophytes: a molecular appraisal of intercontinental disjunctions. *International Journal of Plant Sciences*, **164**, 917–932.
Crawford D.J., Landolt E., Les D.H., Archibald J.K., Kimball R.T. (2005) Allozyme variation within and divergence between *Lemna gibba* and *L disperma*: Systematic and biogeographic implications. *Aquatic Botany*, **83**, 119–128.
Crawford D.J., Landolt E., Les D.H., Kimball R.T. (2006) Speciation in duckweeds (Lemnaceae): phylogenetic and ecological inferences. *Aliso*, **22**, 231–242.
Landolt E., Schmidt-Mumm U. (2009) Lemnaceae. In: *Flora de Colombia* (Betancur J., Galeano G., Aguirre C. J., eds). Universidad Nacional de Colombia, Bogotá, Colombia: 1–54.
Bog M., Baumbach H., Schween U., Hellwig F., Landolt E., Appenroth K.-J. (2010) Genetic structure of the genus *Lemna* L. (Lemnaceae) as revealed by amplified fragment length polymorphism. *Planta*, **232**, 609–619.
Li H., Landolt E. (2010) Lemnaceae. In: *Flora of China* (Wu Z., Raven P. H., Hong D., eds). Science Press, Missouri Botanical Garden, Beijing, China and St. Louis, USA: 80–83.
Landolt E. (2011) Lemnaceae. In: *Flora of Australia - Alismatales to Arales* (Wilson A., ed), ABRS and CSIRO Publishing, Canberra and Melbourne, Australia: 274–282.
Bog M., Schneider P., Hellwig F., Sachse S., Kochieva E. Z., Martyrosian E., Landolt E., Appenroth K.-J. (2013) Genetic characterization and barcoding of taxa in the genus *Wolffia* Horkel ex Schleid. (Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). *Planta*, **237**, 1–13.
Bog M., Lautenschlager U., Landrock M.F., Landolt E., Fuchs J., Sree K.S., Oberprieler C., Appenroth K.J. (2015) Genetic characterization and barcoding of taxa in the genera *Landoltia* and *Spirodela* (Lemnaceae) by three plastidic markers and amplified fragment length polymorphism (AFLP). *Hydrobiologia* **749**, 169-182.

Landolt E., Velásquez J., Laemmler W., Gordon E. (2015) The family of Lemnaceae in Venezuela - La familia Lemnaceae en Venezuela. *Acta Botanica Venezuelica* **38**, 113-158.

Further cited references

Acosta K., Appenroth K.J., Borisjuk L., Edelman M., Heinig U., Jansen M.A.K., Oyama T., Pasaribu B., Schubert I., Sorrels S., et al. (2021) Return of the Lemnaceae: Duckweed as a model plant system in the genomics and postgenomics era. *Plant Cell* **33**, 3207–3234.

Appenroth K.J. (2021) Historical Account: Riklef Kandeler (1927 – 2015). *Duckweed Forum* **9**, 5-8.

Appenroth K.J., Borisjuk N., Lam E. (2013) Telling duckweed apart: genotyping technologies for Lemnaceae. *Chinese Journal of Applied and Environmental Biology* **19**, 1–10.

Appenroth, K. J., Crawford, D. J., and Les, D. H. (2015). After the genome sequencing of duckweed – how to proceed with research on the fastest growing angiosperm? *Plant Biology* **17**, 1–4.

Appenroth K.J., Hertel W., Augsten H. (1990) Phytochrome control of turion formation in *Spirodela polyrhiza* L. Schleiden. *Annals of Botany* **66**, 163-168.

Bog M., Appenroth K.J., Sree K.S. (2019) Duckweed (Lemnaceae): Its molecular taxonomy. *Frontiers in Sustainable Food System* **3**,117.

Bog M., Appenroth K.J., Sree K.S. (2020a) Key to the determination of taxa within the family of Lemnaceae: An update. *Nordic Journal of Botany* **38**, e02658.

Bog M, Lautenschlager U, Landrock MF, Landolt E, Fuchs J, Sree KS, Oberprieler C, Appenroth KJ (2015) Genetic characterization and barcoding of taxa in the genera *Landoltia* and *Spirodela* (Lemnaceae) by three plastidic markers and amplified fragment length polymorphism (AFLP). *Hydrobiologia* **749**, 169–182.

Bog M., Schneider P., Hellwig F., Sachse S., Kochieva E. Z., Martyrosian E., et al. (2013). Genetic characterization and barcoding of taxa in the genus *Wolffia* Horkel ex Schleid. (Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). *Planta* **237**, 1–13.

Bog M., Sree K.S., Fuchs, J., Hoang, P.T.N., Schubert, I., Kuever, J. Rabenstein, A., Paolacci, S., Jansen M.A.K., Appenroth K.J. (2020b) A taxonomic revision of *Lemna* sect. *Uninerves* (Lemnaceae). *Taxon* **69**, 56-66.

Bog M., Xu S., Himmelbach, A., Brandt R., Wagner F., Appenroth K.J., Sree K.S. (2020) Genotyping-by-Sequencing for species delimitation in *Lemna* section *Uninerves* Hegelm. (Lemnaceae) pp. 115-123 In: X. H. Cao et al. (eds.), The Duckweed Genomes, Compendium of Plant Genomes. Springer Nature Switzerland.

Borisjuk N., Chu P., Gutierrez R., Zhang H., Acosta K., Friesen N., et al. (2015). Assessment, validation and deployment strategy of a two barcode protocols for facile genotyping of duckweed species. *Plant Biology* **17**, 42–49.

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

Crawford D.J., Landolt E., and Les, D.H. (1996). An allozyme study of two sibling species of Lemna (Lemnaceae) with comments on their morphology, ecology and distribution. *Bull. Torrey Bot. Club* **123**, 1–6.

Les D.H., Crawford D.J. (1999) *Landoltia* (Lemnaceae), a new genus of Duckweeds. *Novon* **9**, 530-533.

Les D.H., Crawford D.J., Landolt E., Gabel J.D., Kimball, R.T. (2002). Phylogeny and systematics of Lemnaceae, the duckweed family. Systematic Botany **27**, 221–240.

Les D. H., Landolt E., Crawford D. J. (1997). Systematics of the Lemnaceae (duckweeds): inferences from micromolecular and morphological data. *Plant Systematic Evolution* **204**, 161–177.

Koch W. 1932. Beitrag zur Lemnaceen-flora mittel und Suedamerikas. *Berichte der Schweizerischen Botanischen Gesellschaft* **41**, 113–118.

Koch W. 1933. *Spirodela biperforata*, eine neue Teichlinse aus Surinam. *Berichte der Schweizerischen Botanischen Gesellschaft* **42**, 186–189.

Laemmler W., Bogner J. (2014) Elias Landolt and the duckweeds. Aroideana **37**, 80-88.

Martinov I. (1820) Techno-Botanical Dictionary (Техно-Ботанический Словарь); Pechashano v Imperatorskoi Tipografii: Saint Petersburg, Russia.

Olah V., Appenroth K.J., Lam E., Sree K.S. (2023) Sixth International Conference on Duckweed Research and Applications presents Lemnaceae as a model plant system in the genomics and postgenomics era. *Plants* (**submitted**)

Reveal J.L. (1990) The neotypification of *Lemna minuta* Humb., Bonpl. & Kunth, an earlier name for *Lemna minuscula* Herter (Lemnaceae). *Taxon* **39**, 328-330.

Sree K. S., Bog M., Appenroth K.J. (2016) Taxonomy of duckweeds (Lemnaceae), potential new crop plants. *Emirate Journal of Food and Agriculture* **28**, 291–302.

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

Tippery N.P., Les D.H., Crawford D.J. (2015). Evaluation of phylogenetic relationships in Lemnaceae using nuclear ribosomal data. *Plant Biology* **17**, 50–58.

Biographic references about Elias Landolt

Honegger, R. (2013) Elias Landolt (24. Juli 1926 – 1. April 2013) Prof. Dr. sc. nat. ETH. Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich **158**, 105–106.

Laemmler, W., Bogner, J. (2014) Elias Landolt and the duckweeds. *Aroideana* **37**, 80-88.
Urbańska, K. (1986) Elias Landolt – Wissenschaftler, Dozent, Kamerad. In: Krystyna Urbańska (Hrsg.): Aspekte der Geobotanik. Festschrift Elias Landolt. ETHZ, Geobotanisches Institut Zürich, p. 7–16.
Urbanska, K.M., Crawford, D.J., Appenroth K.J., Les, D.H. (2013) Obituary Elias Landolt(July21,1926–April1,2013). *Aquatic Botany* **111**, A1-A2.
https://de.wikipedia.org/wiki/Elias_Landolt_(Botaniker)

Duckweed Research and Applications for the Circular Bioeconomy in Ireland

UCC – June 9, 2023

The duckweed group at University College Cork is pleased to host a small workshop on "Duckweed Research and Applications for the Circular Economy in Ireland". This will be an in-person, one-day workshop with loads of discussion and networking opportunities.

The workshop will take place on Friday June 9th, 2023, at University College Cork, Ireland. This event is FREE but places are limited. Places are allocated on a first come first served basis. If interested, please register in advance by emailing Sandra Jansen – a.jansen@ucc.ie

Further information may be obtained from Prof. Marcel Jansen (m.jansen@ucc.ie)

Programme schedule

9.15	9.25	Marcel Jansen – opening
Introducing Lemnaceae		
9.25	9.50	Klaus-J. Appenroth Friedrich Schiller University, Jena, Germany Cultivation of duckweed under sub-optimal conditions
9.50	10.15	Laura Morello Instituto Biologia e Biotecnologia Agraria, Milano, Italy Family affairs in duckweeds, stories of promiscuous species
10.15	10.55	Coffee / tea break
Duckweed Applications I		
10.55	11.15	Patrick Barrett Department of Agriculture, Food and the Marine Creating and shaping the context of Ireland's bioeconomy
11.15	11.35	Vlastimil Stejskal University of South Bohemia in České Budějovice, South Bohemia, Czechia Mt Lucas; a novel multitrophic concept for the cultivation of fish and duckweed

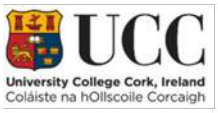
11.35	12.05	Niall O'Leary University College Cork, Ireland Integrating *Lemna* cultivation with microbial bioreactor systems for dairy processing wastewater valorisation
12.05	12.25	Gruffydd Lloyd-Jones Aberystwyth University, Wales Incorporating duckweed into livestock production systems: Optimising yield and nutrient recovery efficiency
12.25	13.35	lunch

The morning programme is funded by the European Regional Development Fund through the Ireland Wales Cooperation Programme

12.25	13.35	lunch
The Art of Growing Duckweed		
13.35	13.55	Finn Petersen Hochschule Osnabrück, Germany Technicalities and functionality of a re-circulating indoor vertical farm for duckweed biomass production
13.55	14.15	Neil Coughlan University College Cork, Ireland Challenges and opportunities for large-scale indoor cultivation of Lemnaceae
14.15	14.35	Reindert de Vlamynck Inagro vzw, Belgium Applying duckweed in the field – lessons from pilot scale cultivation
14.35	14.45	Viktor Oláh University of Debrecen, Debrecen, Hungary To monoculture or to co-culture: is there a difference in biomass productivity?

14.45	15.15	Coffee / tea break
Duckweed Applications II		
15.15	15.35	Paul Fourounjian International Lemna Association, Denville, USA The American way
15.35	15.45	Meritxell Abril Cuevas BETA Technological Centre from the University of Vic-Central University of Catalonia, Barcelona, Spain Use of duckweed to treat and valorise pig manure under natural Mediterranean climate conditions
15.45	16.05	Brijesh Tiwari Teagasc, Ashtown, Ireland A zero waste approach for processing of duckweeds
16.05	16.15	Priya Pollard Bantry Marine Research Station Ltd An unorthodox way towards duckweed preservation: Ensilage
16.15	16.45	Discussion and closure

The afternoon programme is funded by the Environmental Protection Agency (Ireland)



Visit of ISCDRA to Colombo, Sri Lanka

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

[1]Department of Environmental Science, Central University of Kerala, Periye, India
[2]Matthias Schleiden Institute-Plant Physiology, Friedrich Schiller University Jena, Germany

The members of the International Steering Committee on Duckweed Research and Applications (ISCDRA), Klaus-J. Appenroth and K. Sowjanya Sree, visited the Inland Aquatic Resources and Aquaculture Division (IARAD) at the National Aquatic Resources Research and Development Agency (NARA), Colombo, Sri Lanka in December, 2022. Dr. Kamal Tennakoon, Director General, NARA, warmly welcomed the team. Dr. Prajani Heenatigala, Head of the IARAD, organized a workshop on Duckweed research and Applications at NARA in which Klaus-J. Appenroth from Friedrich Schiller University of Jena, Germany, delivered a talk on the topic "Duckweed resurgence as a model plant" and K. Sowjanya Sree from Central University of Kerala, India, delivered a talk on "Recent advances in duckweed research and applications". The workshop was well attended with an active participation of researchers. These two invited talks presented an overview on the research and application of the tiny aquatic plants duckweeds that offer a great potential.

Prajani Heenatigala

K. Sowjanya Sree

Klaus-J. Appenroth

The talks were followed by a visit of the ISCDRA and the NARA teams to the duckweed cultures being grown at NARA and several practical problems about growth and culture of duckweeds were discussed. A few basics about the start of the duckweed research were practically shown in the laboratory, including morphology of duckweeds, isolation of duckweed clones, culturing and inoculation of duckweeds. The literature and publications concerning these and several other broad areas of research in the field of duckweeds were also provided.

The ISCDRA was aware of past duckweed research at NARA especially that from Dr. Soma Ariyaratne who had retired from NARA, Sri Lanka. She had delivered a talk entitled "Duckweed powder as a replacement for fish meal in the feed used in Tilapia fry rearing" at the 3rd International Conference on Duckweed Research and Applications which was held at Kyoto in 2015. Application of duckweed as fish feed was their area of research. Currently efforts are being made to rejuvenate the duckweed research at NARA. Dr. Prajani Heenatigala who is currently heading the IARAD at NARA had obtained her Ph.D., working with duckweeds, from the group of Hongwei Hou, China and had developed efficient protocol for stable and transient gene transformation for *Wolffia globosa* using Agrobacterium which was published in the special issue focused on duckweeds in the journal Frontiers in Chemistry in 2018.

The ISCDRA hopes that the duckweed research at NARA, Colombo, Sri Lanka will be reactivated under the leadership of Dr. Prajani Heenatigala and efforts will be made to utilize the possible collaborative funding that would be available in the near future.

K. Sowjanya Sree demonstrating about duckweed in the lab at NARA, colombo

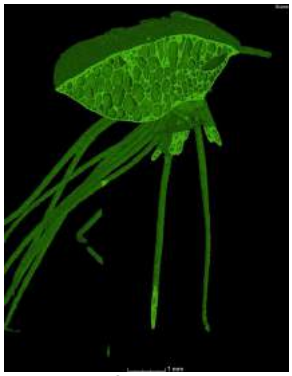
7th ICDRA: Announcement

Student Spotlight: Dylan Jones

Crop Science Centre, University of Cambridge, UK
(Email: dj400@cam.ac.uk)

After doing my undergraduate degree at the University of Nottingham, with a focus on Arabidopsis, I stayed on to do my PhD in the same division. I was first introduced to duckweed during a rotation project when choosing my PhD topic, and was extremely taken with the opportunities it offered and how different it was! As Tony Bishopp had just recently set up the Nottingham duckweed research project, I was able to join this project. I was then able to incorporate my work on duckweed into my thesis, investigating the relationship between monocot anatomy and water. Tony's interest in duckweed fronds from the developmental questions surrounding the loss of root traits, and ultimately the loss of roots entirely that we see across the genera. I was really interested in the vision for duckweed's application as a model for investigating the phenomenon of organ loss and as a tool to investigate other developmental questions.

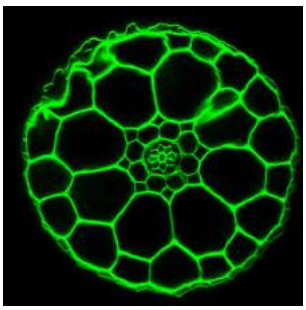
As duckweed represent part of the extreme end of the plant morphological spectrum (especially in the root), and have adapted to an aquatic environment, I found that they made an interesting point of comparison to land and low water environment adapted plants at the other end of this spectrum.



3D render of *Spriodela intermedia* from µCT scanning[1]

There have been several technological advances recently that have enabled high throughput comparisons of plant anatomical traits, facilitating candidate gene identification and functional analysis. These have largely not been applied in investigations of duckweed yet, and during my PhD I aimed to use some of these techniques in duckweed. As my lab was still quite new to duckweed, I was able to be involved in developing the growth and phenotyping systems we would use going forward. We developed methods for aseptic duckweed growth and propagation integrated with growth rate phenotyping, root and frond sectioning, clearing and microscopy, and µCT scanning, which have been used over the past 4 years in our investigations.

With Alex Ware, and other members of Tony's lab, I have used these techniques in investigating the extent to which duckweed roots are vestigial. We conducted a survey of root anatomy, across nearly all rooted duckweeds, and found a significant reduction in all root traits compared to Pistia, and that root traits have been lost between Spirodela, Landoltia, and Lemna sequentially. Interestingly, this was not the case for vascular traits, as they were already massively reduced in Spirodela. We combined this with root function analysis, ionomic, and gene expression analysis to determine the level of vestigialization of duckweed roots.



Lemna gibba root cross section from the root anatomy survey[2]

In addition to the scientific benefits of duckweed research, I found the novelty of this research to be of great interest to colleagues and the general public. The unique properties of duckweed and how common and overlooked they are in the wild presents a great opportunity to engage with a broader audience about the importance of plant research and the potential for novel discoveries. Working with duckweed has been an exciting and rewarding experience for me. The unique characteristics of duckweed presented new challenges and opportunities for research that I had not encountered before. Developing the phenotyping methods used in our investigations was an engaging and fulfilling experience, and I am excited to continue working on novel species and applying these skills in future research projects.

[1]https://www.frontiersin.org/articles/10.3389/fpls.2020.617830/full

[2]https://www.cell.com/current-biology/fulltext/S0960-9822(23)00313-5?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0960982223003135%3Fshowall%3Dtrue

From the Database

Highlights

Loss of ancestral function in duckweed roots is accompanied by progressive anatomical reduction and a re-distribution of nutrient transporters

Ware, A; Jones, DH; Flis, P; Chrysanthou, E; Smith, KE; Kumpers, BMC; Yant, L; Atkinson, JA; Wells, DM; Bhosale, R; Bishopp, A. Current Biology (2023) DOI10.1016/j.cub.2023.03.025

Organ loss occurs frequently during plant and animal evolution. Sometimes, non-functional organs are retained through evolution. Vestigial organs are defined as genetically determined structures that have lost their ancestral (or salient) function. Duckweeds, an aquatic monocot family, exhibit both these characteristics. They possess a uniquely simple body plan, variably across five genera, two of which are rootless. Due to the existence of closely related species with a wide diversity in rooting strategies, duckweed roots represent a powerful system for investigating vestigiality. To explore this, we employed a panel of physiological, ionomic, and transcriptomic analyses, with the main goal of elucidating the extent of vestigiality in duckweed roots. We uncovered a progressive reduction in root anatomy as genera diverge and revealed that the root has lost its salient ancestral function as an organ required for supplying nutrients to the plant. Accompanying this, nutrient transporter expression patterns have lost the stereotypical root biased localization observed in other plant species. While other examples of organ loss such as limbs in reptiles or eyes in cavefish frequently display a binary of presence/absence, duckweeds provide a unique snapshot of an organ with varying degrees of vestigialization in closely related neighbors and thus provide a unique resource for exploration of how organs behave at different stages along the process of loss.

Use of hemicellulose-derived xylose for environmentally sustainable starch production by mixotrophic duckweed

Sun, ZL; Zhao, XY; Xia, ML; Yang, JJ; Chen, Y; Li, XZ; Hou, HW. Sustainable Energy and Fuels (2022) 7: 641-651.

Duckweeds are aquatic plants with considerable potential as substrates for environmentally sustainable production of bioenergy and proteins because of their extremely high starch and protein yields, and nutrient uptake capability from various wastewaters. d-Xylose is a common sugar that exists on earth and the main sugar element of lignocellulosic hydrolysate. Efficient utilization of xylose is necessary to reduce the high cost of duckweed-based biofuel production. In this study, the potential use of d-xylose by duckweed was tested for heterotrophy and mixotrophy. The results showed that duckweed was capable of mixotrophic growth but not heterotrophic growth on d-xylose, which suggested that photosynthesis was necessary for d-xylose metabolism, with 2.8-fold increases in biomass yields compared with that of photoautotrophy. Furthermore, d-xylose markedly boosted starch accumulation up to 9.7 times higher than in photoautotrophic duckweed, and promoted nutrient recovery efficiency. The present study showed that mixotrophic duckweed could use inorganic and organic carbon simultaneously, offering an attractive strategy to enhance the duckweed biomass production with a higher carbon capture efficiency from the surrounding environment, thus boosting starch and protein productivity for bioenergy and food conversion. The method developed in this study demonstrated novel applications in duckweed biomass-based renewable bioenergy production with inorganic and organic carbon capture combined with potential organic carbon wastewater treatment. This will enable the recycling of different carbons for various uses and realize a circular economy.

Metabolic flexibility during a trophic transition reveals the phenotypic plasticity of greater duckweed (*Spirodela polyrhiza* 7498)

Sun, ZL; Zhao, XY; Li, GJ; Yang, JJ; Chen, Y; Xia, ML; Hwang, I; Hou, HW . New Phytologist (2023) DOI10.1111/nph.18844

The greater duckweed (*Spirodela polyrhiza* 7498) exhibits trophic diversity (photoautotrophic, heterotrophic, photoheterotrophic, and mixotrophic growth) depending on the availability of exogenous organic carbon sources and light. Here, we show that the ability to transition between various trophic growth conditions is an advantageous trait, providing great phenotypic plasticity and metabolic flexibility in *S. polyrhiza* 7498. By comparing *S. polyrhiza* 7498 growth characteristics, metabolic acclimation, and cellular ultrastructure across these trophic modes, we show that mixotrophy decreases photosynthetic performance and relieves the CO_2 limitation of photosynthesis by enhancing the CO_2 supply through the active respiration pathway. Proteomic and metabolomic analyses corroborated that *S. polyrhiza* 7498 increases its intracellular CO_2 and decreases reactive oxygen species under mixotrophic and heterotrophic conditions, which substantially suppressed the wasteful photorespiration and oxidative-damage pathways. As a consequence, mixotrophy resulted in a higher biomass yield than the sum of photoautotrophy and heterotrophy. Our work provides a basis for using trophic transitions in *S. polyrhiza* 7498 for the enhanced accumulation of value-added products.

Aquaculture/ Agriculture

A promising ash supplementation strategy in the cultivation of *Spirodela polyrrhiza* plants

Romanowska-Duda, Z; Piotrowski, K; Stepinski, D; Poplonska, K. Cells (2023) 12: 289.

An innovative approach to the management of waste in the form of ash obtained during biomass combustion is justified due to its specific properties, including the presence of macro- and microelements. The aim of the current study was to determine the concentration of ash obtained from *Sorghum* combustion regarding its fertilizer value and its effect on the cytological structures, physiological parameters, growth and development of Lemnaceae plants, thereby demonstrating the possibility of using this waste to supplement culture media. The analyses showed that the use of ash in the in vitro cultivation of Lemnaceae aquatic plants had a dose-dependent effect. The addition of 2% ash favorably affected the condition of plant roots, i.e., meristem elongation and an increase in nucleoli sizes as well as improving the chlorophyll content index, gas exchange parameters, chemical oxygen demand (COD) and plant vigor via PSII, which was confirmed by a chlorophyll fluorescence measurement. On the other hand, too high of a concentration, i.e., 10% ash, adversely affected the plant development and parameters studied. Concluding, the use of ash at a low concentration favorably affected the yielding of *Spirodela polyrrhiza*, whose biomass can be used for energy purposes in the production of bioethanol, plant biogas or the phytoremediation of industrial waters and leachate.

Comment DF: The correct spelling of the species is *Spirodela polyrhiza*

Biochemistry

Physicochemical properties of a new starch from turion of *Spirodela polyrhiza*

Wang, X; Cheng, L; Li, Z; Li, C; Ban, X; Gu, Z; Hong, Y. International Journal of Biological Macromolecules (2022) 223: 1684-1692.

Starch from the turion of *Spirodela polyrhiza* 0196 is a new resource with great development values. The solubility, swelling power, thermogravimetric analysis (TGA), enzyme susceptibility, and in vitro digestibility of *Spirodela* starch, paste clarity, settling volume, and the rheology property of *Spirodela* starch paste, strength, elasticity, and freeze-thaw stability of Spirodela starch gel were studied. The properties of *Spirodela* starch were compared with those of normal corn starch and rice starch. The possible relationship between properties and structure of three kinds of starch was analyzed by principal component analysis (PCA). The results showed that the rapidly digested starch, slowly digested starch and resistant starch were 86.63 %, 4.43 %, and 8.94 % respectively. *Spirodela* starch has higher strength (17.77 g), elasticity (4.98 g/s) of starch paste, and freeze-thaw stability of starch gel than those of normal corn starch and rice starch. The rheological results

showed that the stickiness maintainability of starch paste was normal corn > *Spirodela* > rice and *Spirodela* starch paste had compact paste structures with the tan d between 0.33 and 0.38. This study has significant value in the application of *Spirodela* starch.

Efficient accumulation of amylopectin and its molecular mechanism in the submerged duckweed mutant

Liu, Y; Yan, RT; Li, ZH; Fan, SS; Li, CT; Yu, RK; Liu, HQ; Kong, YZ; Li, HM; Tang, XF; Zhou, GK. International Journal of Molecular Sciences (2023) 24: 2934.

Large-scale use of fossil fuels has brought about increasingly serious problems of environmental pollution, development and utilization of renewable energy is one of the effective solutions. Duckweed has the advantages of fast growth, high starch content and no occupation of arable land, so it is a promising starchy energy plant. A new submerged duckweed mutant (sub-1) with abundant starch accumulation was obtained, whose content of amylopectin accounts for 84.04% of the starch granules. Compared with the wild type (*Lemna aequinoctialis*), the branching degree of starch in sub-1 mutant was significantly increased by 19.6%. Chain length DP 6-12, DP 25-36 and DP > 36 of amylopectin significantly decreased, while chain length DP 13-24 significantly increased. Average chain length of wild-type and sub-1 mutant starches were greater than DP 22. Moreover, the crystal structure and physical properties of starch have changed markedly in sub-1 mutant. For example, the starch crystallinity of sub-1 mutant was only 8.94%, while that of wild-type was 22.3%. Compared with wild type, water solubility of starch was significantly reduced by 29.42%, whereas swelling power significantly increased by 97.07% in sub-1 mutant. In order to further analyze the molecular mechanism of efficient accumulation of amylopectin in sub-1 mutant, metabolome and transcriptome were performed. The results showed that glucose accumulated in sub-1 mutant, then degradation of starch to glucose mainly depends on alpha-amylase. At night, the down-regulated beta-amylase gene resulted in the inhibition of starch degradation. The starch and sucrose metabolism pathways were significantly enriched. Up-regulated expression of SUS, AGPase2, AGPase3, PYG, GPI and GYS provide sufficient substrate for starch synthesis in sub-1 mutant. From the 0H to 16H light treatment, granule-bound starch synthase (GBSS1) gene was inhibited, on the contrary, the starch branching enzyme (SBE) gene was induced. Differential expression of GBSS1 and SBE may be an important reason for the decrease ratio of amylose/amylopectin in sub-1 mutant. Taken together, our results indicated that the sub-1 mutant can accumulate the amylopectin efficiently, potentially through altering the differential expression of AGPase, GBSS1, SBE, and BAM. This study also provides theoretical guidance for creating crop germplasm with high *amylopectin* by means of synthetic biology in the future.

Overexpression of the phosphoserine phosphatase-encoding gene (AtPSP1) promotes starch accumulation in *Lemna turionifera* 5511 under sulfur deficiency

Wang, L; Kuang, YY; Zheng, SY; Tong, YA; Zhu, YR; Wang, Y. Plants (2023) 12: 1012.

Duckweeds are well known for their high accumulation of starch under stress conditions, along with inhibited growth. The phosphorylation pathway of serine biosynthesis (PPSB) was reported as playing a vital role in linking the carbon, nitrogen, and sulfur metabolism in this plant. The overexpression of AtPSP1, the last key enzyme of the PPSB pathway in duckweed, was found to stimulate the accumulation of starch under sulfur-deficient conditions. The growth- and photosynthesis-related parameters were higher in the AtPSP1 transgenic plants than in the WT. The transcriptional analysis showed that the expression of several genes in starch synthesis, TCA, and sulfur absorption, transportation, and assimilation was significantly up- or downregulated. The study suggests that PSP engineering could improve starch accumulation in *Lemna turionifera* 5511 by coordinating the carbon metabolism and sulfur assimilation under sulfur-deficient conditions.

Biotechnology

Structural change kinetics, drying characteristics, antioxidant properties, and the correlation between quality parameters of dried duckweed (*Wolffia arrhiza* (L.) Wimm) affected by different levels of microwave power

Suebsamran, I; Dachyong, A; Tira-Umphon, A; Soubsub, K; Phahom, T. Journal of the Science of Food and Agriculture (2023) DOI10.1002/jsfa.12501.

Duckweed is considered as a future food material due to its fast growth, high yield, high nutritional value, and low impact on land use. However, in its fresh form, it has high moisture content (95% wet basis), resulting in a short shelf life. In this study, microwave drying (MWD) was conducted to produce a shelf-stable duckweed with minimal loss of quality. Drying characteristics and quality aspects of dried duckweed were assessed. Reaction order kinetics, including zero and first order, was applied to describe structural changes during drying process. Hierarchical cluster analysis (HCA) was used to select the appropriate drying conditions. Of five drying models, the Midilli-Kucuk model was the one that best described the drying process. Drying at high microwave power could reduce energy consumption and increase energy efficiency. Increasing both microwave power and drying time increased the structural shrinkage rate as described by first-order reaction kinetics. High correlations among quality parameters were observed using Pearson's correlation. Drying treatments were differentiated into two main clusters by HCA and the results showed that MWD at 720 and 900 W provided samples that were closer in terms of quality to a freeze dried sample (the positive control) than samples that had been subjected to MWD at 450 W. Drying behaviors of duckweed were well-described by the Midilli-Kucuk model. Microwave drying at 900 W gave the lowest energy consumption and displayed the most efficient use of energy. The first-order equation could be used effectively to describe the structural changes in the duckweed. Microwave drying at 720 and 900 W was the appropriate drying condition according to the HCA classification.

The study of co-combustion characteristics of coal and duckweed by single particle and TGA methods

Cao, J; Zhang, R; Shi, BQ; Shi, MZ; Zhang, LJ; Liu, D. Power Technology (2023) 421: 118410

Co-combustion of coal and biomass is a feasible technology to reduce CO2 emissions and coal consumption. In this paper, the co-combustion characteristics of coal and three kinds of duckweed (*Lemna minor*, *Wolffia*, *Spirodela*) were studied by a single particle combustion method and thermogravimetric analysis (TGA) method in air. The effects of blending ratio of the duckweed and fuel properties on the ignition delay time, burnout time and flame temperature were studied. The ignition delay time, burnout time and flame temperature all decrease as the blending ratio of duckweed increases. The different volatile content and composition of duckweed will affect the temperature distribution and combustion rate in the combustion stage. Compared with the ash of coal, the ash of duckweed can promote the combustion of coal. During the combustion of blended fuels with volatile removed, different contents of ash have different effects on char combustion, such as combustion temperature range, combustion rate, and temperature at peak combustion rate.

Biogas effluent treatment by *Landoltia punctata* for starch biomass production

Kirdponpattara, S; Kittiwongwattana, C; Phisalaphong, M; Chanroj, S; Sriariyanun, M. Environmental technology and Inovation (2023) 30: 103049

Due to the high potential of using *Landoltia punctata* in biogas effluent treatment and starch accumulation, three integrated processes were proposed: (1) bioethanol production from duckweed, (2) biogas production from vinasse, and (3) biogas effluent treatment with sequentially increasing starch-rich duckweed biomass. This recent work focuses only on the third process, which involves the treatment of wastewater from biogas effluent and the production of *L. punctata* with high starch content. *L. punctata* showed a significantly higher

TKN removal and biomass production than *Lemna aequinoctialis*. After nine days of wastewater treatment and five days of starch accumulation, *L. punctata* grew by 4.3 g/m^2/day, with 44.4% starch content. NH_3, NH^+_4, and PO^{3-}_4 in the biogas effluent were rapidly utilized in the first three days for *L. punctata*'s growth. The upregulation of AGPase genes in the starch biosynthesis pathway was observed when the environmental conditions changed in both nutrient starvation and population reduction. Production of starch-rich *L. punctata* using biogas effluent is a sustainable feedstock for the bioethanol process. Moreover, L. punctata-based stabilization pond is a promising technique for biogas effluent treatment.

The use of plants from the Lemnaceae family for biofuel production-A bibliometric and in-depth content analysis

Krzywonos, M; Romanowska-Duda, Z; Seruga, P; Messyasz, B; Mec, S. Energies (2023) 16: 2058.

Plants of the Lemnaceae family are becoming increasingly popular among researchers. The goal of the study was to characterize trends in scientific research related to the use of aquatic plants from the Lemnaceae family for energy purposes, especially for the production of biogas, bioethanol, and other biofuels. These plants fit perfectly into the concept of a circular economy. This study performed a bibliometric and in-depth content analysis to review the use of plants from the Lemnaceae family for biofuel production. A set of 666 articles published from 2008 to 2022 was identified from the Scopus and Web of Science databases. Different analytical scientometric tools (topic mapping and overlay visualization networks) were used to analyze 141 articles; the most influential countries, institutions, authors, journals, and articles were identified. Depth content analysis reveals five research areas: (i) development of duckweed growth and starch accumulation; (ii) development of the pretreatment techniques; (iii) development of ethanol fermentation; (iv) hydrothermal liquefaction and bio-oil production; and (v) anaerobic digestion and biogas production.

Improving biorefinery sustainability and profitability by cultivating aquatic plants on ozonized distillery effluents

Dziugan, P; Romanowska-Duda, Z; Piotrowski, K; Cieciura-Wloch, W; Antolak, H; Smigielski, K; Binczarski, M; Witonska, I; Domanski, J. Bioresources (2023) 18: 317-336.

Industrial production of biogas offers a way to manage distillery leachate. The waste is usually subjected to anaerobic digestion for producing biogas. However, the effluent from anaerobic processes has high chemical oxygen demand (COD) and is harmful to the environment. An effective method of lowering COD is ozonation. Effluent from biogas plants after ozonation has the potential for use in breeding grounds for plants of the Lemnaceae family. Thus, they can provide a valuable additional source of biomass for the production of bioethanol. *Lemna minor* L. and *Spirodela polyrhiza* cultures were grown in media with the addition of 2.5% PFE, which had been treated by ozonation for between 6 and 50 min. Using ozonated effluent was an effective cultivation technique in all variants. The analyzed parameters were plant growth, chlorophyll index, fresh plant weight and photosynthetic traits (net photosynthesis, stomatal conductance, transpiration and concentration of intercellular CO2). The best growth of *Lemna minor* L. was observed in the media with PFE treated for 12 min. Similar effects were obtained for *S. polyrhiza*, with ozone treatment for 12 and 25 min. The results show the potential of using ozone-treated post-fermentation leachate as a supplement in culture media.

Wolffia globosa as a biocatalyst in plant-based biofuel cells

Hubenova, Y; Hubenova, E; Mitov, M. Journal of Electrochemical Science and Engineering (2023) DOI10.5599/jese.1547

The rootless duckweed *Wolffia globosa*, not explored toward electrogenicity till now, is investigated as a putative biocatalyst in Plant-based Biofuel Cells (P-BFC) for the electrical current generation and its basic metabolic changes during the polarization are depicted. After a short adaptation period, the open-circuit voltage of P-BFC, utilizing *W. globosa* as an anodic biocatalyst, reaches values of 630 mV. At a connected external resistor of 1 kohm in the electric circuit, stable current densities of 170 ± 10 mA m^{-2} are achieved. The electrical outputs depend on the anodic potential, reaching negative values of ca.-200 mV (vs. SHE). *W. globosa* produces an electrochemically active compound, acting as an electron shuttle. The polarization

intensifies the *W. globosa* metabolism, expressed in a double increased glucose and starch content along with 1.82 times higher specific amylase activity of 70.0 ± 2.8 U g^{-1} wet biomass in the organelle-enriched fractions of the explored as biocatalysts plants compared to the control. The results reveal that *Wolffia globosa* can be utilized as a biocatalyst in P-BFC for simultaneous electricity generation and increased carbohydrate and protein content.

Speciation and transformation of nitrogen in the hydrothermal liquefaction of wastewater-treated duckweed for the bio-oil production

Zhao, KG; Li, WQ; Yu, YY; Chen, GY; Yan, BB; Cheng, ZJ; Zhao, H; Fang, Y. Renewable Energy (2023) 204: 661-670.

Bio-oil production from wastewater-treated duckweed by hydrothermal liquefaction (HTL) is a sustainable development mode. In this study, the effect of reaction conditions on nitrogen transformation during HTL of wastewater-treated duckweed for bio-oil production was investigated. GC-MS, FT-ICR-MS and XPS were used to characterize the nitrogen speciation in each product. The highest bio-oil yield of 34.7 wt% was obtained at 360°C, 60 min. The maximum high heating value (HHV) and energy recovery of bio-oil separately reached 36.41 MJ/kg and 86.11%. As the temperature increased (240°C-360°C), more nitrogen (21.14%-35.50%) migrated to the bio-oil and mainly existed in the form of nitrogen-containing heterocycles and amides. Higher temperatures favored the acylation reaction to produce more amides, while the Maillard reaction was hindered resulting in a decrease in the relative content of nitrogen-containing heterocycles. N2 species such as pyrazines, imidazole were the dominant organic nitrogen species. In the aqueous phase, the nitrogen recovery decreased from 44.7% to 39.3%, and pyridine, pyrimidine and pyrrolidine were the most abundant organic nitrogen forms. The nitrogen distributed to the solid residue decreased from 21.1% to 5.1%, with pyridine-N becoming the dominant nitrogen species at temperatures above 320°C.

Co-fermentation of *Lemna minor* with glucose and Diospyros kaki peels for hydrogen production

Oren, I; Argun, H. Biomass Conversion and Biorefinery (2023) DOI10.1007/s13399-023-03751-7.

Batch hydrogen gas production by co-fermentation of *Lemna minor* with glucose and Diospyros kaki peels was investigated. Box-Wilson statistical experiment design was used to configure the experiments. The aim was to determine the most suitable *Lemna minor*-glucose and *Lemna minor*-Diospyros kaki peel concentration blends resulting in the most convenient hydrogen production yield and rate. Two sets of experiments were performed. For the first set of experiments, most convenient hydrogen yield (77.34 mL H_2/ 10 mL) and rate (10.82 mL H_2/ h) were obtained by blending 40 g/L *Lemna minor* with 21 g/L glucose. Higher glucose concentration than 21 g/L at 40 g/L fixed *Lemna minor* caused substrate inhibition. For the second set of experiments, blending 40 g/L *Lemna minor* with 60 g/L Diospyros kaki peels resulted in maximum hydrogen yield and rate of 125.35 mL H_2/ 10 mL and 26.95 mL H_2/ h, respectively. Increasing the blend concentration of Diospyros kaki peels at fixed *Lemna minor* concentration did have an increasing effect on hydrogen yield and rate. The total sugar concentration did not exceed 21 g/L within the inspected ranges during the second set of experiments; therefore, 40 g/L *Lemna minor* was blended with 123 g/L Diospyros kaki peels which provided 21 g/L total sugar, and the maximum hydrogen yield (142.24 mL H_2/ 10 mL) and rate (21.34 mL H_2/ h) were obtained at this point. Model predictions and experimental results were reasonable. Blending *Lemna minor* with Diospyros kaki peels resulted in more efficient hydrogen production than blending with glucose.

Ecology

Metabolically active angiosperms survive passage through the digestive tract of a large-bodied waterbird

Paolacci, S; Jansen, MAK; Stejskal, V; Kelly, TC; Coughlan, NE. Royal Society Open Science (2023) 10: 230090

Avian vectors, such as ducks, swans and geese, are important dispersers of plant propagules. Until recently, it was thought that small vegetative propagules were reliant on adherence to vectors and are unlikely to survive passage through the avian digestive tract. Here, we conclusively demonstrate that metabolically active angiosperms can survive passage through the digestive tract of a large-bodied waterbird. In addition, we show that extended periods of air exposure for up to 7 days does not inhibit the survival of plantlets embedded in faecal matter. Following air exposure, plantlets (n = 3000) were recovered from 75 faecal samples of mute swans, *Cygnus olor*, with the survival of 203 plantlets. The number of recovered and surviving plantlets did not significantly differ among durations of air exposure. For recovered plantlets, the long-term viability and clonal reproduction of two duckweed species, *Lemna minor* and *L. gibba*, were confirmed following greater than eight months of growth. These data further amplify the key role of waterbirds as vectors for aquatic plant dispersal and demonstrate the internal transport (i.e. endozoochory) of metabolically active plantlets. These data suggest dispersal of vegetative plant propagules by avian vectors is likely to be a common occurrence, underpinning connectivity, range expansion and invasions of some aquatic plants.

Distribution and ecology of *Wolffia arrhiza* (L.) Horkel ex Wimm. in the lowland part of Lower Silesia (Poland)

Pietryka, M; Richter, D; Podlaska, M. Biologia (2023) 79: 971-978.

Genus *Wolffia* Horkel ex Schleiden (Araceae, former Lemnaceae) includes 11 species that are the smallest of known flowering plants. The genus is widespread in the world. In Europe, the only native species is *Wolffia arrhiza*. In Poland, it is considered to be relatively rare and until the end of the 20th century it was known from small number of populations dispersed throughout the country Poland. Our research focused on Lower Silesia population of *Wolffia arrhiza*. The study included the verification of historical sites of this species known from the literature and an inventory of the contemporary occurrence. It also aimed to explain the pattern of *W. arrhiza* occurrence in selected habitats and determine the ecological conditions and phytocoenoses of the reservoirs it inhabits. The research of the species showed extinction of historical sites and the current presence in 12 water reservoirs of different origin, size, depth and management method. The share of W. arrhiza in phytocoenosis patches in the individual reservoirs is very diverse and ranges from 15 to 80%. Presented data supplement the information on the occurrence of *W. arrhiza* in western Poland as well as provide new information on its biology and ecology.

The combination of untargeted metabolomics with response surface methodology to optimize the functional potential of common duckweed (*Lemna minor* L.)

Zhang, LL; Rocchetti, G; Zengin, G; Del Buono, D; Trevisan, M; Lucini, L. Antioxydants (2023) 12: 313.

The present study was designed to evaluate the functional potential of common duckweed (*Lemna minor* L.) as a source of bioactive compounds of nutraceutical interest. The untargeted profiling of the bioactive components of common duckweed was carried out through ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), in parallel with assessing in vitro antioxidant and enzymatic inhibition properties. The optimization of extraction parameters was determined using the response surface methodology (RSM) through a 3-factor central composite design. The process parameters included extraction temperature, % of ethanol, and ultrasound power, while the response variables were the phenolic content (considering each main phenolic class), total glucosinolates, total carotenoids, the antioxidant potential, and enzyme inhibition activities. The results revealed that common duckweed was a rich source of carotenoids and total flavonoids (mainly flavones and flavonols), followed by phenolic acids, low-molecular-weight phenolics, and glucosinolates. Interestingly, the total flavones, total flavonols and total carotenoid equivalents showed the highest and most positive correlation values with the bioactive properties measured. Finally, the combined RSM approach and unsupervised statistics allowed us to point out the pivotal impact of ethanol percentage in the extraction solvent to recover the highest amounts of bioactive compounds efficiently.

Feed & Food

Partial replacement of fishmeal with duckweed (*Spirodela polyrhiza*) in feed for two carnivorous fish species, Eurasian Perch (*Perca fluviatilis*) and Rainbow Trout (*Oncorhynchus mykiss*)

Stadtlander, T; Tschudi, F; Seitz, A; Sigrist, M; Refardt, D; Leiber, F. Aquaculture Research (2023), 6680943

Two four-week feeding trials were conducted with fingerlings of Eurasian perch (Perca fluviatilis, 3.52 ± 0.08 g) and rainbow trout (*Oncorhynchus mykiss*, 1.49 ± 0.05 g) fed with graded levels of dried (DWD) and fermented (DWF) duckweed meal (*Spirodela polyrhiza*). The purpose of these two trials was to evaluate DWD and DWF as replacements for fishmeal. Fishmeal protein was substituted by 12%, 24%, and 35% of duckweed protein and compared to control diets containing 40% (for perch) and 35% (for rainbow trout) fishmeal and no duckweed. The performance of the fish (growth, feed conversion, and protein and lipid utilization) and their whole-body composition were evaluated and compared with the control. While even the lowest inclusion level, regardless of its form (dried or fermented), resulted in significantly reduced performance in Eurasian perch, rainbow trout were able to utilize feed containing duckweed meal considerably well. Compared to the control, at a 12% inclusion level, rainbow trout showed an equal or comparable percent weight gain (PWG; DWD: 377%, DWF: 373%), specific growth rate (SGR; DWD: 4.37%/day, DWF: 4.33%/day), feed conversion ratio (FCR; DWD: 1.11, DWF: 1.12), and protein productive value (PPV; DWD: 21.5%, DWF: 21.2%). Increasing the inclusion levels above 12% of both DWD and DWF resulted in reduced performance in rainbow trout, with the most pronounced effects observed in the DWD35 group. All experimental diets, including control, affected the whole body composition of perch, most notably reducing the lipid content compared to initial fish. Compared to initial, control and DWD rainbow trout increased whole-body protein, lipid, and ash contents. In conclusion, for rainbow trout, fermented and dried S. polyrhiza duckweed meal appears to be a promising feed ingredient when used at a maximum inclusion level of 12%, while for Eurasian perch, it should not be considered as a feed ingredient.

Current status and nutritional value of green leaf protein

Balfany, C; Gutierrez, J; Moncada,; Komarnytsky, S. Nutrients (2023) 15: 1327

Green leaf biomass is one of the largest underutilized sources of nutrients worldwide. Whether it is purposely cultivated (forage crops, duckweed) or upcycled as a waste stream from the mass-produced agricultural crops (discarded leaves, offcuts, tops, peels, or pulp), the green biomass can be established as a viable alternative source of plant proteins in food and feed processing formulations. Rubisco is a major component of all green leaves, comprising up to 50% of soluble leaf protein, and offers many advantageous functional features in terms of essential amino acid profile, reduced allergenicity, enhanced gelation, foaming, emulsification, and textural properties. Nutrient profiles of green leaf biomass differ considerably from those of plant seeds in protein quality, vitamin and mineral concentration, and omega 6/3 fatty acid profiles. Emerging technological improvements in processing fractions, protein quality, and organoleptic profiles will enhance the nutritional quality of green leaf proteins as well as address scaling and sustainability challenges associated with the growing global demand for high quality nutrition.

Impact of pH on the growth and nutritional profile of *Lemna minor* L. as a sustainable alternative for Pakistan's feed sector

Ullah, H; Gul, B; Khan, H; Rehman, KU; Hameed, I; Zeb, U; Roomi, S; Zill-E-Huma. Aquaculture International (2023) DOI10.1007/s10499-023-01063-1

Pakistan's conventional feed supply is rapidly depleting, forcing the country to turn to non-conventional sustainable feed to meet the demand of the livestock and fishery sectors. The current study investigates the effect of pH on the nutritional composition of *Lemna minor* L., a freshwater macrophyte and a potential alternative feed. Fronds were grown in a pH range of 4-10 and replicated three times. The growth rate was highest (90 g m^{-2} day^{-1}) at pH 7 while lowest (40 g m^{-2} day^{-1}) at pH 4. Protein content dropped sharply at pH 4, but the highest (31 g/100 g) was found at pH 7 and 8. The lipid fraction was high at greater pH values (9, 10),

while low in acidic conditions. Carbohydrate was maximum (59.3 g/100 g dw). A higher level of Ca (34, 37, 38 mg/100 g dw) was observed in pH 5 and 6 while low Ca content (24 mg/100 g dw) was recorded for the control group. In contrast, the Mg level slightly increased with increasing pH while the maximum concentration (32 mg/100 g dw) was observed at pH 10 and in the control group. High Fe moiety (936 mg/100 g) was found at pH 5 and 6. Higher Mn (3.0 mg/100 g) was seen at a slightly acidic pH. The lowest level of Mn (1.7 mg/100 and 2.0 mg/100) was recorded at pH 9 and 10, respectively. The highest fraction of Zn (0.08 mg/100 g) was observed in pH 6 and 7 while the lowest Zn came at pH 9. The study concluded that *L. minor* grows best between pH 6 and 10 under the current agroclimatic conditions of Pakistan with an optimal nutritional profile.

Nutritional value of duckweed as protein feed for broiler chickens-digestibility of crude protein, amino acids and phosphorus

Demann, J; Petersen, F; Dusel, G; Bog, M; Devlamynck, R; Ulbrich, A; Olfs, HW; Westendarp, H. Animals (2023) 13: 130

An increasing global population will lead to an increased demand for protein and a protein deficit. The production of soy, the most common protein source in animal nutrition, is often associated with deforestation and long transport distances. In this context, duckweed can be considered an alternative protein source for animal nutrition. The term duckweed describes a group of small plants floating on the water surface with sizes up to 1.5 cm. Three different duckweed batches consisting of different species were tested as feed for broiler chickens. They showed different impacts on feed intake, animal growth, and digestibility. Digestibility describes the share of nutrients resorbed within the digestive system. Possible compounds that inhibited the digestibility were identified. The results suggest that the nutritional value of duckweed and its influence on animal performance are variable. Hence, a stable duckweed biomass quality adapted to the requirements of broilers is needed. Duckweed is gaining attention in animal nutrition and is considered as a potential alternative protein source for broiler chickens. In order to evaluate the nutritional value of duckweed, three individual batches were investigated. They consisted of a mixture of *Lemna minuta* and *Lemna minor* (A, 17.5% crude protein), *Spirodela polyrhiza* (B, 24.6% crude protein) and *Lemna obscura* (C, 37.0% crude protein). Treatment diets contained 50% batch A, 50% batch B, and 25, 50 and 75% of batch C. All diets were fed to broiler chickens (Ross 308) from an age of 21 to 27 days. Diets with a share of 50 and 75% of batch C led to decreased feed intake (109.3 and 74.9 g/day, respectively) compared to the control. Standardized ileal digestibility of crude protein and amino acids differed significantly between duckweed batches, at values for methionine between 49.9 and 90.4%. For all amino acids, batch A consistently had the lowest and batch C the highest digestibility. Batches had different tannin contents of 2943, 2890 and 303 mg/kg for batches A, B and C, respectively. The apparent ileal digestibility of phosphorus differed significantly between all batches (50.8-78.9%). Duckweed can be used as a protein feed for broiler chickens. However, a defined and stable biomass composition optimized for the requirements of broiler chickens is needed.

Lutein-fortified plant-based egg analogs designed to improve eye health: Formation, characterization, in vitro digestion, and bioaccessibility

Vu, G; Xiang, XK; Zhou, HL; McClements, DJ. Foods (2023) 12: 2.

Lutein is a carotenoid found in real eggs that has been reported to have beneficial effects on eye health by reducing the risk of age-related macular degeneration. However, lutein is not often included in plant based (PB) egg analogs. It would, therefore, be advantageous to fortify PB eggs with this health-promoting carotenoid. Moreover, lutein is a natural pigment with a bright red to yellowish color depending on its concentration and environment. It can, therefore, also be used as a plant-based pigment to mimic the desirable appearance of egg yolk. Some of the main challenges to using lutein as a nutraceutical and pigment in PB foods are its poor water-solubility, chemical stability, and bioavailability. In this study, we encapsulated lutein in oil-in-water emulsions, which were then utilized to formulate whole egg analogs. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) protein isolated from a sustainable plant-based source (duckweed) was used to mimic the thermally irreversible heat-set gelling properties of globular egg proteins, with the aim of obtaining a similar cookability and texture as real eggs. The lutein content (80 mg/100 g) of the egg analogs

was designed to be at a level where there should be health benefits. The protein (12.5 wt.%) and oil (10 wt.%) contents of the egg analogs were selected to match those of real egg. The effects of oil droplet size and oil type on the bioaccessibility of the encapsulated lutein were examined using the INFOGEST in vitro digestion model. For the emulsions formulated with long chain triglycerides (LCTs, corn oil), lutein bioaccessibility significantly increased when the initial droplet diameter decreased from around 10 to 0.3 μm, which was attributed to more rapid and complete digestion of the lipid phase for smaller droplets. For medium chain triglycerides (MCTs), however, no impact of droplet size on lutein bioaccessibility was observed. A high lutein bioaccessibility (around 80%) could be obtained for both LCTs and MCTs emulsions containing small oil droplets. Thus, both types of oil can be good carriers for lutein. In summary, we have shown that lutein-fortified PB eggs with good digestibility and bioaccessibility can be created, which may play an important role in ensuring the health of those adopting a more plant-based diet.

Interaction with other organisms

Endophytic microbiota of floating aquatic plants: recent developments and environmental prospects

Pramanic, A; Sharma, S; Dhanorkar, M; Prakash, O; Singh, P. World Journal of Microbiology and Biotechnology (2023) 39: 96

Plant-associated microorganisms play a critical role in plant survival and functional attributes. There are many studies on the taxonomical and functional aspects of microorganisms associated with terrestrial plants. However, the microbiome of aquatic plants is not much explored. This work details the studies on microbiomes and diversity in microbial communities inhabiting the three common free-floating aquatic plants of tropical regions viz. duckweed, water hyacinth and water lettuce, widely implicated for their bioremediation potential. Studies conducted till date reveal the prevalence and dominance of different *Bacillus* sp. Other genera, including *Rhodanobacter, Pseudomonas, Rhizobium, Achromobacter, Serratia, Actinobacteria, Proteobacter, Klebsiella* and *Acidobacteria*, have also been prominently reported. This lesser explored niche offers great bioprospecting opportunities to obtain taxonomically diverse and functionally distinct microorganisms. Bacterial endophytes from these aquatic plants have been primarily studied for their ability to produce indole acetic acid and degrade phenol. Limited studies reveal some fungal endophytes to have promising herbicidal effect. Not much is known on other functional attributes and hence microbial studies on these plants holds much promise for obtaining novel isolates or isolates with novel functions that would impact both aquatic and terrestrial ecosystems. This study proposes the need for exploring the role of endophytes as biocontrol agents and their potential to provide a pragmatic and robust solution to the aquatic weed menace in freshwater bodies. Bioprospecting of this lesser studied ecological niche hence is a promising field of research that has both environmental and economic potential.

Non-negligible effect of native *rhizobacteria* on cooperation with plant growth regulators improve tolerance to cadmium: A case study using duckweed *Spirodela polyrhiza* as indicating plant

Mu, DM; Lin, WT; Luo, JF. Journal of Plant Growth Regulation (2023) DOI10.1007/s00344-023-10954-9

Plant growth regulators (PGRs) are well known for their ability to improve plants' tolerance to heavy metals. However, in these treatments, the contribution of native rhizobacteria on heightened tolerance to heavy metals is usually not considered, and thus PGR ability is overestimated. In this study, using duckweed *Spirodela polyrhiza* as indicating plant and according to the responses of it grown under nonsterile and sterile conditions, native *rhizobacteria* were able to cooperate with PGRs and thereby improved duckweed's tolerance to Cd stress. All the randomly selected bacterial isolates and salicylic acid exerted synergistic effects that improved Cd tolerance, suggesting that active *rhizobacteria* are not necessarily plant growth-promoting *rhizobacteria* (PGPRs). Comparative physiological and transcriptomic analyses showed that the cooperation between the native bacterium *Pseudomonas* and salicylic acid can greatly reduce Cd accumulation and decrease oxidative stress, thus lowering the requirement of antioxidant defense in duckweed; moreover, the synthesis of

flavonoids that facilitates reactive oxygen species scavenging in duckweed was significantly induced. This study recommends that the contribution of native *rhizobacteria* on improved plant tolerance to heavy metals should be considered when applying PGRs.

Optimization of molecular methods for detecting duckweed-associated bacteria

Acosta, K; Sorrels, S; Chrisler, W; Huang, WJ; Gilbert, S; Brinkman, T; Michael, TP; Lebeis, SL; Lam, E. Plants (2023) 12: 872.

The bacterial colonization dynamics of plants can differ between phylogenetically similar bacterial strains and in the context of complex bacterial communities. Quantitative methods that can resolve closely related bacteria within complex communities can lead to a better understanding of plant-microbe interactions. However, current methods often lack the specificity to differentiate phylogenetically similar bacterial strains. In this study, we describe molecular strategies to study duckweed-associated bacteria. We first systematically optimized a bead-beating protocol to co-isolate nucleic acids simultaneously from duckweed and bacteria. We then developed a generic fingerprinting assay to detect bacteria present in duckweed samples. To detect specific duckweed-bacterium associations, we developed a genomics-based computational pipeline to generate bacterial strain-specific primers. These strain-specific primers differentiated bacterial strains from the same genus and enabled the detection of specific duckweed-bacterium associations present in a community context. Moreover, we used these strain-specific primers to quantify the bacterial colonization of duckweed by normalization to a plant reference gene and revealed differences in colonization levels between strains from the same genus. Lastly, confocal microscopy of inoculated duckweed further supported our PCR results and showed bacterial colonization of the duckweed root-frond interface and root interior. The molecular methods introduced in this work should enable the tracking and quantification of specific plant-microbe associations within plant-microbial communities.

Microbial population dynamics in Lemnaceae (duckweed)-based wastewater treatment system

Singh, P; Jani, K; Sharma, S; Rale, V; Souche, Y; Prakash, S; Jogdeo, P; Patil, Y; Dhanorkar, MN. Current Microbiology (2023) 80: 56.

The dynamic microflora associated within, and in the surrounding aquatic environment, has been found to be responsible for the functional properties of many aquatic plants. The aim of the current work was to evaluate the effectiveness of Lemnaceae-based wastewater treatment system under tropical conditions and investigate the changes in the aquatic microflora upon plant growth. A biological wastewater treatment system was designed and investigated using mixed Lemnaceae culture comprising *Lemna minor* and *Spirodela polyrhiza* in a batch mode. A significant reduction in total solids (31.8%), biochemical oxygen demand (93.5%), and chemical oxygen demand (73.2%) was observed after seven days of duckweed growth using a low inoculum. A preliminary study on the change in the microbial population diversity and functionality, in the wastewater before and after treatment, revealed an increase in the denitrifying microflora in wastewater post-Lemnaceae treatment. Dominance of 10 bacterial phyla, contributing for 98.3% of the total bacterial communities, was recorded, and similar to 50.6% loss of diversity post-treatment of wastewater was revealed by the Shannon Index. Among 16 bacterial families showing relative abundance of ≥ 1% in untreated wastewater, Methylobacteriaceae, Pseudomonadaceae, Brucellaceae, Rhodobacteraceae, and Acetobacteraceae prevailed in the water post-treatment by duckweeds. This is a novel work done on the dynamics of aquatic microflora associated with Lemnaceae under tropical Indian conditions. It confirms the application of Lemnaceae-based wastewater treatment system as effective biofilter and calls for further studies on the active involvement of the endophytic and aquatic microflora in the functions of these plant.

Live under strong power: A third plant species alters interspecific interactions between two plant species

Peng, L; Xue, W; Yu, FH. Ecological indicators (2023) 146: 109758.

Organisms from higher tropical levels (e.g., herbivores) can mediate indirect competitive interactions between two target plant species, but such apparent competition may also be mediated by a third plant species and thus may vary depending on the size and competitive ability of the target plant species and the third plant species. We set up an outdoor experiment with four aquatic plant species with similar niches but differing greatly in size, i.e., *Lemna minor* (the smallest), *Spirodela polyrhiza* (the second smallest), *Salvinia natans* (the second largest) and *Pistia stratiotes* (the largest). We grew *L. minor* and *S. polyrhiza* alone or in mixture and in the absence of any third plant species, in the presence of a small, third species *S. natans* or in the presence of a large, third species *P. stratiotes*. In the absence of the third species or in the presence of *S. natans*, the growth of *L. minor* was greatly inhibited by *S. polyrhiza*, but in the presence of *P. stratiotes*, the inhibiting effects of *S. polyrhiza* disappeared completely and the growth of *L. minor* greatly increased. By contrast, the presence of *L. minor* had no effect on the growth of *S. polyrhiza*, and this effect did not depend on the presence or absence of the third plant species, although the presence of the third species, particularly *P. stratiotes*, decreased its growth. We conclude that the presence of a third plant species can regulate plant-plant interactions, but such an impact depends on the size and competitive ability of the competing species as well as the third species. Our findings highlight the role of complexity in plant-plant interactions, and suggest that apparent competition between plants can also occur at the same trophic level. These results have important implications for the explanations of species coexistence and biodiversity maintenance.

Mitigating the growth of plant pathogenic bacterium, fungi, and nematode by using plant-mediated synthesis of copper oxide nanoparticles (CuO NPs)

Khan, M; Khan, AA; Parveen, A; Min, KM; Yadav, VK; Khan, AU; Alam, M. Green Chemistry Letters and Reviews (2023) 16: 2177520.

Nanoparticles of copper oxide were synthesized from the *Spirodela polyrrhiza* (greater duckweed) plant. Ultraviolet spectroscopy absorbance of around 400 nm was used for nanoparticle characterization. The presence of copper and oxygen in the biogenic nanoparticles was analyzed by the EDX technique. Based on scanning, and transmission electron microscopy (SEM and TEM) results, copper oxide nanoparticles (CuO NPs) are evenly distributed and spherical, and their sizes are in the 100 nm range. The synthesized nanoparticles of copper oxide were tested for antibacterial, antifungal, and nematicidal effectiveness against plants pest and pathogens. Antifungal and nematicidal activity against pests and plant pathogens was observed, with fungal mycelium reduced by 45-50% and eggs hatching. The findings suggest that plant-based copper oxide nanoparticles could be a potential source of plant pathogen inhibition. From this study, we concluded that synthesized CuO NPs have the potential to serve as a safer alternative for plant disease management.

Comment DF: The correct spelling of the species is *Spirodela polyrhiza*

Diazotrophic bacterium *Azotobacter vinelandii* as a mutualistic growth promoter of an aquatic plant: *Lemna minor*

Shuvro, SK; Jog, R; Morikawa, M. Plant Growth regulation (2023) DOI10.1007/s10725-022-00948-0

Lemnaceae plants, commonly referred to as duckweeds, are small planktonic terrestrial freshwater plants that live in symbiosis with various microbial communities. *Azotobacter vinelandii* are typical free-living nitrogen fixing soil bacteria that indirectly benefit plants by providing nitrogen compounds. In this study, *Lemna minor* RDSC 5512 and *A. vinelandii* ATCC 12837 = NBRC 13581 were co-cultured under gnotobiotic conditions. The growth of *L. minor* colonized by *A. vinelandii* was accelerated in both nitrogen-containing and nitrogen-free water conditions. The growth promotion effect is attributed to several plant growth promotion factors produced by the bacterium as well as biological nitrogen fixation in nitrogen-free condition. Moreover, *L. minor* elevated the nitrogen fixing activity of *A. vinelandii* and the cell number of *A. vinelandii* on *L. minor* increased continuously over 30 d. These observations indicated that *L. minor* provides a favorable environment for *A. vinelandii* colonization, allowing them to mutually benefit and flourish through syntrophism.

Molecular Biology

A non-cell-autonomous circadian rhythm of bioluminescence reporter activities in individual duckweed cells

Watanabe, E; Muranaka, T; Nakamura, S; Isoda, M; Horikawa, Y; Aiso, T; Ito, S; Oyama, T. Plant Physiology (2023). DOI10.1093/plphys/kiad218

The circadian clock is responsible for the temporal regulation of various physiological processes in plants. Individual cells contain a circadian oscillator consisting of a clock gene circuit that coordinates physiological rhythms within the plant body in an orderly manner. The coordination of time information has been studied from the perspective of cell-cell local coupling and long-distance communication between tissues based on

the view that the behavior of circadian oscillators represents physiological rhythms. Here, we report the cellular circadian rhythm of bioluminescence reporters that are not governed by the clock gene circuit in expressing cells. We detected cellular bioluminescence rhythms with different free-running periods in the same cells using a dual-color bioluminescence monitoring system in duckweed (*Lemna minor*) transfected with *Arabidopsis* CIRCADIAN CLOCK ASSOCIATED 1::luciferace+(AtCCA1::LUC+) and Cauliflower mosaic virus 35S::modified click-beetle red-color luciferase (CaMV35S::PtRLUC) reporters. Co-transfection experiments with the two reporters and a clock gene-overexpressing effector revealed that the AtCCA1::LUC+rhythm, but not the CaMV35S::PtRLUC rhythm, was altered in cells with a dysfunctional clock gene circuit. This indicated that the AtCCA1::LUC+rhythm is a direct output of the cellular circadian oscillator, whereas the CaMV35S::PtRLUC rhythm is not. After plasmolysis, the CaMV35S::PtRLUC rhythm disappeared, whereas the AtCCA1::LUC+rhythm persisted. This suggests that the CaMV35S::PtRLUC bioluminescence has a symplast/apoplast-mediated circadian rhythm generated at the organismal level. The CaMV35S::PtRLUC-type bioluminescence rhythm was also observed when other bioluminescence reporters were expressed. These results reveal that the plant circadian system consists of both cell-autonomous and non-cell-autonomous rhythms that are unaffected by cellular oscillators.

Polyploidy impacts population growth and competition with diploids: multigenerational experiments reveal key life-history trade-offs

Anneberg, TJ; O'Neill, EM; Ashman, TL; Turcotte, MM. New Phytologist (2023) 238: 1294-1304.

Ecological theory predicts that early generation polyploids ('neopolyploids') should quickly go extinct owing to the disadvantages of rarity and competition with their diploid progenitors. However, polyploids persist in natural habitats globally. This paradox has been addressed theoretically by recognizing that reproductive assurance of neopolyploids and niche differentiation can promote establishment. Despite this, the direct effects of polyploidy at the population level remain largely untested despite establishment being an intrinsically population-level process. We conducted population-level experiments where life-history investment in current and future growth was tracked in four lineage pairs of diploids and synthetic autotetraploids of the aquatic plant *Spirodela polyrhiza*. Population growth was evaluated with and without competition between diploids and neopolyploids across a range of nutrient treatments. Although neopolyploid populations produce more biomass, they reach lower population sizes and have reduced carrying capacities when growing alone or in competition across all nutrient treatments. Thus, contrary to individual-level studies, our population-level data suggest that neopolyploids are competitively inferior to diploids. Conversely, neopolyploid populations have greater investment in dormant propagule production than diploids. Our results show that neopolyploid populations should not persist based on current growth dynamics, but high potential future growth may allow polyploids to establish in subsequent seasons.

The immediate effects of polyploidization of *Spirodela polyrhiza* change in a strain-specific way along environmental gradients

Bafort, Q; Wu, T; Natran, A; De Clerck, O; Van de Peer, Y. Evolution Letters (2023) 7: 37-47.

The immediate effects of plant polyploidization are well characterized and it is generally accepted that these morphological, physiological, developmental, and phenological changes contribute to polyploid establishment. Studies on the environmental dependence of the immediate effects of whole-genome duplication (WGD) are, however, scarce but suggest that these immediate effects are altered by stressful conditions. As polyploid establishment seems to be associated with environmental disturbance, the relationship between ploidy-induced phenotypical changes and environmental conditions is highly relevant. Here, we use a common garden experiment on the greater duckweed *Spirodela polyrhiza* to test whether the immediate effects of WGD can facilitate the establishment of tetraploid duckweed along gradients of two environmental stressors. Because successful polyploid establishment often depends on recurrent polyploidization events, we include four genetically diverse strains and assess whether these immediate effects are strain-specific. We find evidence that WGD can indeed confer a fitness advantage under stressful conditions and that the environment affects ploidy-induced changes in fitness and trait reaction norms in a strain-specific way.

Examination of the metallothionein gene family in Greater Duckweed *Spirodela polyrhiza*

Pakdee, O; Tshering, S; Pokethitiyook, P; Meetam, M. Plants (2023) 12: 125.

Duckweeds are aquatic plants that proliferate rapidly in a wide range of freshwaters, and they are regarded as a potential source of sustainable biomass for various applications and the cost-effective bioremediation of heavy metal pollutants. To understand the cellular and molecular basis that underlies the high metal tolerance and accumulation capacity of duckweeds, we examined the forms and transcript profiles of the metallothionein (MT) gene family in the model duckweed *Spirodela polyrhiza*, whose genome has been completely sequenced. Four *S. polyrhiza* MT-like genes were identified and annotated as SpMT2a, SpMT2b, SpMT3, and SpMT4. All except SpMT2b showed high sequence homology including the conserved cysteine residues with the previously described MTs from flowering plants. The *S. polyrhiza* genome appears to lack the root-specific Type 1 MT. The transcripts of SpMT2a, SpMT2b, and SpMT3 could be detected in the vegetative whole-plant tissues. The transcript abundance of SpMT2a was upregulated several-fold in response to cadmium stress, and the heterologous expression of SpMT2a conferred copper and cadmium tolerance to the metal-sensitive increment cup1 strain of *Saccharomyces cerevisiae*. Based on these results, we proposed that SpMT2a may play an important role in the metal detoxification mechanism of duckweed.

Studying whole-genome duplication using experimental evolution of *Spirodela polyrhiza*

Wu, T; Natran, A; Prost, L; Aydogdu, E; Van de Peer, Y; Bafort, Q. Methods in Molecular Biology (2023): 373-390.

In this chapter, we present the use of *Spirodela polyrhiza* in experiments designed to study the evolutionary impact of whole-genome duplication (WGD). We shortly introduce this duckweed species and explain why it is a suitable model for experimental evolution. Subsequently, we discuss the most relevant steps and methods in the design of a ploidy-related duckweed experiment. These steps include strain selection, ploidy determination, different methods of making polyploid duckweeds, replication, culturing conditions, preservation, and the ways to quantify phenotypic and transcriptomic change.

Rapid and highly efficient genetic transformation and application of interleukin-17B expressed in duckweed as mucosal vaccine adjuvant

Tan, X; Chen, S; Fang, Y; Liu, PH; Hu, ZB; Jin, YL; Yi, ZL; He, KZ; Li, X; Zhao, LY; Wang, HN; Zhao, H. Biomolecules (2022) 12: 1881.

Molecular farming utilizes plants as a platform for producing recombinant biopharmaceuticals. Duckweed, the smallest and fastest growing aquatic plant, is a promising candidate for molecular farming. However, the efficiency of current transformation methods is generally not high in duckweed. Here, we developed a fast and efficient transformation procedure in *Lemna minor* 0403, requiring 7-8 weeks from screening calluses to transgenic plants with a stable transformation efficiency of 88% at the DNA level and 86% at the protein level.

We then used this transformation system to produce chicken interleukin-17B (chIL-17B). The plant-produced chIL-17B activated the NF-kappa B pathway, JAK-STAT pathway, and their downstream cytokines in DF-1 cells. Furthermore, we administrated chIL-17B transgenic duckweed orally as an immunoadjuvant with mucosal vaccine against infectious bronchitis virus (IBV) in chickens. Both IBV-specific antibody titer and the concentration of secretory immunoglobulin A (sIgA) were significantly higher in the group fed with chIL-17B transgenic plant. This indicates that the duckweed-produced chIL-17B enhanced the humoral and mucosal immune responses. Moreover, chickens fed with chIL-17B transgenic plant demonstrated the lowest viral loads in different tissues among all groups. Our work suggests that cytokines are a promising adjuvant for mucosal vaccination through the oral route. Our work also demonstrates the potential of duckweed in molecular farming.

Physiology & Stress

Maleic hydrazide prompting growth and delaying senescence of mother frond in *S. polyrriza* 7498

Wang, J; Gao, T; Hu, H; Sun, L; Ban, S; Tan, X; Li, X; Zhu, M; Tong, Y; Zhu, Y; Wang, Y. Journal of Plant Physiology (2023) 284, 153966. DOI10.1016/j.jplph.2023.153966

The effect and function mechanism of maleic hydrazide on the growth of mature leaves is unclear. Duckweed is widely used as a model plant to study the effect of compounds on plant growth. The observation of section and ultrastructure of the fronds, the comparation of SOD enzyme activity and related-gene transcriptional expression level showed that 75 μg/mL maleic hydrazide could prompt the growth of the mother fronds in *Spirodela polyrriza* 7498. The half-mother fronds (without meristematic tissue, cut from the mother fronds) with little meristematic tissue could repair themselves and delay their senescence by 75 μg/mL MH. The mother fronds turned more greener with 50 μg/mL MH and exogenous 0.1 μmol/L 6-BA (a kind of cytokinin) treatment, as well as with the increasing of fresh and dry weight in *S. polyrriza* 7498. RNA-Seq data found that the happy growth of the mother fronds caused by MH, was probably resulted from up-regulating the expression of gene related to the synthesis and signaling transduction of cytokinin in *S. polyrriza* 7498. which are responsible for the maintaining membrane system integrate and transport protein function. The work gives lights to the study of function mechanism of MH prompting mature leaves growth and delaying mature leaves senescence in plant. And it provides a strategy to increase biomass with the application of low concentration MH and 6-BA in the same time in agriculture.

Comment DF: The correct spelling is *Spirodela polyrhiza* (*S. polyrhiza*)

Rate enhancement of plant growth using Ormus solution: optimization of operating factors by response surface methodology

Samimi, M; Mohammadzadeh, E; Mohammadzadeh, A. International Journal of Phytoemediation (2023) DOI10.1080/15226514.2023.2179014

The Ormus solution was applied for the first time to increase plant growth. In addition, the operating factors, including the pH of the medium, the residence time in the Ormus preparation process, and the concentration of Ormus solution, were optimized using the RSM based on a BBD model. In this study, Ormus was introduced under optimal conditions as a suitable additive to increase the proliferation rate of plants. Phytoremediation is an economical technique for the biological treatment of soil and water contamination. Improving the growth of plants used in this technique leads to greater efficiency of the process. Herein, *Lemna minor* plant used in phytoremediation was collected from Sarab-e Nilufar region of Kermanshah province, Iran, and maintained in a culture medium. This study focused on the preparation of Ormus solution based on crystalline salt extracted from the salt mountains of Karmowstaj region in Fars province, Iran, to increase the proliferation of *Lemna minor*. The optimal growth conditions, including the pH of the medium, the residence time in the Ormus preparation process, and the concentration of Ormus solution were analyzed using the response surface methodology (RSM) based on a Box-Behnken design (BBD) model. Numerical optimization based on the

quadratic model was carried out to achieve the maximum growth percentage. The percentage of the proliferation of *Lemna minor* varied from 34.6% to 117.3%, while growth was highest at a medium pH value of 10.75, a residence time of 72 h in the Ormus preparation process, and an Ormus concentration of 17 $g.L^{-1}$. The results revealed that Ormus, as a supplemental fertilizer, under optimal conditions can significantly increase the rate of plant reproduction.

A novel cultivation platform of duckweed (Lemna minor) via application of beeswax superhydrophobic coatings

Chua, MX; Cheah, YT; Tan, WH; Chan, DJC. Environmental Research (2023) 224: 115544.

Conventional establishment of laboratory cultures of duckweed *Lemna minor* are prepared in beakers, Erlenmeyer flasks or Schott bottles. These conventional cultivation methods limit the available surface area for growth which then causes layering of fronds that reduces the efficiency of plants in sunlight capturing. Here, acrylic sheets were spray-coated with a superhydrophobic (SHP) beeswax suspension and these coated acrylic sheets were used as a novel cultivation platform for *L. minor*. *L. minor* was grown for 7 days in conventional glass jar which acted as the control and were compared to SHP coated acrylic (SHPA) and SHP coated acrylic with aluminium mesh centrally placed (SHPAM) at similar duration and cultivation conditions. Addition of mesh was to entrap the plantlets and fixed the plantlets' position on the growing platform. The effects of cultivation platforms on growth rate and biochemical compositions of *L. minor* were monitored. The highest biomass growth was obtained from SHPA cultivation where the relative growth rate (RGR) was 0.0909 ± 0.014 day^{-1} and the RGR was 2.17 times higher than the control. Moreover, *L. minor* harvested from SHPA displayed the highest values in total protein content, total carbohydrates content and crude lipid percentage. The values were 156.04 ± 12.13 mg/g, 94.75 ±- 9.02 mg/g and 7.09 ± 1.14% respectively. However, the control showed the highest total chlorophyll content which was 0.7733 ± 0.042 mg/g FW. Although SHPA obtained a slightly lower chlorophyll content than the control, this growing platform is still promising as it displayed the highest growth rate as well as other biochemical composition. Hence, this study proved that the proposed method that applied superhydrophobic properties in cultivation of *L. minor* provided a larger surface area for *L. minor* to grow, which then resulted in a greater biomass production while simultaneously maintaining the quality of the biochemical compositions of duckweeds.

Overwintering and re-emergence in *Lemna minor*

Jewell, MD; Bell, G. Aquatic Botany (2023) 186: 103633

In cold climates, aquatic plants employ a variety of strategies to survive winter, usually by either going to seed, or by producing resistant vegetative tissue that can withstand freezing. Although *Lemna minor*, the common duckweed, reproduces almost exclusively by asexual vegetative propagation and lacks any additional specific overwintering structure, it thrives over a vast geographic distribution including in northern climates. It is often thought that populations are subjected to a seasonal bottleneck with only a small proportion of plants surviving to found the following year's population. In this study we use experimental enclosures to measure re-emergence of *L. minor* in a forested pond in Quebec, Canada, after four months of ice cover. Frond recruitment as surface re-emergence and relative growth rate were estimated and compared to the pre-winter population. To our surprise, 92% of fronds survived the winter, indicating the virtual absence of any bottleneck. *Lemna minor* is then essentially perennial, which despite having a life span of just a few weeks in favourable conditions, can easily endure several months under surface ice cover.

Melatonin treatment enhances the growth and productivity of useful metabolites in the in vitro culture of *Spirodela polyrhiza*

Ko, JH; Ryu, JE; Noh, SW; Choi, HK. Journal of Agricultural and Food Chemistry (2023) DOI10.1021/acs.jafc.2c07147

Spirodela polyrhiza (Araceae family) is a duckweed species that serves as a potential resource for feed, food, bioremediation, and pharmaceutical applications. In this study, we assessed the effects of different concentrations of melatonin (0, 0.1, 1, and 10 μM) on the growth of *S. polyrhiza* during in vitro culture and the

metabolic profiles and productivities of useful metabolites using gas chromatography-mass spectrometry coupled with multivariable statistical analysis. We found that exogenous melatonin significantly improved the total dry weight and altered the metabolic profiles of *S. polyrhiza* cultures. Melatonin significantly enhanced the cellular production of useful metabolites, such as γ-aminobutyric acid, dopamine, threonine, valine, and phytosterols. The volumetric productivities (mg/L) of γ-aminobutyric acid, dopamine, campesterol, fi-sitosterol, and stigmasterol were the highest in the presence of 10 μM melatonin on day 12. Moreover, the productivities of ascorbic acid and serotonin were the highest in the presence of 1 μM melatonin on day 12. Therefore, melatonin could be used to enhance the production of biomass and useful metabolites during large-scale *S. polyrhiza* cultivation in cosmetic, food/feed, and pharmaceutical industries.

Effects of parental age on salt stress tolerance in an aquatic plant

Chmilar, SL; Laird, RA. Oikos (2023) DOI10.1111/oik.09218

Parental age influences components of offspring fitness in many species. The ability to tolerate stress also affects fitness, but less is known regarding changes in offspring stress tolerance with increasing parental age, especially in plants. We examined first and fifth-born clonal offspring (using birth order as a proxy for parental age), and compared their fitness in several sub-lethal concentrations of salt (NaCl), to investigate the interactive effects of birth order and salt stress on the offspring of the aquatic plant *Lemna minor* L. We found that increasing salt concentration reduced reproduction particularly at early ages, which detrimentally affected fitness, as measured by the intrinsic rate of natural increase. Fifth offspring had greater fitness than first offspring, potentially due to the hump-shaped relationship between offspring fitness and birth order observed in other studies on *Lemna*, with fifth offspring near the peak of the hump. We found no interactive effect of birth order and salt concentration on offspring fitness; however, there were interactive effects on the time to first reproduction and the size of fronds. Specifically, first offspring exposed to increasing salt concentrations exhibited longer delays to first reproduction and grew to a greater size, while fifth offspring showed little change in either variable with increasing salt concentration. Thus, variation in birth order affected offspring response to salt stress, although not in terms of fitness. These results help illuminate factors impacting the age-specific strength of natural selection and stress responses, and may be environmentally relevant in the context of environmental salinization.

Clonal transgenerational effects transmit for multiple generations in a floating plant

Zhang, XM; Jin, Y; Xue, W; Gao, JQ; Lei, NF; Chen, JS; Yu, FH. Phyton (2023) 92: 1589-1601.

Environmental conditions of a parent plant can influence the performance of their clonal offspring, and such clonal transgenerational effects may help offspring adapt to different environments. However, it is still unclear how many vegetative generations clonal transgenerational effects can transmit for and whether it depends on the environmental conditions of the offspring. We grew the ancestor ramets of the floating clonal plant *Spirodela polyrhiza* under a high and a low nutrient level and obtained the so-called 1st-generation offspring ramets of two types (from these two environments). Then we grew the 1st-generation offspring ramets of each type under the high and the low nutrient level and obtained the so-called 2nd-generation offspring ramets of four types. We repeated this procedure for another five times and analyzed clonal transgenerational effects on growth, morphology and biomass allocation of the 1st- to the 6th-generation offspring ramets. We found positive, negative or neutral (no) transgenerational effects of the ancestor nutrient condition on the offspring of *S. polyrhiza*, depending on the number of vegetative generations, the nutrient condition of the offspring environment and the traits considered. We observed significant clonal transgenerational effects on the 6th generation offspring; such effects occurred for all three types of traits (growth, morphology and allocation), but varied depending on the nutrient condition of the offspring environment and the traits considered. Our results suggest that clonal transgenerational effects can transmit for multiple vegetative generations and such impacts can vary depending on the environmental conditions of offspring.

Modelling of *Lemna minor* L. growth as influenced by nutrient supply, supplemental light, CO_2 and harvest intervals for a continuous indoor cultivation

Schmidt, KM; Goldbach, HE. Heliyon (2022) 8: DOI10.1016/j.heliyon.2022.e12194

Given the proper conditions, *Lemna* spp. rapidly produce a high amount of valuable biomass which is considered as an alternative source for feed and food. For a continuous and long-term indoor production under controlled conditions, environmental and harvest parameters have to be optimized to suppress algal growth and constantly yield a high-quality product. Experimentally assessing the effect of a larger number of parameters on the growth rate ri is impossible due to the theoretically high number of parameter combinations. Thus, a SIMILE (R) -based model has been developed. This enables production parameters to be assessed individually for its effect on the growth rate ri by a differential equation. Start values for numerical integration were taken from measured data and analytical solutions of the differential growth equation. At 400 ppm CO_2, the regrowth rate ri in an optimized laboratory set-up amounted to 216 g FM m^{-2} d^{-1}, harvesting one third of the biomass at intervals of 5 days. In up -scaled set-ups, lower regrowth rates ri of about 173 g FM m^{-2} d^{-1} (Kalkar) and 190 g FM m^{-2} d^{-1} (Berlin) were obtained, because temperature and light conditions were below optimum. At 3,500 ppm CO_2, the regrowth rate ri in laboratory set-up increased to 323 g FM m^{-2} d^{-1} by shortening the harvest interval to three days. Maximum growth rates ri were obtained with an NH_4 /NO_3-ratio of 1/9 at 1.14 mM total N concentration. The results indicate how to optimize culture conditions and harvest intervals. Model runs closely match the experimental data taken from the three different approaches and thus confirm the validity of the model.

Assessment of the effect of heavy metal salts on the photosynthetic activity of aquatic plants

Lobkova, GV; Tikhomirova, EI; Simonova, ZA. Biology Bulletin (2022) 10: 1801-1805

The status of the photosynthetic pigments in the aquatic plants *Lemna minor* L. and *Elodea canadensis* Michx. under the action of Ni^{2+}, Co^{2+}, Cu^{2+} and Pb^{2+} acetates in concentrations of 5.00, 2.50, 1.25, 0.62, 0.31, 0.15, 0.07, and 0.03 mg/L was assessed by changes in the fluorescence intensity of chlorophyll a and b and their ratio. It was established that nickel acetate in original solutions in all the above concentrations caused an increase in the fluorescence intensity of chlorophylls a and b in *L. minor* in relation to the control while the lead salt suppressed it. Co^{2+} acetate inhibited the fluorescence of chlorophyll a with concentrations in the initial solutions of 0.03 to 0.15 and 2.50 mg/L and that of chlorophyll b at all concentrations, except 0.62, 1.25, and 2.50 mg/L. For *E. canadensis*, it was found that the salts of all metals at all concentrations caused a reduction of the fluorescence intensity of chlorophyll a relative to the control and increased it in the case of chlorophyll b. The exception was the effect of copper and lead acetates with a concentration of 1.25 mg/L, when the fluorescence intensity of chlorophyll b was maintained at the control level. The presence of Ni^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+} acetates in all concentrations in the culture medium influenced the quantitative and qualitative characteristics of chlorophyll a and b, which indicated a violation of the photosynthesis process. Our data on the change in the chlorophyll a/b ratio led to a conclusion about degradation of chlorophyll a relative to chlorophyll b after the effect of heavy metals.

Phytoremediation

Removal of antibiotics from pharmaceutical wastewater using *Lemna aoukikusa (duckweed)*

Habaki, H; Thyagarajan, N; Li, ZH; Wang, SY; Zhang, J; Egashira, R Separation Science and Technology (2023) DOI10.1080/01496395.2023.2195544

Duckweed (*Lemna aoukikusa*), a common aquatic plant found worldwide, was used to treat a solution polluted with the model antibiotics, ciprofloxacin (fluoroquinolone group) and sulfamethoxazole (sulfonamide group), which were selected because they were frequently present in pharmaceutical wastewater. The plant could grow even in an aqueous solution with a high concentration of both antibiotics, and illumination enhanced its

growth. The presence of antibiotics in the solution inhibited the growth, and the inhibitory effect of sulfamethoxazole was greater than that of ciprofloxacin. Ciprofloxacin was effectively reduced by hydrolysis, photolysis, and uptake by the duckweed. Although sulfamethoxazole was resistant to hydrolysis and photolysis, its uptake by duckweed under illumination conditions could remove both antibiotics from the solution, even at high concentrations. Regarding the contribution of the respective types of antibiotic removal rates to total antibiotic removal, the uptake rate of antibiotics was the highest, and the treatment of wastewater using duckweed was proposed as a promising treatment method.

Comment DF: The correct species name is Lemna aequinoctialis

Physiological responses and antibiotic-degradation capacity of duckweed (*Lemna aequinoctialis*) exposed to streptomycin

Huang, WJ; Kong, R; Chen, LJ; An, YX. Frontiers in Plant Science (2022) 13: 1065199.

Aquatic plants are constantly exposed to various water environmental pollutants. Few data on how antibiotics affect duckweed health and its removal ability. The aim of this study was to investigate the impact of streptomycin on the physiological change and uptake capability in duckweed (*Lemna aequinoctialis*) after exposure at different time points (0, 5, 10, 15 and 20 days). Duckweeds were exposed to streptomycin at a range of concentrations (0.1-10 mM). Results indicated that the high streptomycin concentrations (≥ 1 mM) resulted in a lower duckweed biomass (21.5-41.5%), RGR (0.258-0.336 g d^{-1}), decrease in total Chl and increase in carotenoids. Antioxidative enzymes, including CAT (18-42.88 U mg $protein^{-1}$), APX (0.41-0.76 U mg $protein^{-1}$), and SOD (0.52-0.71 U mg $protein^{-1}$) were found to accumulate in the streptomycin groups in comparison to the control group. The significant reduction (72-82%) in streptomycin content at 20 d compared to the control (40-55%) suggested that duckweed has a high ability in removing streptomycin. Transcriptome analysis showed that the secondary metabolic pathways including phenylpropanoid biosynthesis and flavonoid biosynthesis were significantly upregulated in the streptomycin setup compared to the control. Therefore, our findings suggested that duckweed can contribute to the streptomycin degradation, which should be highly recommended to the treatment of aquaculture wastewater and domestic sewage.

Spatial optimization of nutrient recovery from dairy farms to support economically viable load reductions in the Chesapeake Bay Watershed

Femeena, PV; Costello, C; Brennan, RA. Agricultural Systems (2023), 207: 103640

To promote circularity in agricultural systems, the utilization of aquatic vegetation for ecological wastewater treatment is a potential mechanism to capture and upcycle nutrients. Agricultural wastewater is an excellent growing medium for aquatic plants like duckweed, offering opportunities for wastewater treatment and conversion of harvested biomass into bio-based products, including protein-rich livestock feed, which can potentially replace conventional soil-based crops such as alfalfa. We hypothesize that nitrogen (N) and phosphorus (P) loadings to the Chesapeake Bay Watershed (CBW) can be reduced via replacing alfalfa cultivation with manure-grown duckweed by: a) reducing excess manure application on agricultural fields; b) reducing synthetic fertilizer application on alfalfa croplands; and c) decreasing the release of fixed N back into the environment from the decomposition of alfalfa crop residue. This study developed an optimization framework to identify locations where alfalfa-to-duckweed replacement could be theoretically employed to minimize N and P loads into the CBW. A relative effectiveness (RE) indicator representing landscape-specific nutrient delivery capacity was included within the frame-work. Using county-level data on alfalfa yields, cropping area, and nutrient inputs from alfalfa croplands and dairy manure, we identified alfalfa cultivation areas that could be removed and replaced with full or partial duckweed cultivation and land conservation for optimal benefits.

Phytoremediation of toxic chemicals in aquatic environment with special emphasis on duckweed mediated approaches

Thakuria, A; Singh, KK; Dutta, A; Corton, E; Stom, D; Barbora, L; Goswami, P. International Journal of Phytoremediation (2023) DOI10.1080/15226514.2023.2188423

The discharge of toxic chemicals into water bodies and their linked detrimental effects on health is a global concern. Phytoremediation, an environment-friendly plant-based technology, has gained intensive interest over the last decades. For the aquatic phytoremediation process, the commonly available duckweeds have recently attracted significant attention due to their capacity to grow in diverse ecological niches, fast growth characteristics, suitable morphology for easy handling of biomass, and capacity to remove and detoxify various potential toxic elements and compounds. This review presents the progress of duckweed-assisted aquatic phytoremediation of toxic chemicals. A brief background of general phytoremediation processes, including the different phytoremediation methods and advances in understanding their underlying mechanisms, has been described. A summary of different approaches commonly practiced to assess the growth of the plants and their metal removal capacity in the phytoremediation process has also been included. A vast majority of studies have established that duckweed is an efficient plant catalyst to accumulate toxic heavy metals and organic contaminants, such as pesticides, fluorides, toxins, and aromatic compounds, reducing their toxicity from water bodies. The potential of this plant-based phytoremediation process for its downstream applications in generating value-added products for the rural economy and industrial interest has been identified.

The combined effect of three floating macrophytes in domestic wastewater treatment

Akowanou, AVO; Deguenon, HEJ; Balogoun, KC; Daouda, MMA; Aina, MP. Scientific African (2023) 20: 01630.

We conducted this study to assess the efficiency of the combination of three floating macrophytes (*Eichhornia crassipes* (water hyacinth), *Pistia stratiotes* L . (water lettuce), and *Lemna minor* L. (duckweed)) for the treatment of domestic wastewater. The experiments were performed on a pilot scale consisting of four basins aligned in series: one anaerobic pond, two facultative ponds (containing water hyacinth and water lettuce respectively), and one maturation pond (containing duckweed). The study showed that combining the different macrophytes studied led to significantly reduced pollution in domestic wastewater. Based on inlet pollution loads, water hyacinth pond better eliminate carbon pollution. The water lettuce pond was good at removing nitrogen pollution, and the duckweed pond better-eliminated phosphorus. The overall yield for TSS, COD, BOD5, TKN, and PO_4^{3-} was 92%, 98%, 98%, 87%, and 80% respectively. Based on the results, combining different floating macrophytes in a domestic wastewater treatment plant could improve its overall treatment performance.

Waste removal efficiencies of floating macrophytes for restoration of polluted stream: An experimental analysis

Mahajan, B; Shastri, S; Londhe, S. Urban Science (2023) 7: 27.

Freshwater sources are affected by a diverse range of pollutants, which increases the demand for effective remediation. Aquatic phytoremediation is a nature-based solution. It has the potential to provide efficient, adaptable, and multi-targeted treatment of polluted waters. The aim of this research is to evaluate non-mechanized, low-cost onsite treatment of waste water intrusions. It includes an experimental set up with three replicates. Each consists of a modified flow pattern under outdoor conditions. Experimental set up A and B were provided with macrophytes, water lettuce and duckweed, respectively, with plant coverage at 50% and 90%. Experimental set up C was a controlled set up without macrophytes. The highest removal of BOD, COD and Total solids by using water lettuce were observed to be 89%, 77% and 38.5%, respectively. By using duckweed, the highest removal of BOD, COD and Total solids were observed at 88%, 66% and 27.59%, respectively. Removal was also observed in Set up C for BOD, COD and Total solids; its efficiency was 48%, 47% and 25%, respectively. Set up A can be recommended for treating wastewater intrusion, so that wastewater will purify to a to satisfactory to disposal standard level before mixing in river water. The area available in the stream itself can be used as a treatment zone.

Soil heavy metal absorption potential of *Azolla pinnata* and *Lemna gibba* with arbuscular mycorrhizal fungi in rice (*Oryza sativa* L.) farming

Herath, BM; Bamunuarachchige, C; Stephenson, SL; Elgorban, AM; Asad, S; Kumla, J; Suwannarach, N; Karunarathna, SC. Sustainability (2023) 15: 4320.

This study assessed the potential uptake of soil-contaminated heavy metals by *Azolla pinnata* and *Lemna gibba* in combination with and without arbuscular mycorrhizal fungi (AMF) in traditional and improved rice varieties. Total levels of cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) in soil, rice roots, shoots, grains, *A. pinnata*, and *L. gibba* were estimated using ICP-MS. The percentage colonization in AMF-inoculated and AMF-uninoculated rice varied from 1.13-30.67% and 1.33-5.42%, respectively. These findings suggested that AMF provide protection to rice plants against the combined toxicity of Cd, As, Pb, and Hg in rice field soil. The combined interaction of AMF, organic fertilizer, and *A. pinnata* decreased heavy metal accumulation in rice roots, shoots, and grains in both tested varieties. The intake and subsequent accumulation of Cd, As, Pb, and Hg in the rice grains differed significantly ($p < 0.05$) between the two rice varieties. Furthermore, it was revealed that the AMF-inoculated rice plants reduced the translocation of heavy metals from root to shoot. Therefore, it can be concluded that heavy metal absorption and accumulation in rice can be reduced by the application of AMF, organic fertilizer, and *A. pinnata* together in rice farming.

Phytoremediation of contaminated water using aquatic plants, its mechanism and enhancement

Kristanti, RA; Hadibarata, T. Current Opinion in Environmental Science and Health (2023) 32: 100451. DOI10.1016/j.coesh.2023.100451

The contamination of natural resources, including land and water with organic pollutants, has become a major concern due to present intensive agricultural methods and industrialization. Despite being used for many years, phytoremediation is still a relatively new technology. Using a variety of mechanisms, aids that can increase the efficacy of phytoremediation processes, and strengths and limits associated with the application of this technology, this review article synthesizes the most recent research on aquatic plant species suited for use in phytoremediation. Water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Duckweed (*Lemna minor*) are common accumulator plants for remediating polluted water. The phytoremediation's potential can be enhanced by genetic engineering, natural microbial stimulation, and chemical and natural additives. Phytoremediation can be a dependable option for a sustainable and affordable remediation of water from the organic and inorganic pollutants due to its low cost, sustainability linked with plants, and use of renewable energy.

Phytoremediation of mercury from water by monocultures and mixed cultures pleustophytes

Sitarska, M; Traczewska, T; Filarowska, W; Holtra, A; Zamorska-Wojdy, D; Hanus-Lorenz, B. Journal of Water Process Engineering (2023) 52: 103529.

Mercury pollution is a global environmental problem. An alternative to the chemical method of removing mercury from the aquatic environment is the phytoremediation process, based on phytofiltration, phytoextraction, and phytovolatilization techniques. The study showed the effectiveness of phytoremediation with plants *Lemna minor* and Salvinia natans carried out in monoculture systems and mixed cultures. We used Hoagland's mercury-contaminated liquid medium: 0.15 mg Hg/dm^3, 0.20 mg Hg/dm^3 and 0.30 mg Hg/dm^3. The relative growth rate of plants in the control samples was 0.04-0.07 g/gd. In the presence of mercury for monocultures, observed a toxic effect in the form of growth stimulation, and the relative growth rate reached 0.12 g/gd. The bioconcentration factor was the highest on the 7th day of mercury exposure and was 216-856. The efficiency of mercury removal from the substrate in the phytoremediation process was 96 %. The total protein was increased for *Lemna minor* by 34 %, *Salvinia natans* by 84 %, and in mixed culture by up to 99 %. Also, the total chlorophyll increased for *Lemna minor* by 14 % and for the mixed culture by up to 60 %. For *Salvinia natans*, the total chlorophyll decreased by 53 %. The biochemical changes may have been a toxic effect of mercury in the environment.

Lowering pH enables duckweed (*Lemna minor* L.) growth on toxic concentrations of high-nutrient agricultural wastewater

Jones, G; Scullion, J; Dalesman, S; Robson, P; Gwynn-Jones, D. Journal of Cleaner Production (2023) 395: 136392.

The use of duckweed species to remediate nutrient-rich wastewater has grown as a field of research and in industry; however, the need to dilute wastewater to the low ammoniacal-N concentrations tolerated by duckweed represents a barrier to commercially implementing these systems in agriculture. This study investigated the potential for acidifying anaerobically digested cattle slurry (digestate), shifting the NH_4^+:NH_3 equilibrium towards the less toxic ionised form, thus allowing the growth of *Lemna minor* on less dilute wastewater. First, a study was conducted to identify the ammoniacal-N concentrations tolerated by *L. minor* and to confirm the positive effect of lower pH on growth in high nutrient solutions using modified Hutner's solutions at two pH levels (8.2 and 6.5). In Hutner's solution at a pH of 8.2, *L. minor* growth was highest at the lowest ammoniacal-N concentration of 10 mg L^{-1} and decreased with increasing concentrations. At a pH of 6.5, *L. minor* growth remained unaffected with increasing ammoniacal-N up to a concentration of 250 mg L^{-1}. *L. minor* was then grown in digestate concentrations ranging from 5% (65 mg L^{-1} ammoniacal-N) to 30% (350 mg L^{-1} ammoniacal-N), based on its growth in Hutner's solutions. It was hypothesised, that growth would decrease as the digestate concentration increased at pH 8.2, and that acidifying digestate to pH 6.5 would alleviate this effect. On unamended digestate (pH 8.2), *L. minor* growth was prevented even in the most dilute treatment (5%); however, on acidified digestate (pH 6.5), growth rates remained positive and significantly higher than the unamended controls up to the 20% dilution (239.3 mg L^{-1} ammoniacal-N). Higher growth rates in the Hutner's solutions compared to digestate, particularly at pH 8.2 where no growth was recorded in digestate, suggest the presence of additional inhibitory factors in complex, high-nutrient wastewaters, and potentially sub-optimal concentrations of some of the nutrients provided in Hutner's solution. Nevertheless, correlation matrix analysis of digestate chemical properties highlighted the importance of acidification, with a strong negative correlation between pH and *L. minor* growth rate. For the first time, we demonstrate that by lowering pH, *L. minor* could be grown on dilutions of nutrient-rich agricultural wastewater that were otherwise toxic, and which make it feasible as a nutrient removal method. These findings could have important implications for implementing duckweed-based remediation systems in agriculture, increasing water-and land use efficiency, and thus, their commercial viability.

The potential of common duckweed (*Lemna minor*) in phytoremediation of phenanthrene and pyrene

Zazouli, MA; Asghari, S; Tarrahi, R; Lisar, SYS; Babanezhad, E; Dashtban, N. Environmental Engineering Research (2023) 28: 210592

The distribution of polycyclic aromatic hydrocarbons (PAHs) as a group of toxic and persistent aromatic pollutants in the environment is rapidly enhancing. These compounds have adverse impacts on the health of living organisms. Hence, in the present study, we investigated the potential of duckweed (*Lemna minor*) as an aquatic plant species for uptake, accumulation, and biodegradation of phenanthrene and pyrene under controlled conditions. *L. minor* plants were treated with 10 and 20 mg L^{-1} concentrations of phenanthrene and pyrene at the experimental duration of ten days. According to the results obtained, the toxicity of phenanthrene and pyrene contaminants on *L. minor* was influenced by the different initial PAHs concentrations. An increase in phenanthrene and pyrene concentration significantly decreased all studied growth parameters such as fresh weight, dry weight, and RFN and also photosynthetic pigment contents of the plant. Phenanthrene and pyrene concentrations were measured using high-performance liquid *chromatography* (HPLC) technique after 10 days of exposure to the PAHs. The results revealed that *L. minor* species could bioaccumulate effectively both typical PAHs. Furthermore, the gas chromatography-mass spectroscopy (GC/MS) technique explained the biological degradation of phenanthrene and pyrene by *L. minor* in the present research, and accordingly, several intermediate by-products were identified.

Duckweeds for phytoremediation of polluted water

Zhou, YZ; Stepanenko, A; Kishchenko, O; Xu, JM; Borisjuk, N. Plants (2023) 12: 589.

Tiny aquatic plants from the Lemnaceae family, commonly known as duckweeds, are often regarded as detrimental to the environment because of their ability to quickly populate and cover the surfaces of bodies of water. Due to their rapid vegetative propagation, duckweeds have one of the fastest growth rates among flowering plants and can accumulate large amounts of biomass in relatively short time periods. Due to the high yield of valuable biomass and ease of harvest, duckweeds can be used as feedstock for biofuels, animal feed, and other applications. Thanks to their efficient absorption of nitrogen- and phosphate-containing pollutants, duckweeds play an important role in the restorative ecology of water reservoirs. Moreover, compared to other species, duckweed species and ecotypes demonstrate exceptionally high adaptivity to a variety of environmental factors; indeed, duckweeds remove and convert many contaminants, such as nitrogen, into plant biomass. The global distribution of duckweeds and their tolerance of ammonia, heavy metals, other pollutants, and stresses are the major factors highlighting their potential for use in purifying agricultural, municipal, and some industrial wastewater. In summary, duckweeds are a powerful tool for bioremediation that can reduce anthropogenic pollution in aquatic ecosystems and prevent water eutrophication in a simple, inexpensive ecologically friendly way. Here we review the potential for using duckweeds in phytoremediation of several major water pollutants: mineral nitrogen and phosphorus, various organic chemicals, and heavy metals.

Textile wastewater phytoremediation using *Spirodela polyrhiza* (L.) Schleid. assisted by novel bacterial consortium in a two-step remediation system

Parihar, A; Malaviya, P. Environmental Research (2023) 221: 115307.

The study aims at developing a phyto-microremediation system for textile wastewater treatment using *Spirodela polyrhiza* (L.) Schleid. and a consortium of bacterial strains isolated from textile wastewater-contaminated matrices and rhizosphere of *S. polyrhiza*. The sequential phyto-microremediation of textile wastewater was carried out utilizing two-stage phyto-microremediation systems I [phytoremediation system (Stage 1) preceded microremediation system (Stage 2)] and II [microremediation system (Stage 1) preceded phytoremediation system (Stage 2)]. *Pseudomonas stutzeri*, *Janibacter anophelis, Bacillus safensis, Bacillus pumilus, Bacillus thuringiensis*, and *Bacillus cereus* constituted the bacterial consortium that was involved in the microremediation of textile wastewater. Biochemical characterization of *Spirodela* on exposure to untreated textile wastewater showed cadmium and nickel uptake as 26.03 and 22.99 mg g^{-1} dw^{-1}. *S. polyrhiza* exhibited anatomical changes like distortion in the structure of the xylem, phloem, lower epidermis, and increased aerenchyma formation when remediating textile wastewater. The textile wastewater bioremediation in phyto-microremediation system I gives final reduction of COD 77.36%, color 91.70%, calcium 61.65%, iron 69.41%, nickel 89.30%, cadmium 88.37%, nitrate 70.83%, phosphate 73.11%, and sulfate 75.49%. Further, LC-MS analysis of treated wastewater from phyto-microremediation system I have shown biotransformation of metabolites into simpler compounds like 2-{Bis [4-(2-cyanophenoxy)phenyl]methyl}benzoic acid (C34H22N2O4). The FTIR spectrum of bacterial biomass exposed to textile wastewater exhibits substantial shifts of various bands in the IR region for functional groups such as alcohol, alkene, esters, azide, and amine as compared to non-exposed biomass.

Wastewater treatment of solid waste leachate and production of proteinaceous biomass using duckweed vegetation (*Lemna minor*)

Tekogul, H. Journal of Costal Research (2023) DOI10.2112/JCOASTRES-D-22-00072.1

Phytoremediation provides cost-effective wastewater treatment and recovery of wastewater as rich biomass by aquatic plants and produces bioenergy and various important by-products. This study was carried out to treat solid waste leachate with *Lemna minor* L. and convert wastewater nutrients into protein-rich biomass by this aquatic plant. To create the experimental setups, *L. minor* was produced in wastewater at six dilution ratios: 100% (T1), 75% (T2), 50% (T3), 25% (T4), Hoagland medium with *Lemna* (T5c), and 100% wastewater without *Lemna* (T6). At the end of the 45-day study, the pH 7.00 (T5c) and 8.81, the protein yield rate (crude protein) in the trial groups was found to be T1 = 40.10%, T2 = 67.53%, T3 = 75.24%, T4 = 42.72%, and T5c = 85.00%. In the study, the T3 group showed the best performance in terms of initial weight, final weight, total

biomass, specific growth rate, relative growth rate, and protein ratio in wastewater groups. The highest and lowest removal rates in leachate ranged between 85.40% (T4) and 56.50% (T1) for the NH_4^+-N value, 14.20% (T4) and 4.73% (T2) for NO_2^--N, 16.96% (T4) and 6.53% (T3) for NO_3^--N, and 23.10% (T3) and 16.08% (T1) for PO_4^{-3}-P. The total biomass of the trial groups showed an average 50.55% increase from the baseline level. Although 46% biomass was obtained in the Hoagland medium with vegetative growth, a very high biomass of 61% was obtained in the T3 group of solid waste leachate containing dilute wastewater. As a result, phytoremediation is recommended as a solid option for the management of wastewater resources. This efficiency has shown that aquatic plants can be used in the treatment of wastewater and the obtained high protein biomass can be used in various ways, such as animal feed and bioenergy.

Cupric ions inducing dynamic hormesis in duckweed systems for swine wastewater treatment: Quantification, modelling and mechanisms

Li, CX; Lin, Y; Li, X; Cheng, JJ; Yang, CP. Science of the Total Environment (2023) 866: 161411.

Hormesis has attracted close attention of environmental and toxicological communities over the past decades. Most studies focused on the hormesis induced by stressors in the aspect of their biotoxicity to organisms, while little research was conducted on hormesis in the aspect of biological wastewater treatment process. In this study, removal of NH_4^+-N and Cu^{2+} by *S. polyrrhiza* under long-term Cu^{2+} exposure at environmentally relevant concentrations in swine waste-water was investigated. Removal efficiencies of NH_4^+-N by duckweeds at 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/L Cu^{2+} were 81.6 %, 83.7 %, 89.4 %, 74.9 %, 61.8 % and 45.1 % on day 28, however, during the initial period of cultivation (0-4 days), such hormetic effect was not observed, indicating time-dependent feature of hormesis in NH_4^+-N removal. The modified logistic growth model was applied to describe long-term hormesis induced by Cu^{2+} on NH_4^+-N removal and it suggested that the optimal copper exposure for ammonium removal was 0.48 mg/L. More importantly, it was found that previous exposure to low doses of Cu^{2+} (0-1 mg/L) could enhance NH_4^+-N removal performance under the second exposure. Cu^{2+} above 1 mg/L could switch copper bioaccumulation pattern from the Langmiur-irreversible type to reversible one, indicating risk of secondary pollution. Six components including freshly -produced humic-like substances, lignin, fulvic acid-protein complex, free amino acid-like substances, tyrosine-like substance and soluble amino acid-like substances in duckweeds were detected by parallel factor (PARAFAC) model de-tected. Principle component analysis (PCA) conducted on PARAFAC components suggested that enhanced synthesis of protein and growth factors intracellularly at low dose stimulation improved ammonia uptake from the environment. This study provided a novel strategy to improve treatment performance of duckweeds for copper contaminated waste-water and helped understand biochemical responses and their roles in evolutionary adaptive strategies to stresses.

Short and long-term phytoremediation capacity of aquatic plants in Cu-polluted environments

Enochs, B; Meindl, G; Shidemantle, G; Wuerthner, V; Akerele, D; Bartholomew, A; Bulgrien, B; Davis, A; Hoyt, K; Kung, L; Molina, M; Miller, E; Winship, A; Zhang, Y; Graney, J; Collins, D; Hua, J. Heliyon (2023) 9: 12805.

Freshwater ecosystems face numerous threats from human populations, including heavy metal contamination. Phytoremediation, the use of plants to remediate contaminated soils and sediments, is an effective and low-cost means of removing chemical contaminants, including heavy metals, from polluted environments. However, key questions remain unanswered in the application of this technology in aquatic environments, such as the long-term fate of pollutants following plant uptake. In this study, using two common wetland plant species (duckweed and tape grass), we first examined the capacity of plants to remove copper (Cu) from polluted water. Next, we evaluated the leaching potential of plant tissues following decomposition and how it is affected by a simulated freeze-thaw cycle. Using phytoremediated water and leachates from senesced plants we assessed phytoremediation success and Cu leaching potential by conducting standard toxicity assays using pond snails (Physa acuta), a species with known Cu sensitivity. We found that duckweed outperformed tape grass as a phytoremediator at low Cu concentrations. In addition, for plants grown in low concentrations of Cu, leaching from decaying plant material did not negatively impact snail survival, while at high concentrations of Cu, leaching did result in toxicity. Lastly, we found that a simulated freeze-thaw cycle

increased the release of Cu from plant tissue in the presence of high Cu concentrations only, resulting in reduced snail survival. Our results indicate that in moderately Cu-polluted environments, some aquatic plants can remove contaminants without a long-term risk of leaching. In contrast, phytoremediation in highly polluted environments will likely require removal of plant tissue to prevent leaching of previously accumulated metals. Land managers must not only consider plant species and degree of contamination, but also geographic location, as freeze-thaw cycles may enhance plant decomposition and increase the likelihood of contaminant leaching following phytoremediation efforts in aquatic ecosystems.

Urban surface water quality and the potential of phytoremediation to improve water quality in peri-urban and urban areas in Sub-Saharan Africa - a review

Schnabel, B; Wright, S; Miller, R; Bryant, LD; Kjeldsen, TR; Maconachie, R; Gbanie, SP; Bangura, KS; Kamara, AJ. Water Supply (2022) 22: 8372-8404.

In 2017, 400 million people in Sub-Saharan Africa (SSA) were still using unimproved drinking water sources, 80 million of whom relied on untreated surface water. Urban areas are vastly expanding all over the continent and many larger cities in SSA struggle to provide safely managed drinking water. Phytoremediation implemented in constructed wetlands (CWs) is a low-cost and sustainable alternative to highly costly and energy consuming wastewater treatment plants. In addition, CWs offer the potential to be integrated into farming and aquaculture systems and can therefore improve food quality and production. The most prominent pollutants in urban SSA surface waters and the pollutant removal efficiencies for microbial and chemical contaminations of different plant species were identified from the literature and the accumulation rates for Pb, Cr, and Cd were compared to each other. A strong focus was given to studies conducted in SSA or other (sub)tropical regions. This review identified a range of potential phytoremediators to treat contaminated surface water and highlights the need for further in-situ studies in SSA. Plant species such as *Lemna minor*, *Ipomoea aquatica*, *Spirodela polyrhiza* and *Brachiaria mutica* show a high potential to phytoremediate the heavy metals Pb, Cr and Cd from surface water.

Phytotoxicity

Clay-catalyzed ozonation of organic pollutants in water and toxicity on *Lemna minor*: Effects of molecular structure and interactions

Wembe, ENF; Benghafour, A; Dewez, D; Azzouz, A. Molecules (2023) 29: 222.

The use of clays as adsorbents and catalysts in the ozonation of organic pollutants (Atrazine, bis-Phenol A, Diazinon, and Diclofenac sodium) allowed simulating their natural oxidative degradation in clay soils and to evaluate the ecotoxicity of mixtures partially oxidized on the species *Lemna minor*, a biodiversity representative of plants in the aquatic environment. Kinetic data showed that the adsorption of organic pollutants on clay particles obeys the pseudo-second-order model, while the adsorption isotherms satisfactorily fit the Langmuir model. Adsorption reduces the dispersion of the organic pollutant in the environment and prolongs its persistence and its natural degradation probability. Measurements of the Zeta potential and particle size as a function of pH demonstrate that the catalytic activity of clay depends on its cation, its silica/alumina ratio, and therefore on its permanent and temporary ion exchange capacities. These factors seem to govern its delamination and dispersion in aqueous media, its hydrophilic-hydrophobic character, and its porosity. Tests conducted on *Lemna minor* in contact with ozonation mixtures revealed that the toxicity could be due to pH decrease and to the toxicity of the intermediates yielded. Ecotoxicity would depend on the structure of the organic molecules, the chemical composition of the clay surface and ozonation time, which determines the oxidation progress. These results are of great importance for further research because they allow concluding that the negative impact of the persistence of an organic molecule in clay-containing media depends on the type and composition of the very clay mineral.

Toxicological effects resulting from co-exposure to nanomaterials and to a beta-blocker pharmaceutical drug in the non-target macrophyte species *Lemna minor*

Silva, PMM; Alkimin, GD; Camparotto, NG; Prediger, P; Nunes, B. Environmental Pollution (2023) 322: 121166

The wide use of carbon-based materials for various purposes leads to their discharge in the aquatic systems, and simultaneous occurrence with other environmental contaminants, such as pharmaceutical drugs. This co-occurrence can adversely affect exposed aquatic organisms. Up to now, few studies have considered the simultaneous toxicity of nanomaterials, and organic contaminants, including pharmaceutical drugs, towards aquatic plants. Thus, this study aimed to assess the toxic effects of the co-exposure of propranolol (PRO), and nanomaterials based on cellulose nanocrystal, and graphene oxide in the aquatic macrophyte *Lemna minor*. The observed effects included reduction of growth rate in 13% in co-exposure 1 (nanomaterials+PRO 5 μgL^{-1}), and 52-64% in co-exposure 2 (nanomaterials+PRO 51.3 mgL^{-1}), fresh weight reduction of 94-97% in co-exposure 2 compared to control group, and increased pigment production caused by co-exposure treatments. The analysis of PCA showed that co-exposure 1 (nanomaterials+PRO 5 μgL^{-1}) positively affected growth, and fresh weight, and co-exposure 2 positively affected pigments content. The results suggested that the presence of nanomaterials enhanced the overall toxicity of PRO, exerting deleterious effects in the freshwater plant *L. minor*, suggesting that this higher toxicity resulting from co-exposure was a consequence of the interaction between nanomaterials and PRO.

Phytotoxicity of microplastics to the floating plant *Spirodela polyrhiza* (L.): Plant functional traits and metabolomics

Wang, Y; Bai, J; Wen, Li; Wang, W; Zhang, L; Liu, Z; Liu, H. Environmental Pollution (2023) 322: 121199

Freshwater ecosystems are gradually becoming sinks for terrestrial microplastics (MPs), posing a potential ecological risk. Although the effects of MPs on plankton and aquatic animals in freshwater ecosystems have been given increasing attention, the toxicity of MPs to the metabolism of aquatic plants remains unclear. Here, the model aquatic plant *Spirodela polyrhiza* (L.) Schleid. (*S. polyrhiza*) was exposed to polyvinyl chloride (PVC; 0, 10, 100 and 1000 mg/L) MPs, and changes in the plant functional traits and physiological metabolism were monitored. The results showed that the high dose of PVC MPs decreased the adventitious root elongation ratio by 41.68% and leaf multiplication ratio by 61.03% of *S. polyrhiza*, and resulted in the decrease in anthocyanin and nitrogen contents to 63.45% and 84.21% of the control group, respectively. Moreover, the widely targeted metabolomics analysis results showed 37 differential metabolites in the low-dose treatment and 119 differential metabolites in the high-dose treatment. PVC MPs interfered with organic matter accumulation by affecting carbon metabolism, nitrogen metabolism, amino acid metabolism and lipid metabolism, and *S. polyrhiza* resists PVC MP stress by regulating the synthesis and metabolism of secondary metabolites. PVC MPs had concentration-related toxicological effects on plant functional traits, inhibited plant growth and reproduction, affected plant nutrient metabolism, and exhibited profound effects on the nitrogen fate of aquatic plant habitats. Overall, we systematically summarized the metabolic response mechanisms of aquatic plants to PVC MP stress, providing a new perspective for studying the effects of MPs on plant trait function and ecological risks.

Polystyrene microplastics reduce Cr(VI) and decrease its aquatic toxicity under simulated sunlight

Zhang, J; Wei, JT; Hu, T; Du, L; Chen, ZJ; Zhang, Y; Zhang, WC. Journal of Hazardous Materials (2023) 445: 130483

Microplastics (MPs) serve as vectors for chromium (Cr), influencing its fate and toxicity in aquatic environments, and have attracted much attention recently. However, it is still unknown whether MPs mediate Cr species transformation under sunlight irradiation. This study confirmed that polystyrene (PS) MPs could reduce Cr(VI) to Cr(III) under sunlight irradiation, with a photoreduction rate constant of 0.0023 h^{-1}. PS MPs-mediated Cr(VI) reduction was predominantly dependent on $O^{\cdot-}_2$ and simultaneously suppressed by $^1O_2$, ·OH and $^3PS^*$. Aged PS MPs were exposed to simulated sunlight irradiation for 0, 200, 500, and 800 h, and Cr(VI) reduction was hindered by increased O-1(2) and center dot OH formation and light-screening effects

(decreased photon absorption). The size, functional groups and concentration of PS MPs and environmental factors (e.g., humic acid, pH, Mg2+, Fe3+ and O-2) strongly affected Cr(VI) reduction. Furthermore, Cr(VI) reduction induced by PS MPs could occur in reservoir water, and the reduction rate was faster than that in double distilled (DD) water. Correspondingly, PS MPs (1 mg/L) decreased the oxidative stress induced by Cr(VI) to Lemna minor in reservoir water after 96 h of sunlight irradiation. This study provided deep insight into how PS MPs affect Cr species transformations and hazardous effects in realistic aquatic environments under sunlight conditions.

Ambiguous changes in photosynthetic parameters of *Lemna minor* L. after short-term exposure to naproxen and paracetamol: Can the risk be ignored?

Zezulka, S; Kummerova, M; Smeringai, J; Babula, P; Triska, J. Aquatic Toxicology (2023), 259: 106537

Non-steroidal anti-inflammatory drugs (NSAID) are recently monitored in the aquatic environment. Naproxen (NPX), paracetamol (PCT) and their transformation products can influence the biochemical and physiological processes at the sub-cellular and cellular levels taking part in the growth and development of plants. This study aimed to compare the effects of NPX and PCT, drugs with different physico-chemical properties, on the growth and photosynthetic processes in *Lemna minor* during a short-term (7 days) exposure. Although duckweed took up more than five times higher amount of PCT as compared to NPX (275.88g/g dry weight to 43.22g/g when treated with 10mg/L), only NPX limited the number of new plants by 9% and 26% under 1 and 10mg/L, respectively, and increased their dry weight (by 18% under 10mg/L) and leaf area per plant. A considerable (by 30%) drop in the content of photosynthetic pigments under 10mg/L treatment by both drugs did not significantly affect the efficiency of the primary processes of photosynthesis. Values of induced chlorophyll fluorescence parameters (F0, FV/FM, PhiII, and NPQ) showed just a mild stimulation by PCT and a negative effect by NPX (by up to 10%), especially on the function of photosystem II and electron transport in both intact duckweed plants and isolated chloroplasts. Lowered efficiency of Hill reaction activity (by more than 10% under 0.1 - 10mg/L treatments) in isolated chloroplasts suspension proved the only inhibition effect of PCT to primary photosynthetic processes. In intact plants, higher treatments (0.5 - 10mg/L) by both NPX and PCT induced an increase in RuBisCO content. The results prove that the potential effect of various drugs on plants is hard to generalise.

The effect of quinolones on Common Duckweed *Lemna minor* L., a hydrophyte bioindicator of environmental pollution

Sikorski, L; Bes, A; Warminski, K. International Journal of Environmental Research and Public Hedalth (2023) 20: DOI10.3390/ijerph20065089

Plant growth and the development of morphological traits in plants are inhibited under exposure to pharmaceuticals that are present in soil and water. The present study revealed that moxifloxacin (MOXI), nalidixic acid (NAL), levofloxacin (LVF) and pefloxacin (PEF) at concentrations of >0.29, >0.48, >0.62 and >1.45 mg * L^{-1}, respectively, inhibited the growth (Ir) of duckweed plants and decreased their yield (Iy). In the current study, none of the tested quinolones (QNs) at any of the examined concentrations were lethal for common duckweed plants. However, at the highest concentration (12.8 mg * L^{-1}), LVF increased Ir and Iy values by 82% on average and increased the values of NAL, PEF and MOXI by 62% on average. All tested QNs led to the loss of assimilation pigments. In consequence, all QNs, except for LVF, induced changes in chlorophyll fluorescence (Fv/Fm), without any effect on phaeophytinization quotient (PQ) values. The uptake of NAL, MOXI, LVF by *Lemna minor* during the 7-day chronic toxicity test was directly proportional to drug concentrations in the growth medium. Nalidixic acid was absorbed in the largest quantities, whereas in the group of fluoroquinolones (FQNs), MOXI, LVF and PEF were less effectively absorbed by common duckweed. This study demonstrated that biosorption by *L. minor* occurs regardless of the plants' condition. These findings indicate that *L. minor* can be used as an effective biological method to remove QNs from wastewater and water and that biosorption should be a mandatory process in conventional water and wastewater treatment.

Joint effects of gamma radiation and zinc on duckweed *Lemna minor* L.

Bodnar, IS; Cheban, EV. Aquatic Toxicology (2023) 257: 106438

When assessing the consequences of combined chemical and radiation pollution on bodies of water, it is important to take into account the interaction of different factors, especially the possible synergistic increase in the toxic effect on growth, biochemical and physiological processes of living organisms. In this work, we studied the combined effect of gamma-radiation and zinc on freshwater duckweed *Lemna minor* L. Irradiated plants (doses were 18, 42, and 63 Gy) were placed on a medium with an excess of zinc (3.15, 6.3, 12.6 μmol/L) for 7 days. Our results showed that the accumulation of zinc in tissues increased in irradiated plants when compared to non-irradiated plants. The interaction of factors in assessing their effect on the growth rate of plants was most often additive, but there was also a synergistic increase in the toxic effect at a zinc concentration of 12.6 μmol/L and irradiation at doses of 42 and 63 Gy. When comparing the combined and separate effects of gamma radiation and zinc, it was found that a reduction in the area of fronds (leaf-like plates) was caused exclusively due to the effects of radiation. Zinc and gamma-radiation contributed to the enhancement of membrane lipid peroxidation. Irradiation stimulated the production of chlorophylls a and b, as well as carotenoids.

Physiological response of nutrient-stressed *Lemna gibba* to pulse colloidal silver treatment

Varga, M; Pfeiffer, TZ; Begovic, L; Mlinaric, S; Horvatic, J; Miloloza, T; Camagajevac, IS. Plants (2023) 12: 1367.

Wastewater is a source of many environmental pollutants and potentially high concentrations of essential plant nutrients. Site-specific nutrient levels may influence the response of exposed plants to a chemical stressor. In the present study, we focused on the responses of model aquatic macrophyte swollen duckweed (*Lemna gibba* L.) to a short pulse exposure and a commercially available colloidal silver product as a potential environmental chemical stressor, combined with two levels of total nitrogen and phosphorus nutrition. Treatment with the commercially available colloidal silver product caused oxidative stress in *L. gibba* plants under both high and low nutrient levels. Plants grown and treated under high nutrient levels showed lower levels of lipid peroxidation and hydrogen peroxide accumulation, as well as higher levels of photosynthetic pigment content in comparison to treated plants under low nutrient levels. Higher free radical scavenging activity for plants treated with silver in combination with high nutrient levels resulted in better overall protection from silver-induced oxidative stress. The results showed that external nutrient levels significantly affected the *L. gibba* plant's response to the colloidal silver presence in the environment and that nutrient levels should be considered in the assessment of potential environmental impact for contaminants.

Silver inhibits *Lemna minor* growth at high initial frond densities

Tran, IT; Heiman, JA; Lydy, VR; Kissoon, L. Plants (2023) 12: 1104.

Silver nanoparticles (AgNPs) are the most popular engineered nanomaterials in consumer products due to their antimicrobial properties. They enter aquatic ecosystems via insufficient purified wastewaters from manufacturers or consumers. AgNPs inhibit growth of aquatic plants, including duckweeds. Growth media nutrient concentration and initial duckweed frond density can affect growth. However, it is not well understood how frond density affects nanoparticle toxicity. We investigated the toxicity of 500 μg/L AgNPs and $AgNO_3$ on *Lemna minor* at different initial frond densities (20, 40, and 80 fronds per 28.5 cm^2) over 14 days. Plants were more sensitive to silver at high initial frond densities. Growth rates based on frond number and area were lower for plants at 40 and 80 initial frond density in both silver treatments. AgNPs had no effect on frond number, biomass, and frond area at 20 initial frond density. However, $AgNO_3$ plants had lower biomass than control and AgNP plants at 20 initial frond density. Competition and crowding at high frond densities resulted in reduced growth when silver was present, therefore plant density and crowding effects should be considered in toxicity studies.

Microplastic toxicity and trophic transfer in freshwater organisms: Ecotoxicological and genotoxic assessment in *Spirodela polyrhiza* (L.) Schleid. and *Echinogammarus veneris* (Heller, 1865) treated with polyethylene microparticles

Iannilli, V; Passatore, L; Carloni, S; Lecce, F; Sciacca, G; Zacchini, M; Pietrini, F. Water (2023) 15: 921.

The widespread occurrence of microplastics (MPs) has resulted in their interaction with biological processes. Thus, there is a great concern about the potential toxicity of MPs on animal and plant cells and on the possibility that MPs reach humans through the food web. In order to shed light on both issues, laboratory assays were performed for evaluating the effects of polyethylene (PE) microparticles on the aquatic plant *Spirodela polyrhiza* (L.) Schleid. and the gammarid *Echinogammarus veneris* (Heller, 1865). Moreover, a stock of MP-treated Spirodela plants was used to feed gammarid individuals, and the presence of MP particles in their digestive tracts was analyzed. Results evidenced the lack of toxic effects of MPs on plants, evaluated at growth and physiological level by biometric parameters, pigment content, and photosynthetic performance estimated by chlorophyll fluorescence imaging through the ETPT (EcoTox Photosystem Tool). Only a slight reduction in pigment-related indices in MP-treated plants was observed. A remarkable genotoxic effect was instead highlighted by Comet assay in the hemocytes of gammarid individuals exposed to MPs, with three times more DNA damage (expressed as Tail Moment) in MP-treated individuals compared to control ones. Finally, the gut content of the gammarids fed with MP-treated plants revealed the presence of 7.6 MP particles/individual, highlighting the occurrence of trophic transfer of MPs among freshwater ecosystem organisms. Novel indications about the potential impact of the PE microparticles in the aquatic compartment are provided. Notably, the transfer of MP particles between primary producer and primary consumer organisms of the freshwater trophic chain and the genotoxic effects associated with the ingestion of such particles by gammarids are issues of concern for the aquatic ecosystem and the food web leading to the human diet.

Multigenerational effects of microplastic fragments derived from polyethylene terephthalate bottles on duckweed *Lemna minor*: Size-dependent effects of microplastics on photosynthesis

Cui, R; Kwak, JI; An, YJ. Science of the Total Environment (2023) 872: 162159.

The 2019 global coronavirus disease pandemic has led to an increase in the demand for polyethylene terephthalate (PET) packaging. Although PET is one of the most recycled plastics, it is likely to enter the aquatic ecosystem. To date, the chronic effects of PET microplastics (MPs) on aquatic plants have not been fully understood. Therefore, this study aimed to investigate the adverse effects of PET MP fragments derived from PET bottles on the aquatic duckweed plant *Lemna minor* through a multigenerational study. We conducted acute (3-day exposure) and multigenerational (10 generations from P0 to F9) tests using different-sized PET fragments (PET0-200, < 200 μm; PET200-300, 200-300 μm; and PET300-500, 300-500 μm). Different parameters, including frond number, growth rate based on the frond area, total root length, longest root length, and photosynthesis, were evaluated. The acute test revealed that photosynthesis in *L. minor* was negatively affected by exposure to small-sized PET fragments (PET0-200). In contrast, the results of the multigenerational test revealed that large-sized PET fragments (PET300-500) showed substantial negative effects on both the growth and photosynthetic activity of *L. minor*. Continuous exposure to PET MPs for 10 generations caused disturbances in chloroplast distribution and inhibition of plant photosynthetic activity and growth. The findings of this study may serve as a basis for future research on the generational effects of MPs from various PET products.

Toxicological effects resulting from co-exposure to nanomaterials and to a B-blocker pharmaceutical drug in the non-target macrophyte species *Lemna minor*

da Silva, PMM; de Alkimin, GD; Camparotto, NG; Prediger, P; Nunes, B. Environmental Pollution (2023) 322: 121166.

The wide use of carbon-based materials for various purposes leads to their discharge in the aquatic systems, and simultaneous occurrence with other environmental contaminants, such as pharmaceutical drugs. This co-occurrence can adversely affect exposed aquatic organisms. Up to now, few studies have considered the simultaneous toxicity of nanomaterials, and organic contaminants, including pharmaceutical drugs, towards aquatic plants. Thus, this study aimed to assess the toxic effects of the co-exposure of propranolol (PRO), and nanomaterials based on cellulose nanocrystal, and graphene oxide in the aquatic macrophyte *Lemna minor*. The observed effects included reduction of growth rate in 13% in co-exposure 1 (nanomaterials + PRO μg L^{-1}), and 52-64% in co-exposure 2 (nanomaterials + PRO 51.3 mg L^{-1}), fresh weight reduction of 94-97% in co-exposure 2 compared to control group, and increased pigment production caused by co-exposure treatments. The analysis of PCA showed that co-exposure 1 (nanomaterials + PRO 5 μg L^{-1}) positively affected growth, and fresh weight, and co-exposure 2 positively affected pigments content. The results suggested that the presence of nanomaterials enhanced the overall toxicity of PRO, exerting deleterious effects in the freshwater plant *L. minor*, suggesting that this higher toxicity resulting from co-exposure was a consequence of the interaction between nanomaterials and PRO.

Phytotoxic action of silver nanoparticles on *Lemna minor*: Multi-parameter analysis of different physiological processes

Ljubimir, KG; Domijan, AM; Brkanac, SR. Plants (2023) 12: 343.

Considering the widespread use of silver nanoparticles (AgNPs) and their consequent build-up in waterways, there is a concern about the hazardous effect of AgNPs for aquatic ecosystems. The aim of this study was to clarify the mechanism of the action of AgNPs on duckweed (*Lemna minor* L.) by evaluating multiple parameters in different physiological processes. Duckweed was treated with AgNPs in a concentration range of 0.5 to 5 mg/L over a 7-day period. The analysis revealed that the AgNP-treated duckweed accumulated Ag in accordance with increasing AgNP concentrations. Furthermore, higher concentrations (2 and 5 mg/L) of AgNPs negatively affected N, P and especially K and Mg levels in the plant tissue. Accordingly, the plant growth and photosynthetic parameters were more inhibited in response to higher concentrations of AgNPs. Nanosilver significantly increased the generation of ROS at higher concentrations, although lipid peroxidation was significant even at the lowest concentration of AgNPs. However, defense mechanisms were able to counteract AgNP-induced oxidative stress and balance the intracellular redox status, as evidenced by increased activities of the main detoxification enzymes. With this experimental setting, AgNPs exhibited a relatively weak phytotoxicity at 0.5 and 1 mg/L; nevertheless, silver in a nano form poses a hazard for plants, considering its continuous release into aquatic environments.

Species- and metal-specific responses of the ionome of three duckweed species under chromate and nickel treatments

Olah, V; Irfan, M; Szabo, ZB; Sajtos, Z; Ragyak, AZ; Donczo, B; Jansen, MAK; Szabo, S; Meszaros, I. Plants (2023) 12: 180.

In this study, growth and ionomic responses of three duckweed species were analyzed, namely *Lemna minor*, Landoltia punctata, and *Spirodela polyrhiza*, were exposed for short-term periods to hexavalent chromium or nickel under laboratory conditions. It was found that different duckweed species had distinct ionomic patterns that can change considerably due to metal treatments. The results also show that, because of the stress-induced increase in leaf mass-to-area ratio, the studied species showed different order of metal uptake efficiency if plant area was used as unit of reference instead of the traditional dry weight-based approach. Furthermore, this study revealed that μXRF is applicable in mapping elemental distributions in duckweed fronds. By using this method, we found that within-frond and within-colony compartmentation of metallic ions were strongly metal- and in part species-specific. Analysis of duckweed ionomics is a valuable approach in exploring factors that affect bioaccumulation of trace pollutants by these plants. Apart from remediating industrial effluents, this aspect will gain relevance in food and feed safety when duckweed biomass is produced for nutritional purposes.

Duckweeds. Quick guide

Mateo-Elizalde, C; Lynn, J; Ernst, E; Martienssen, R. Current Biology (2023) 33: R85-R97.

Lemnaceae, commonly known as duckweeds, constitute a family of monocotyledonous aquatic floating plants that live in freshwater ponds and lakes all over the world. This family includes two subfamilies (Lemnoideae and Wolffioideae), 5 genera (*Spirodela*, *Lemna*, *Landoltia, Wolffia* and *Wolffiella*), and 36 currently recognized species, varying in size from 1.5 centimeters to less than one millimeter. The most exceptional trait of the duckweeds is that they are the smallest and fastest growing angiosperms known.

Melatonin involved in protective effects against cadmium stress in *Wolffia arrhiza*

Chmur, M; Bajguz, A. International Journal of Molecular Sciences (2023) 24: 1178.

Melatonin (MT) is a new plant hormone that protects against adverse environmental conditions. In the present study, the responses of *Wolffia arrhiza* exposed to cadmium (Cd) and MT were analyzed. Quantitative analysis of MT and precursors of its biosynthesis was performed using LC-MS-MS. The photosynthetic pigments and phytochelatins (PCs) contents were determined using HPLC, while protein and monosaccharides, stress markers, and antioxidant levels were determined using spectrophotometric methods. Interestingly, the endogenous level of MT and its substrates in *W. arrhiza* exposed to 1-100 µM Cd was significantly higher compared to the control. Additionally, the application of 25 µM MT and Cd intensified the biosynthesis of these compounds. The most stimulatory effect on the growth and content of pigments, protein, and sugars was observed in plants treated with 25 µM MT. In contrast, Cd treatment caused a decrease in plant weight and level of these compounds, while the application of 25 µM MT mitigated the inhibitory effect of Cd. Additionally, Cd enhanced the level of stress markers; simultaneously, MT reduced their content in duckweed exposed to Cd. In plants treated with Cd, PC levels were increased by Cd treatment and by 25 µM MT. These results confirmed that MT mitigated the adverse effect of Cd. Furthermore, MT presence was reported for the first time in *W. arrhiza*. In summary, MT is an essential phytohormone for plant growth and development, especially during heavy metal stress.

Effects of microplastic contamination on the aquatic plant *Lemna minuta* (Least Duckweed)

Ceschin, S; Mariani, F; Di Lernia, D; Venditti, I; Pelella, E; Iannelli, MA. Plants (2023) 12: 207.

Microplastics are widely spread in aquatic environments. Although they are considered among the most alarming contaminants, toxic effects on organisms are unclear, particularly on freshwater plants. In this study, the duckweed *Lemna minuta* was grown on different concentrations (50, 100 mg/L) of poly(styrene-co-methyl methacrylate) microplastics (MP) and exposure times (T0, T7, T14, T28 days). The phytotoxic effects of MP were investigated by analyzing several plant morphological and biochemical parameters (frond and root size, plant growth, chlorophyll, and malondialdehyde content). Observations by scanning electron microscope revealed MP adsorption on plant surfaces. Exposition to MP adversely affected plant growth and chlorophyll content with respect to both MP concentrations and exposure times. Conversely, malondialdehyde measurements did not indicate an alteration of oxidative lipid damage in plant tissue. The presence of MP induced root elongation when compared to the control plants. The effects of MP on *L. minuta* plants were more evident at T28. These results contribute to a better understanding of MP's impact on aquatic plants and highlight that MP contamination manifests with chronic-type effects, which are thus detectable at longer exposure times of 7 days than those traditionally used in phytotoxicology tests on duckweeds.

Ammonium uptake, mediated by ammonium transporters, mitigates manganese toxicity in duckweed, *Spirodela polyrhiza*

Kishchenko, O; Stepanenko, A; Straub, T; Zhou, YZ; Neuhaeuser, B; Borisjuk, N. Plants (2023) 12: 208.

Nitrogen is an essential nutrient that affects all aspects of the growth, development and metabolic responses of plants. Here we investigated the influence of the two major sources of inorganic nitrogen, nitrate and ammonium, on the toxicity caused by excess of Mn in great duckweed, *Spirodela polyrhiza*. The revealed

alleviating effect of ammonium on Mn-mediated toxicity, was complemented by detailed molecular, biochemical and evolutionary characterization of the species ammonium transporters (AMTs). Four genes encoding AMTs in *S. polyrhiza*, were classified as SpAMT1;1, SpAMT1;2, SpAMT1;3 and SpAMT2. Functional testing of the expressed proteins in yeast and *Xenopus oocytes* clearly demonstrated activity of SpAMT1;1 and SpAMT1;3 in transporting ammonium. Transcripts of all SpAMT genes were detected in duckweed fronds grown in cultivation medium, containing a physiological or 50-fold elevated concentration of Mn at the background of nitrogen or a mixture of nitrate and ammonium. Each gene demonstrated an individual expression pattern, revealed by RT-qPCR. Revealing the mitigating effect of ammonium uptake on manganese toxicity in aquatic duckweed *S. polyrhiza*, the study presents a comprehensive analysis of the transporters involved in the uptake of ammonium, shedding a new light on the interactions between the mechanisms of heavy metal toxicity and the regulation of the plant nitrogen metabolism.

Taxonomy & Geobotany

Hybrid between Lemna minor and *L. turionifera* (*L. x japonica*, Lemnaceae) in East Europe is more frequent than parental species and poorly distinguishable from them

Volkova, PA; Nachatoi, VA; Bobrov, AA. Aquatic Botany (2023) 14: 103593. DOI10.1016/j.aquabot.2022.103593

Biodiversity studies and practical use of duckweeds are impossible without reliable species identification. Despite of clear genetic differentiation of *L. minor*, *L. turionifera* and their hybrid *L. x japonica*, their morphological discrimination is challenging even for professional botanists; consequently, the distribution of the two latter taxa is not precisely known. Although numerous determination keys exist, none of them is based on quantitative analysis of the morphological variation observed in nature. Thus, we aimed to study variation of morphological characteristics most frequently proposed for discrimination between *L. minor*, *L. turionifera* and *L. x japonica* (frond shape and pigmentation, papule presence along the median nerve) on specimens collected in the wild, which were taxonomically identified using molecular tools (intron length polymorphism in the 8-tubulin gene). We focused on samples from the poorly studied East Europe, including some from the Russian Far East for comparison. *Lemna x japonica* appeared to be quite abundant in different regions of East Europe. The Far Eastern occurrences of *L. minor* and *L. x japonica* should be carefully verified. Even our limited sampling demonstrated that morphological characters conventionally used for delimitation of *L. minor, L. turionifera* and *L. x japonica* do not work in wild populations. Additional studies should be performed for verification of diagnostic value of generative characters. Large-scale genetic screening of Lemna populations in nature is also indispensable to clarify distribution of taxa from the *L. minor* complex.

Aquatic vascular plants in the mires of the Volga Upland: Distribution, occurrence, and ecology

Grishutkin, OG; Ershkova, EV; Sokolova, IS. Inland Water Biology (2022) 15: 773-783.

Information on 15 species of aquatic vascular plants growing in the mires of the Volga upland is provided. It is revealed that *Lemna minor*, *Persicaria amphibia*, and *Utricularia vulgaris* are common representatives of the flora of fens and, less often, transitional mires. *Hydrocharis morsus-ranae* and *Potamogeton natans* have a slightly smaller distribution, which are confined mainly to disturbed mires. *Utricularia intermedia* and *U. minor* are rare species in most regions of the Volga upland; they grow mainly in transitional mires, where their occurrence is estimated as sporadic. *Potamogeton gramineus* and *Salvinia natans* are included in the regional Red Data Books; most of the finds are known from the reaches of the Volga reservoirs, but these species are characterized by habitats on some subtypes of mires. Six species are common on the Volga upland for lakes, but they are rare in mires: *Lemna trisulca* and *Spirodela polyrhiza* only in the most mineral-rich fens, *Nuphar lutea* and *Nymphaea candida* in coastal strips of intracoastal reservoirs, and *Ceratophyllum demersum* and *Stratiotes aloides* in mires of the initial stage of formation from mainly ancient lakes.

DNA barcoding and biomass accumulation rates of native Iranian duckweed species for biotechnological applications

Taghipour, E; Bog, M; Frootan, F; Shojaei, S; Rad, N; Arezoumandi, M; Jafari, M; Salmanian, AH. Frontiers in Plant Science (2022) 13: 1034238.

The Lemnaceae family (duckweed) consists of at least three recognized genera with six reported species in Iran that are distributed in wetlands. Duckweeds are the simplest and smallest flowering aquatic monocots with free-floating fronds that can reproduce asexually every 2-3 days. Duckweed could be a major source of balanced amino acids and high protein content, which is increasingly promising for biotechnological applications. For molecular classification and species identification of the collected samples, DNA barcoding was performed using two standard chloroplast markers, the spacer region between the ATP synthase subunits F and H (atpF-atpH) and the intron region of the ribosomal protein S16 (rps16). The results confirm the presence of four species belonging to the two genera *Lemna* and *Spirodela*. In addition, *L. turionifera* was detected for the first time in Iran. Due to the high growth rates of duckweed, measurement of biomass accumulation and doubling time are important factors in determining growth potential, especially for native species. The relative growth rates (RGR), doubling times (DT), biomass accumulation, and relative weekly yields (RY) of 40 distinct duckweed clones were determined under standard cultivation conditions. The dry weight-based RGR ranged from 0.149 to more than 0.600 per day, DT from 1.12 to 9 days, and RY from 7 to 108.9 per week. All values are comparable with previous studies. RGR and RY of selected clones are higher than the growth potential for a wide range of wild plants and common crops. These data support that native duckweed has high productivity value and should be further investigated as a potentially rich protein source for alternative human food, livestock feed, and recombinant protein production.

Instructions to Contributors for the Duckweed Forum

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

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

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

Presubmissions

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

Copyright and co-author consent

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

Formatting requirements:

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

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

Title:

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

Authors:

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

Image resolution and submission:

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

Links for Further Reading

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

http://www.InternationalLemnaAssociation.org/ Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://thecharmsofduckweed.org Comprehensive site on all things duckweed-related, By Dr. John Cross, maintained by Paul Fourounjian.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

Community Resources - Updated Table for Duckweed Collections in the Community

For information related to the location, collection size and contact email for duckweed collections in our community, please access the website of the RDSC (Rutgers Duckweed Stock Cooperative) under the heading "List of Worldwide Duckweed Collections". This Table will be updated as new entries for duckweed collections are being supplied to members of the International Steering Committee for Duckweed Research and Applications (ISCDRA). We also plan to publish the updated table in the first issue of each Duckweed Forum newsletter volume starting in 2021.

Note to the Reader

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

Please let us know via email to the Chair of ISCDRA, Prof. Eric Lam: ericL89@hotmail.com