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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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Milano, Italy

New home of the Landolt Duckweed Collection

Cover page

Milano, Italy: New home of the Landolt Duckweed Collection

This montage introduces the new home for the famed Landolt collection that has recently been moved from Switzerland to Italy, at the Istituto di Biologia e Biotecnologia Agraria in Milano. This important resource for the duckweed community will be under the care of Laura Morello and her colleague at the Institute. Their recent demonstration of natural interspecific hybrids in the *Lemna* genus, represented in the top middle illustration, helped to resolve the long-standing confusion in identifying certain *Lemna* species. Clockwise from the upper right panel: The entrance to their building; one view of their block in the Institute are shown on the bottom row; the Duomo Cathedral, a major landmark of Milano.

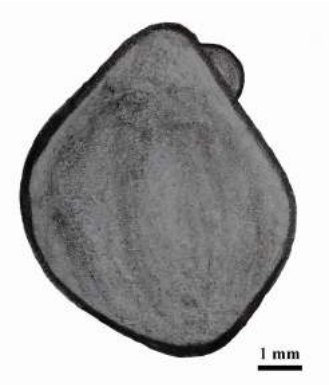
In this issue

The 4th International Steering Committee on Duckweed Research and Applications Members

- **Chair: Prof. Eric Lam**, Rutgers, The state University of NJ, New Brunswick, USA; ericL89@hotmail.com
- **PD Dr. Klaus-J. Appenroth**, Friedrich Schiller University of Jena, Germany; Klaus.Appenroth@uni-jena.de
- **Dr. K. Sowjanya Sree**, Central University of Kerala, Periye, India; ksowsree9@cukerala.ac.in
- **Dr. Yubin Ma**, Ocean University of China, Qingdao, China; mayubin@ouc.edu.cn
- **Dr. Tsipi Shoham**, GreenOnyx Ltd., Tel Aviv, Israel; tsipi@greenonyx.biz

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

Science meets art: *Spirodela intermedia* W.Koch



In contrast to *Spirodela polyrhiza*, the tip of fronds of *S. intermedia* is more roundish whereas the general shape of the fronds (ratio length / width) is practically the same. *Spirodela intermedia* occupies a rather restricted area which is mainly in subtropical and tropical regions of South and Central America. One reason might be that *S. intermedia* does not form turions that would help it to survive unfavourable environmental conditions like low temperature seasons. Although there are hardly differences in genome size, the chromosomal structure and even the number of chromosomes of both species are quite different (Hoang et al., Scientific Reports 10: 19230 (2020)). Legend by Dr. Klaus-J. Appenroth, Friedrich Schiller University, Jena, Germany; Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the Editor:

Jan. 29th, 2022

Dear *Duckweed Forum* readers,

Happy New Year of 2022. Not only it is January of 2022, it will also soon be the Year of the Tiger on the Lunar Calendar in 3 days. Tiger is a symbol of vigor and good health, and it is an appropriate time to wish everyone a happy and healthy 2022. Let us look forward to moving beyond the COVID pandemic and to a new normal this year.

An important event for our community this year will be the resumption of our International Conference on Duckweed Research and Applications. As you will see in the announcement among these pages of the newsletter, this postponed conference is scheduled to take place as planned at the end of May this year. Registration is now open on its website and the requirements for attendance during this global health crisis situation can be found on the "Registration" page. I encourage everyone to check this out as soon as possible and register for attendance as well. I believe there will be many exciting new development in the duckweed field to be presented in this Conference in addition to many opportunities to network with fellow duckweed enthusiasts. Thus, the additional steps to enable everyone to attend the meeting safely will be well worth the extra effort.

There are several exciting advances in the duckweed research field that I would like to point out here. As we have expressed in the recent *Plant Cell* review article (Acosta *et al*., 2021), one of the key area to develop in the duckweed field is a robust genetic platform for both research and practical applications. The recent publication of Yoshida *et al*. (Front. in Pl. Sci. 2021) reported a thorough first analysis of flowering genes' expression in the clone *L. aequinoctialis* Nd. Using the model plant Arabidopsis' rich genetic resources, they demonstrated the diverse functions of FT (Flowering Locus T) like homologs from duckweed to promote or inhibit flowering. This work, and others like this in the future, should help usher in our molecular understanding of flowering induction for duckweeds in general. Two other items in this DF issue converge with this Database Highlight. First, an Obituary to the passing of J.P. Khurana ably summarized his many contributions to the early days of flowering studies in the duckweed field. While they were mostly pharmacological experiments, they nevertheless established the foundational knowledge that can now be extended with molecular and genomic tools. Secondly, the recent work on molecular systematics of the *Lemna* complex using the TBP barcoding approach, as summarized by Braglia and Morello, demonstrated the natural formation of hybrids between *L. minor* with partners of *L. gibba* or *L. turionifera*. This important finding not only resolved the difficult task of correctly identifying these morphologically similar clones, it also corrected many of the misidentified clones in existing collections. The RDSC is already taking steps to revise the designation of the clones that were affected in its collection, as an example. Even more importantly, this work showed that it should be possible to create crosses and hybrids of duckweeds with parental clones of defined genetic makeup and with the right flowering induction signals. The future thus looks bright for the day when duckweed genetics will be routine and we can add this missing tool to our field.

With this positive thought, I wish all of our readers a productive and prosperous year. Last but not least, a big thanks to all the contributors to the *Duckweed Forum* over the past year as well as to the members of our International Steering Committee. Their effort and dedication is greatly appreciated and key to the successful completion of each of the past issues for this community newsletter.

Sincerely,

Eric Lam

Chair, ISCDRA

Duckweed evolution: unveiling a history of hybridizations

Laura Morello and Luca Braglia

Institute for Agricultural Biology and Biotechnology, Milan, Italy (Email: morello@ibba.cnr.it)

The duckweed (Lemnaceae) family includes the five genera *Landoltia, Spirodela*, *Lemna*, *Wolffia* and *Wolffiella*. Phylogenetic trees clearly told that their evolutionary history proceeded toward a morphologic simplification and a reduction in size of the body plan, despite a progressive increase in genome size. *Spirodela* is the most ancestral genus, with two species having large fronds and many roots, while the tiny, rootless Wolffia and *Wolffiella*, are the most recently diverged genera. However, little is known about adaptive radiation and speciation mechanisms in these mainly asexual plants. Divergence of peripheral isolates seems a common geographical mode of speciation in duckweeds, through adaptation to different climates. In several cases, closely related species differ in the ability to survive extreme conditions such as cold temperature and desiccation, through formation of turions or drought resistant seeds (e.g. *Spirodela polyrhiza/Spirodela intermedia; Lemna perpusilla/Lemna aequinoctialis*). It is then supposed that morphologically similar species may differ ecologically, and that these ecological differences limit their distributions. (Crawford et al., 2006).

Interspecific hybridization in the genus *Lemna* originated at least two hybrid species, recently revealed (Figure 1). This suggests that hybrid speciation could have been another mechanism of sympatric speciation, despite the rarity of flowering observed in most duckweed species. How this finding could be extended to other genera such as *Wolffia* and *Wolffiella* is not known but is worth to be investigated.

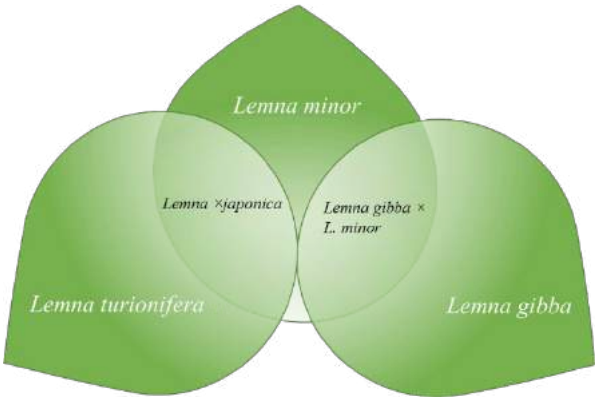


Figure 1: Diagram representing the formation of the two hybrid Lemna species – From Braglia et al., 2021b

Lemna ×japonica

Less than two years ago, by testing the power of the TBP molecular marker for species delimitation in duckweeds, we found evidence that the species described by E. Landolt in 1980 as *Lemna japonica* (Landolt, 1980) was in fact a hybrid between two species L. *minor* and *L. turionifera* (Braglia et al., 2021a). This was in perfect agreement with the original description of *L. japonica* "The species combines characteristics of *L. minor* and *L. turionifera*. It looks similar to *L. minor* but under certain conditions it may turn red and slightly gibbous underneath. Some strains can form turion-like fronds […]. The flowers are similar to those of *L. turionifera*, the fruits are unknown" (Landolt, 1980). Not only all clones classified as *L. japonica* were found to be hybrids, but also a large number

of *L. minor* clones, which were misclassified for their morphologic similarity, suggesting a wider geographic distribution of the species.

But this was just the beginning of the story, since how and when such hybridization took place is still under investigation. Genomic and chromosomal structure of the hybrid have to be elucidated and the complexity of the taxonomic relationships between these three closely related species is not unraveled, yet. In fact, both *L. minor* and *L. japonica* (and probably also the less investigated *L. turionifera*) show a wide spectrum of variation at the genetic and genome size level (Hoang et al., 2019), which is confirmed by subclustering of each species by TBP molecular marker analysis (Braglia et al., 2021b). The three species could form a species complex, a term which describes a group of closely related organisms that are so similar in appearance that the boundaries between them are often unclear, despite genetic difference.

Hybridization and/or introgression may cause reticulate evolution which makes the establishment of limits among species difficult. Species complexes originated by hybridization may represent early stages of speciation, when the absence or looseness of reproductive barriers between species still allow partial gene flow.

In the case of duckweeds, however, the prevalence of asexual reproduction can itself reduce the selective pressure towards reproductive isolation, which can eventually be provided by geographic or ecological barriers. The integrity of the parent species is likely granted by the prevalent asexual reproduction and the occasional occurrence of flowering in many species. Fast agamic propagation may allow hybrid speciation to proceed fast, even in case of hybrid sterility, thus limiting the possibility of backcrossing.

Investigation of species complexes requires a multidisciplinary approach including molecular markers, genomics, cytogenetics, anatomy, ecology, and reproductive biology (Pinheiro et al., 2018).

Another hybrid: *Lemna gibba* × *Lemna minor*

By continuing our genotyping analysis of the genus *Lemna*, we came across another interspecific hybrid. Also in this case one of the parent was *Lemna minor* whereas the other was *Lemna gibba*. The identification of the new hybrid species was revealed once again by the perfect overlapping of the hybrid TBP profile with those of the two parental species, further confirmed by intron sequence analysis. This is reported in our last publication in the recently published special Issue in *Plants* (Braglia et al., 2021b).

We found six clones belonging to this hybrid species among 23 putative *L. gibba* analyzed clones, which means that all were recorded as *L. gibba* by morphological analysis: 9562, 7641, 6861, 7320, 9425a and 9248. All six clones were from Europe and the Mediterranean basin (Italy, Egypt, Israel), where both parents are widely distributed, sometimes in the same community. No hybrid was found, up to now, among American clones of *L. gibba*. Conversely to what was found in *L. ×japonica*, in which plastid markers were always found to be inherited from *Lemna minor*, in the case of *Lemna gibba* × *Lemna minor*, both reciprocal crosses were identified, with two out of six clones having *L. gibba* as the plastid donor, therefore the female parent. This provides a nice model to study the maternal effect on the phenotype of the progeny, as well as to investigate heterosis on important traits such as growth rate and stress tolerance for applicative purposes.

The gross morphology of this new cryptic species is not different from *L gibba*, although pronounced gibbosity was never observed, *in vitro*. The six clones are therefore under investigation for subtler morphological traits. Flowering induction will probably help for this purpose, as flowers and fruits, if

present, may provide important distinctive traits. Therefore, the hybrid *Lemna gibba* × *Lemna minor* is still waiting for a description and a name.

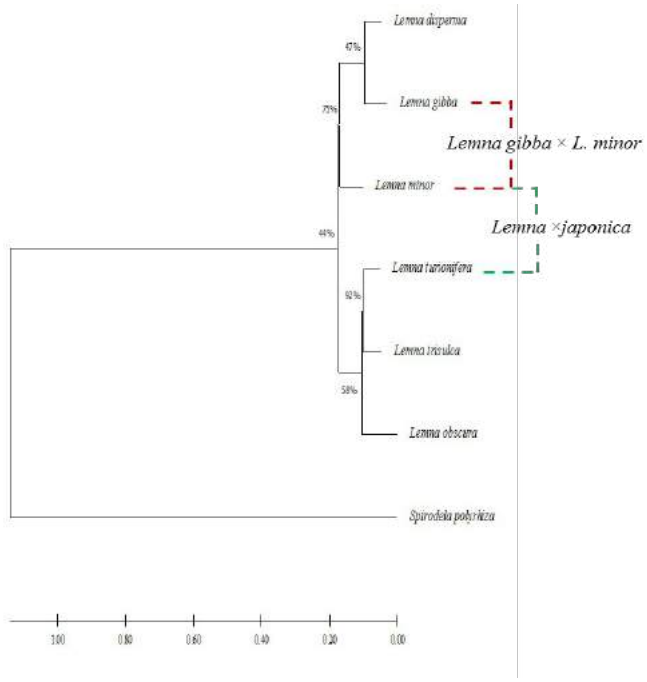


Figure 2: Suggested phylogeny of the Lemna section Lemna. From Braglia et al., 2021b

The finding of the two hybrid species led us to partially redraw the phylogenetic tree of the *Lemna* section *Lemna*, as shown in Figure 2.

The secret life of *Lemna symmeter*: the story of a rediscovered manuscript

Although *Lemna gibba* is generally discriminated from *L. minor* for the characteristic ventral gibbosity that gives it the name, 'flat forms' with intermediate phenotype, and not easily assigned to any of the two species, have been reported in the literature, particularly in old papers of the seventies in the last century (De Lange, 1975; Landolt, 1975). These flat forms may occur in the same water body with gibbous *L. gibba* and are often mistaken for *L. minor*.

One of such papers, cited by Kandeler (1975) as well as in Landolt's monography (Landolt, 1986) was authored by Giuseppe Giuga in 1973 under the title "Vita segreta di Lemnacee I. *Lemna symmeter* G. Giuga-species nova" (The secret life of Lemnaceaea. *Lemna symmeter* G. Giuga-new species). As from its title, the manuscript reports the description of a new *Lemna* species, namely *L. symmeter*, closely related to *L. gibba* and identified in Southern Italy.

Suspecting that the newly discovered hybrid could be related to "*Lemna symmeter*", we were interested in the full description of this species and its distinctive taxonomical traits. We than searched the original text in a number of scientific journal online repositories, where many publications of the same age are recorded as xerox copies. Nothing at all. We were completely unsuccessful also by inquiring to duckweed researchers in Europe, to the Landolt collection and even contacting the libraries of the University of Naples and the Botanical Gardens. We could neither find any other publication by this Author, nor information about its affiliation. We resigned to give up when a colleague in Naples suggested us: "why don't you try on eBay"? This was the right way and luck smiled at us: in a few days a second-hand bookseller in Naples sent us its only copy of the booklet for 10 €, and the description of *L. symmeter* was in our hands. It is a small pamphlet, published in Naples and written in Italian, except for the Abstract that is also translated in English. The booklet, including 19 typed pages and 9 Tables (two drawings and nine b/w pictures), reports an accurate description of *L. gibba* populations found in different places of the Campania region (Southern Italy), documenting observations on flowering in the wild, as well as on the cultivation of collected specimens.

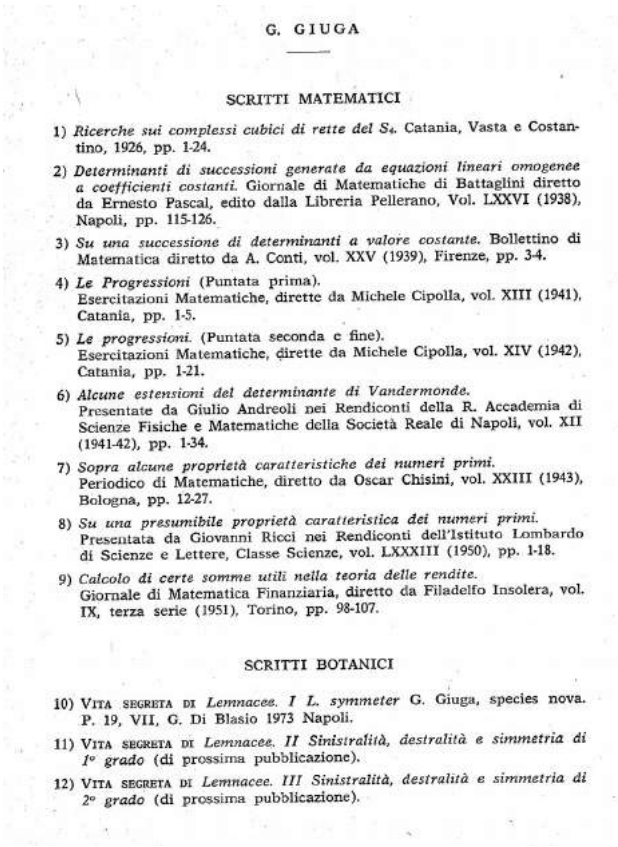
The fundamental distinctive trait of *L. symmeter*, according to Giuga, is flower development, differing from that of *L. gibba* for the symmetrical (simultaneous) growth of anthers who appear some days after the stigma. Flowers are reported to be sterile.

However, the description of the novel species couldn't be considered as valid since it was missing the diagnosis in Latin, requested at the time. Subsequent taxonomic revisions of the family, including

Landolt monography and more recently by Sree et al. (2016), reported *L. symmeter* as one of the synonyms of *L. gibba*, and the species name is now mostly forgotten.

Who was Giuseppe Giuga? When maths meets botany

As we mentioned, a curious fact about Giuga is that his findings about the new species were not published in a national or international botanical journal, nor printed by any Academic publisher or scientific society, but published by the Author himself. Moreover, we couldn't find any other publication by him either on duckweeds or any other plant species. However, we know that he has been in touch with eminent botanists such as Prof. Kandeler in Vienna (see a dedicated article by K J Appenroth in Duckweed Forum 32: 5-8, 2021) who wrote: "we received living material from Professor Giuga collected in the Botanical Garden of Naples, which belongs to *L. obscura* because of the very small fronds" (Kandeler, 1975).



G. GIUGA

SCRITTI MATEMATICI

1) *Ricerche sui complessi cubici di rette del S₄.* Catania, Vasta e Costantino, 1926, pp. 1-24.

2) *Determinanti di successioni generate da equazioni lineari omogenee a coefficienti costanti.* Giornale di Matematiche di Battaglini diretto da Ernesto Pascal, edito dalla Libreria Pellerano, Vol. LXXVI (1938), Napoli, pp. 115-126.

3) *Su una successione di determinanti a valore costante.* Bollettino di Matematica diretto da A. Conti, vol. XXV (1939), Firenze, pp. 3-4.

4) *Le Progressioni* (Puntata prima). Esercitazioni Matematiche, dirette da Michele Cipolla, vol. XIII (1941), Catania, pp. 1-5.

5) *Le progressioni.* (Puntata seconda e fine). Esercitazioni Matematiche, dirette da Michele Cipolla, vol. XIV (1942), Catania, pp. 1-21.

6) *Alcune estensioni del determinante di Vandermonde.* Presentate da Giulio Andreoli nei Rendiconti della R. Accademia di Scienze Fisiche e Matematiche della Società Reale di Napoli, vol. XII (1941-42), pp. 1-34.

7) *Sopra alcune proprietà caratteristiche dei numeri primi.* Periodico di Matematiche, diretto da Oscar Chisini, vol. XXIII (1943), Bologna, pp. 12-27.

8) *Su una presumibile proprietà caratteristica dei numeri primi.* Presentata da Giovanni Ricci nei Rendiconti dell'Istituto Lombardo di Scienze e Lettere, Classe Scienze, vol. LXXXIII (1950), pp. 1-18.

9) *Calcolo di certe somme utili nella teoria delle rendite.* Giornale di Matematica Finanziaria, diretto da Filadelfo Insolera, vol. IX, terza serie (1951), Torino, pp. 98-107.

SCRITTI BOTANICI

10) VITA SEGRETA DI *Lemnacee. I L. symmeter* G. Giuga, species nova. P. 19, VII, G. Di Blasio 1973 Napoli.

11) VITA SEGRETA DI *Lemnacee. II Sinistralità, destralità e simmetria di 1° grado* (di prossima pubblicazione).

12) VITA SEGRETA DI *Lemnacee. III Sinistralità, destralità e simmetria di 2° grado* (di prossima pubblicazione).

Figure 3: Last page of the booklet published by G. Giuga

We than began to wonder who might have been the mysterious Giuseppe Giuga? Maybe a young scientist early in his career? The answer was readily found in the last page of his booklet, where nine of his publications were listed under the title "Mathematical writings" (Figure 3). Yes, that's it, Giuseppe Giuga wasn't a botanist, rather a mathematician! By browsing the web, we finally found out that he is known for his work on prime numbers reported as the so-called "Giuga's conjecture", published in 1950 (Giuga, G. 1950). The Giuga numbers are therefore prime numbers fulfilling the requirements of his conjecture on primality. His theorem was later confirmed by a Japanese scientist (Agoh, 1995; https://en.wikipedia.org/wiki/Agoh%E2%80%93Giuga_conjecture;https://mathworld.wolfram.com/GiugasConjecture.html) and shown by the Canadian mathematician Peter Borwein to be true for all numbers with up to 13800 digits (about 5 complete pages of digits in 12-point courier font!) (Borwein et al., 1996).

This is all what we presently know about G. Giuga, we couldn't find any other information (although we are still investigating) about his life. Therefore, Giuga was very likely a Professor of mathematics at the University of Naples, but surely also a talented, self-taught botanist, well updated about the botanical literature and with international contacts. We can imagine him as a duckweed enthusiast, who likely spent years in observing and growing these fascinating aquatic plants, even without any official title, reminding us that science has no boundaries when led by passion. Since his first mathematical publication dates back to 1926, we can also imagine that he cultivated his passion for duckweeds until the last years of his life.

Interestingly, beside the list of mathematical writings, Giuga's booklet also mentioned as "Botanical writings" two further volumes about *Lemna*, reported as "under publication", and with the following titles:

Secret life of Lemnaceae. II. right-handedness, left-handedness and first degree symmetry
Secret life of Lemnaceae. III. right-handedness, left-handedness and second degree symmetry

These titles refer to the emergence of the daughter fronds and their development.

Symmetry of the body plan is indeed fundamental to the development of organisms, and it has been since long investigated in plants and even in duckweeds (Doss, 1978; Witzum, 1979). This seems a perfect subject for a mathematician fascinated by plants. Unfortunately, no trace of these or other botanical studies by Giuga was found. As far as we know these two manuscripts have never been published, possibly because Giuseppe Giuga didn't live enough to publish them.

We believe that, if the hybrid species that we identified will come out to correspond to "*Lemna symmeter*" as we hypothesize, it will represent a posthumous tribute to the memory of this 'cryptic' Italian plant scientist. For this reason, it will be fundamental to induce flowering in the identified clones. There is an Italian equivalent of the saying "time will tell", which can be translated as: "if they're rose, they'll bloom". We can just conclude: If they're *symmeter*, they'll bloom.

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6th ICDRA- 2022: Update

Dear Duckweed Researchers and all who are interested in Duckweed Research and Applications,

As announced in the Duckweed Forum Issue No. 35 (2021), at http://www.ruduckweed.org/, the 6th International Conference on Duckweed Research & Applications (ICDRA-6), which has been postponed due to pandemic, will now take place from

May 29 to June 01, 2022 in Gatersleben, Germany

hosted by the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben. You are cordially invited to participate in this conference which is being organised for the first time in Europe.

The homepage of the conference: https://icdra-2022.ipk-gatersleben.de/ is now open for registration, abstract submission (both until March 31, 2022) and booking of accommodation. There you will find also information about Covid19 safety measures.

We are looking forward to welcome you in Gatersleben !

For formal invitations or answers to any question, do not hesitate to contact us.

With kind regards
The organizers of the 6th ICDRA

Ingo Schubert
schubert@ipk-gatersleben.de

Manuela Nagel
nagel@ipk-gatersleben.de

Klaus J. Appenroth
Klaus.Appenroth@uni-jena.de

Duckweed Research at the University of Jena, Germany

Klaus-J. Appenroth[1], Helmut Augsten[2], K. Sowjanya Sree[3]

[1] Matthias Schleiden Institute – Plant Physiology, University of Jena, 07743 Jena, Germany; klaus.appenroth@uni-jena.de

[2] von-Hase-Weg 8, 07743 Jena, Germany; helmut.augsten@freenet.de

[3] Department of Environmental Science, Central University of Kerala, Periye, India. ksowsree@gmail.com; ksowsree9@cukerala.ac.in



Duckweed research in Jena started after one of the present authors (H.A.) was appointed as Executive Director of the Institute of Botany, Director of the Department of General Botany and Chair of "General Botany" in the University of Jena. Coming from the University of Greifswald, he started his position at the 1st of September 1966. At that time, the Chair had been vacant for about one year. The predecessor, Hans Wartenberg, left the University on 18th September 1965. H. Wartenberg's research was mainly focused on physiology and pathology of wine grapes and other crop plants connected with his private vineyard near Jena in Rossbach. Microbiology and Plant Physiology were not yet separated at that time into different departments. Consequently, microbiological processes of fermentation was one research topic. According to the political rules at that time (Jena was located in the communist "German Democratic Republic" founded in 1949), the newly appointed Head of the Institute had no influence on selecting possible scientific co-workers. And, all existing co-workers were fixed on their previous research topics which delayed a new start in the Institute. The Institute building was located at von Hase-Weg 3, a former private hospital, not very fit for an Institute of Plant Physiology (Fig. 1, left).

Figure 1: Department of Plant Physiology. Left: von Hase-Weg 3 until 1997; Right: Dornburger Strasse 159 since 1997.

1. Duckweed Research from 1966 to 1993

Duckweeds were introduced as research plants mainly because of their small size and the fast growth and also considering that no suitable climatic chambers or green houses existed that would have been required for research with terrestrial plants that were commonly used. This reasoning is quite similar to those that motivated A. Pirson to start research with duckweeds after the second world war (Appenroth & Sree, 2020). Consequently, the practical investigations required some time for re-structuring the Institute before they can commence. The first duckweed species introduced in 1967 in sterile culture was *Wolffia arrhiza*, clone WJ (now clone 9528). In the following two years a

stock collection was established by the curator F. Jungnickel comprised of the following clones: *Lemna gibba* G1 in 1968 (this clone was lost around the year 2000), *Lemna minor* St in 1969 (now clone 9441; see Appenroth & Sree, 2020), *Lemna trisulca* TJ in 1969 (now clone 9529) and *Spirodela polyrhiza* SJ in 1969 (now clone 9500). The duckweed stock collection from that time is shown in Fig. 2. The first duckweed research paper was published in 1969 by M. Eichhorn and H. Augsten

Figure 2: The head of the department, Helmut Augsten and Klaus J. Appenroth in front of the duckweed collection in 1991.

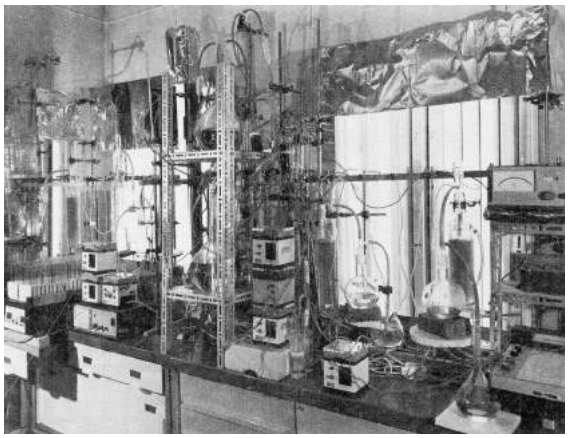

Figure 3: Chemostat for continuous cultivation of *Wolffia arrhiza* under sterile conditions.

(Eichhorn & Augsten 1969). Eichhorn had established sterile, continuous cultivation of *W. arrhiza* under steady-state conditions, (called chemostate culture; Fig. 3) and used this technique in several other experiments (e.g. Eichhorn and Augsten, 1977, 1988). The significance of nutrient media for the cultivation of duckweed species was investigated by F. Jungnickel (Jungnickel, 1986). He discovered that formation of turions of *S. polyrhiza* can be induced by low phosphate concentrations. The influence of light on the activity of nitrate reductase was investigated by Hubald and Augsten (1979), and further studied in more details by Appenroth et al. (1992). The effect of algal bloom on duckweed in nature was investigated by H. Liebert (Loth et al., 1995) who also examined the effect of microcystin-RR produced by cyanobacteria on *Lemna minor* (Weiss et al., 2000). The influence of phytohormones and herbicides on the pigment spectrum of green and de-etiolating plants of *L. gibba* G1 was described by R. Wejnar in a number of publications (Wejnar and Gundermann, 1987; Wejnar et al. 1994) and part of these results were reviewed by Augsten (1984). Numerous application-directed investigations were initiated in this period for wastewater cleaning, wastewater evaluation and tests with herbicides (e.g. Augsten et al., 1989). It is not possible to separate the research activities clearly in the periods before and after 1994 as many activities continued much longer. Fig. 4 shows an active duckweed research group in 1988.

Figure 4: Duckweed Research group in 1988. From left to right: Klaus J. Appenroth, Babara Liebermann, Waltraud Hertel, Helmut Augsten and Reingard Horn.

This period of duckweed research in Jena was characterized by a large number of scientific co-workers in permanent position, associated with numerous technical assistants. However, it should be mentioned that the time and effort dedicated to teaching in that time was much higher than it is nowadays.

2. Duckweed Research from 1994 to 2014

In 1993/1994 the Ordinarius of the department of Plant Physiology (H.A.) retired. The successors, P. Galland and R. Scheuerlein, investigated fungi and ferns, respectively. Moreover, they stayed only for a short time in Jena. From 1997 onward, R. Oelmüller has been the Head for the Department Plant Physiology as a full professor. With his start in Jena, the Institute moved from von Hase-Weg 3 to the present location, Dornburger Str. 159 (Fig. 1, right) doubling the available area. Some of the former scientific co-workers of H.A., however, continued working with duckweeds, mainly with the species *Wolffia arrhiza* (M. Eichhorn), *Lemna gibba* (H.P. Liebert, R. Wejnar) and *Spirodela polyrhiza* (K.J.A.).

As a consequence of the German unification in 1991, the situation at the University of Jena improved dramatically in the subsequent years. After a stay as Alexander von Humboldt fellow in the lab of Hans Mohr in Freiburg, Germany, one of the authors (K.J.A.) received funding by the Germany Research Foundation. On this basis, a research group could be constituted including one technical assistant (Barbara Liebermann until 1997, later Gabriele Lenk until 2004) and PhD students, Diploma students (Master's students), Magister students and teacher students, changing over time (Fig.5 and 6). The focus of duckweed research in the beginning was to continue the investigations of turions of *Spirodela polyrhiza* (Augsten et al., 1988). It was shown that germination of turions depends on a light signal mediate by the photoreceptor phytochrome (Appenroth et al., 1992) and that calcium is involved in the signal transduction chain (Appenroth et al., 1999). The research activities were

Figure 5: Duckweed Research group in 2007: Front row from left to right: Stephanie Franke, Steffi Thomas, Christiane Topp; second row: Eileen Seewald, Katja Kuehdorf, Nelly Bunge, Rebekka Henke; Back row: Maik Vogel, Ute Holtzegel, Klaus J. Appenroth.

Figure 6: Duckweed Research group in 2009. From left to right: Klaus J. Appenroth, Barbara Eichhoff, Philipp Schneider, Katja Kuehdorf, Manuela Bog.

continued in two directions: the formation of turions was investigated to learn about the mechanism, especially in nature. It was shown that limitation of phosphate plays a key role in nature in addition to interaction with low temperature (Appenroth, 2002; Appenroth and Nickel, 2010). The capacity of turion formation (= specific turion yield) is inherited and stable even after long times of stock cultivation under laboratory conditions (Kuehdorf et al., 2014).

Stimulated by M. Steup, University of Potsdam, starch degradation was investigated in turions of *S. polyrhiza* (Reimann et al., 2002, 2004). It was shown that starch degradation in germinating turions is

regulated by phytochrome and also depends on the mineral supply (Dolger et al., 1997; Appenroth and Ziegler, 2008).

Another topic that was kick-started in this period was phytotoxicity. Duckweeds were accepted as valuable test system for toxic compounds in aquatic systems. We investigated the effect of several heavy metals on *L. minor*, clone 9441 as suggested by the standard method ISO20079 (Naumann et al., 2007). Extending these investigations, we realized that applying heavy metals not only inhibit growth but also induced accumulation of starch (Kaszycki et al., 2005; Appenroth et al., 2010). This stimulated further research activities to find out ways to increase starch accumulation in duckweed for use in biotechnology. This research with biotechnological relevance was continued in the following years (review in Appenroth et al., 2021).

Two events in the period between 1994 and 2014 were important for subsequent development. One of the authors (K.J.A.) had the opportunity to meet E. Landolt several times in Zurich and cooperate with him until he passed away in 2013. Originally focused on only a few duckweed species (mainly *S. polyrhiza* 9550 and *L. minor* 9441), the world of K.J.A. was changed by the first meeting with E. Landolt at the ETH Zurich around 1998, where he first encountered more than 1000 identified clones of duckweeds in one place. Moreover, Landolt offered that we could get whatever clones we wanted from him for our research activity. This encouraged us to enter the field of phytotaxonomy by extending our research collection from a few clones to the whole family of Lemnaceae (Tippery et al., 2021). This topic continued well over the next period of duckweed research at the University of Jena.

The second essential event happened during the Second International Conference of Duckweed Research and Applications (ICDRA-2) in 2013 at Rutgers University, NJ, USA. Following the initiative of Eric Lam from this University, the International Steering Committee on Duckweed Research and Applications (ISCDRA) was founded. One of the authors (K.J.A.) served as the Chair for this committee over the first two election cycles (2013-2015, 2015-2017) and serves until now as a member of the ISCDRA. Another author (K.S.S.) became a member of this committee in 2017 and also continues to serve since that time. The ISCDRA assists in organizing a biennial international conference on “Duckweed Research and Applications” and edits four times a year the newsletter *Duckweed Forum* that is published online with open access (http://www.ruduckweed.org/). This organization created a network, which was the basis for duckweed research for the next period at the University of Jena.

3. Duckweed Research from 2014 onward

One of the authors (K.J.A.) retired in 2014 but received the opportunity to continue with duckweed research at the University of Jena. The disadvantage was that no official support by technical assistants or students at any levels was possible anymore. Thus, locally it became a one-man-show. However, because of the by then existing network of duckweed researchers on the basis of cooperation, investigations of duckweeds could be continued. Pillars for that were and still are the cooperation with K.S.S. who was at the beginning of her career at the Amity University Noida, India and later at the Central University of Kerala as Assistant Professor. Until now, this cooperation resulted in 29 papers in peer-reviewed journals according to Web_of_Science. Dr. Manuela Bog at the University of Greifswald in permanent position, formerly a student at the University of Jena, is another important member of this informal network. She brought mainly competence in molecular taxonomy into the cooperation. From all the papers that we published together (13 until now), two reviews are cited here as examples (Bog et al., 2019, 2020). Without the cooperation with E. Landolt before and the extension of the Jena Duckweed Stock Collection thereafter to approximately 600 accessions, this work would not have been possible.

In contrast to the situation in the communist GDR, cooperation all over the world became possible, especially made possible within the network of the ISCDRA. These cooperations are another important factor that shaped duckweed research in Jena. We will mention only a few of the results.

Together with P. Ziegler we investigated growth rate of a large number of duckweed species and clones (Ziegler et al., 2015; see also Sree et al. 2015). In the cooperation with E. Lam (Rutgers University NJ, USA), T. P. Michael (Salk Institute Biological Studies, La Jolla, USA) and I. Schubert (Inst Plant Genetics & Crop Plant Research, Gatersleben, Germany) we contributed to several whole genome sequencing projects (Hoang et al., 2018; Michael et al., 2020) and in cooperation with S. Xu and M. Huber (Xu e al., 2019) 68 clones of *S. polyrhiza* from all over the world were sequenced. In the genus *Lemna*, interspecific hybridization events were detected opening a new chapter on the evolutionary study of duckweeds (Braglia et al., 2021). In another cooperation with different partners we advocated for keeping duckweed at the level of a plant family (Lemnaceae) – in contrast to the suggestion of the Angiosperm Phylogeny Group (Tippery at al., 2021). More recently, cooperation was possible within the University of Jena with G. Jahreis from Institute of Nutritional Science investigating nutritional properties of duckweed species for human consumption (Sree et al., 2019, and references therein). Additionally, we profited from the cooperation with the morphologists A. Keresztes and K. Boka, Budapest, Hungary (Sree et al., 2015) and the very experienced duckweed researchers S.C. Maheshwari and J.P. Khurana in India (Sree et al., 2015). A great part of the results obtained in this period has been summarized in Acosta et al. (2021) and the legacy of the duckweed research in Jena continues.

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Election of the 5th ISCDRA: Now open

Election of members to the 5th International Steering Committee on Duckweed Research and Applications is now open until April 30, 2022.

Link to the e-ballot:

https://forms.gle/E7ahPkcPhS8umByR6

Eligibility for voting: You must belong to at least one of the categories listed below to be eligible to vote.

- Attended any of the past two ICDRA meetings.
- Will attend ICDRA 2022 in Germany.
- A principal investigator who is working with duckweed in his/her laboratory.
- A researcher/postdoc/student who is working with duckweed in their research (i.e. not just reading about it, but actually doing a project).
- An entrepreneur who is working to commercialize a duckweed-based technology and/or product.
- A research scientist who is working on a commercial venture that aims to develop a duckweed-based technology and/or product.
- A venture capital principle who has invested significantly into duckweed-based technology and/or product.
- A worker in a commercial venture involved with a duckweed-based technology and/or product.
- An administrator in a University or Funding Agency who has sufficient interest in the duckweed community to provide funding for one or more duckweed-based activity/project/venture.

List of candidates for the election to 5th ISCDRA is the following:

Eric Lam (New Brunswick, USA)

Sowjanya Sree K (Kerala, India)

Klaus Appenroth (Jena, Germany)

Tsipi Shoham (Tel Aviv, Israel)

Marcel Jansen (Cork, Ireland)

Jiaming Zhang (Hainan, China)

Ingo Schubert (Gatersleben, Germany)

Duckweeds: The underlying engine of the New Circular Economy

Paul Skillicorn[1], Rebecca Torres[2]

[1]Skillicorn Technologies LLC, Austin, Texas, USA

[2]Department of Geography and The Environment, University of Texas, Austin, Texas, USA

Abstract:

The term Circular Economy is shown to be more an aspirational semantic fiction than a practical reality. Here, we reflect on the reasons why this is so, and review advanced methods whereby energy and nutrients can be extracted from municipal and agricultural wastes as a primary mechanism by which to leverage the value of those extracts to create The New Circular Economy. Specifically, we outline an approach to achieve a New Circular Economy by drawing centrally on duckweed-related nutrient-stripping of a highly digested blend of municipal solid wastes and wastewater volatile solids as well as the wastewater itself. The circle is closed with value-added, zero-wastes monetization of harvested duckweeds to produce locally-grown, absolutely fresh proteinaceous foods, vegetables, herbs and vine fruit as well as advanced, renewable materials. The potential for widespread application of this approach in Africa, in particular, could be enabled by the lack of entrenched engineering firms and regulatory agencies, as well as the absence of an existing capital-intensive installed base comprising decades-old wastes treatment and elimination technologies and attendant expensive support services. We conclude that implementation of The New Circular Economy is feasible and practicable, and that it can help to transform the circumstances of communities that adopt its use.

Note: The full text of the article will be soon published as a supplement to the Duckweed Forum.

Obituary: Jitendra P. Khurana

(30.10.1954 – 27.10.2021)

Jitendra P. Khurana during the 4th International Conference of Duckweed Research and Applications organized at the Central University of Kerala in 2017 where he served as Co-President.

Professor Dr. Jitendra P. Khurana, an internationally highly acclaimed Indian scientist, passed away at the age of 66.

Jitendra P. Khurana did B.Sc. from Shahid Bhagat Singh College, affiliated to University of Delhi, India in 1973. He then moved to Kurukshetra University, India in 1973-1975 for his post-graduation (M.Sc Botany). His friends at Kurukshetra University fondly called him Paul, describing him as an affable colleague. He entertained them with melodious songs. He was a passionate photographer, who would click his classmates using his Yashica Camera, but rarely himself.

JP Khurana third from left, looking at the algal collections at Rameshwaram, Tamil Nadu, during their botanical excursion as a student of Kurukshetra University, ca. 1974.

A group photo with his Masters' classmates from Kurukshetra University. JP Khurana is in the last row, third from left, ca. 1975.

In 1975 he joined the Department of Botany, University of Delhi as a doctoral student with Prof. Satish C. Maheshwari. During his Ph.D. (1975-1982), he was strongly influenced by his academic teacher S. C. Maheshwari to focus on duckweeds.

In his first research projects, he investigated the species *Lemna aequinoctialis* (then referred to as *Lemna paucicostata*), especially clone 6746 (a short-day plant from California, which he obtained from R. Cleland, USA) and LP6 (now registered under the ID 9601; a day-neutral plant, which he collected in 1976 in Delhi). Under otherwise non-inductive conditions, salicylic acid or aspirin application resulted in 90 – 100 % flowering in both clones mentioned above (Khurana and

Maheshwari, 1978). Importantly, high concentrations of Ca^{2+} were found to be required for these effects (Khurana et al., 1988a). In clone 9601, they observed an additive effect of benzylaminopurine (a cytokinin) and the chelator EDTA without changing the critical day length (Khurana and Maheshwari, 1983a, b). Application of 8-hydroxyquinoline (a copper chelating agent) to the clone 9601 induced flowering, and this effect was independent of the day length. Interestingly, they found that the content of Cu within the plants increased strongly under these conditions (Khurana and Maheshwari, 1983b). In the short-day clone 6746, he showed that the application of 8-hydroxyquinoline reduced the critical dark period by at least two hours. Analysis indicated that the contents of both Cu and Fe were remarkably increased in these plants (Khurana and Maheshwari, 1984). Subsequently, increasing the Fe concentration applied to clone 9601 to tenfold resulted in flower induction, especially in the presence of the strong Fe-chelator EDTA, which improves the Fe availability for plants. Also, in *L. aequinoctialis* 9601, there was a 12% increase in flowering when Fe and EDTA increased by about tenfold (Khurana et al., 1986c). However, they discovered that replacing EDTA with equimolar concentrations of EDDHA resulted in a dramatic increase to 90 % flowering. Strong effects were also observed after applying some cytokinins in the presence of high Fe concentrations (10^{-4} M). At normal levels of Fe, however, cytokinins did not have any effect on flowering, regardless of the presence or absence of EDTA (Khurana and Maheshwari, 1986a). Researching with *L. aequinoctialis* 6746, they found that flowering could be induced by tannic acid (10^{-5} M) and some catecholamines (10^{-4} M) under strictly non-inductive photoperiods, even in continuous light (Khurana and Maheshwari, 1986b; Khurana et al., 1987). Similar effects, i.e., induction of flowering in the short-day plant 6746 even in continuous light, were observed after cAMP application, whereas 5'-AMP and 5'-ATP had very weak effects (Khurana et al., 1988c). The level of cAMP has been estimated in this clone (Gangwani et al., 1991) and cyclic nucleotide phosphodiesterase have also been isolated from clone 6746. Jitendra P. Khurana together with others showed that its enzymatic activity depends on calmodulin which could explain the effect of the calcium concentration during flowering (Gangwani et al., 1994). The role of NO in the induction of flowering was confirmed using pharmacological approaches: NO donors sodium nitroprusside (SNP), S-nitroso-N-acetyl penicillamine (SNAP), and 3-morpholinosydnonimine (SIN-1) induced flowering in *L. aequinoctialis* 6746 (a short-day strain) and in *L. aequinoctialis* LP6 (a photoperiod-insensitive strain) under non-inductive conditions (Khurana et al., 2011).

J. P. Khurana together with S. C. Maheshwari at the University of Delhi South Campus in 2014.

The influence of several amino acids on flowering induction was investigated by Prof. Khurana with clone 9601. The strongest effects (80 % flowering) were observed after applying glutamate or aspartate. Effective concentrations were between 5 x 10^{-7} and 10^{-5} M, indicating that they likely have a regulatory role rather than simply acting as metabolites (Khurana et al., 1988b).

Also, in *S. polyrhiza* (clone SP20), JPK found that flowering was induced by salicylic acid (5x10^{-5} M), most probably the first report about this species flowering in vitro (Khurana and Maheshwari, 1980). However, an optimal flowering response is

obtained only when compounds such as EDDHA or benzoic acid are supplied. Salicylic acid induced flowering only in the simultaneous presence of EDTA (Khurana and Maheshwari, 1986d).

Professor Khurana also investigated flowering in the duckweed species *Wolffiella hyalina* 7378 (Egypt; collected by M. Mahdi ca. 1960, Landolt Duckweed Collection) and *Wolffia microscopica* 9276 (Delhi, India; collected by Khurana/ Maheshwari).

First investigations of *W. hyalina* by JPK indicated that this clone does not flower when cultivated in a broad spectrum of common nutrient media and in any light regime and even in the presence of several chelators like EDTA. However, the application of 10^{-5} M salicylic acid under short-day conditions (< 13 h daylight) induced profuse flowering in this clone. It was concluded that the action of salicylic acid is hormonal in nature rather than via chelation of metal ions (Tamot et al., 1987).

Wolffia microscopica 9276 is a short-day plant, but Dr. Khurana showed that flowering can be induced under long-day conditions by exposure to salicylic acid and similar compounds in the presence of EDTA. It has been concluded that the effect cannot be due simply to the chelation of metal ions and perhaps the salicyl moiety itself exerts a specific effect (Khurana and Maheshwari, 1983c). Flowering in *W. microscopica* could also be induced, under non-inductive long days, by 8-hydroxyquinoline resulting in 75% flowering at 5×10^{-6} M (Khurana et al., 1986c). Unfortunately, clone 9276 was lost later on, but the species was rediscovered and further investigations were started together with J.P. Khurana and S.C. Maheshwari (Sree et al., 2015). Moreover, a historical overview of this species was published a month before Prof. Khurana passed away (Sree et al., 2021). Prof. Khurana's love for flowers was not only limited to laboratory research on microscopic flowers of duckweeds. He always took special care and interest, over the years, to keep up the floral beauty of the South Campus, University of Delhi.

J.P. Khurana (left) together with KSS and KJA hunting *Wolffia microscopica* in Manouli, Haryana, India in October, 2014 in an effort to replace the lost clone 9276.

The research activity of J.P. Khurana was not completely restricted to duckweed species (Tyagi and Sopory, 2021). In 1985 he moved to the Smithsonian Institute, Washington, USA, as a post-doctoral fellow in the lab of Charles Cleland. In 1986 he went to Michigan State University to work with Kenneth Poff in the newly emerging area of physiological analysis of Arabidopsis mutants. At MSU, he isolated a phototropism deficient mutant JK224 that Winslow Briggs' laboratory had later used to isolate the phototropin 1 photoreceptor. He then returned to India in 1988 to join the newly established department of Plant Molecular Biology at University of Delhi, with which he was associated till his demise. He was a key faculty to establish this department as a leading center of Plant Molecular Biology for teaching and research in India. Then, his lab mainly focused on functional genomics of plants, using Arabidopsis, wheat, and rice. However, he kept his interest in duckweed alive and painstakingly maintained all clones in a stock collection by regularly culturing

them for all these years. One of the authors, KSS is fortunate to now host his stock collection comprised of clones from all five genera of duckweeds. His interest in duckweeds was also demonstrated when he served as the co-president of the 4th International Conference on Duckweed Research and Applications organized in Kerala in 2017.

He played a key role in sequencing three important crop genomes, rice, tomato, and wheat, as an Indian partner in International consortia. His work on cryptochromes in mustard and rice flowering and identifying stress- and auxin-related gene families is well recognized and well-cited by the international community. Even during the last year, despite ill-health, he was busy finalizing his research papers on developmental aspects of rice. In total, he published 23 papers about duckweeds with the most recent one published in September 2021, 30 with wheat, 52 with rice, and 68 with *Arabidopsis thaliana* (Web_of_Science).

Dr. Khurana also tirelessly worked on Institution building and promoting and nurturing plant science in India. He served as a member and chairman of several DBT, CSIR, and DST committees in India. He was actively involved with all National Science Academies and served as vice-president of INSA, Delhi. He also served as the Pro-Vice-Chancellor for the University of Delhi (2016-19). Most importantly, his Alma Mater recognized his seminal work with the Goyal Prize in Life Sciences by Kurukshetra University, Kurukshetra, and Nishtha Dhriti Satyam Samman by University of Delhi, India.

He was a very good friend, mentor, and Scientist. Despite attaining very high positions and being busy with administration and research, he remained in contact with his teachers, students, and friends. One could reach out to him for help, and he would do his best to help. Any one who would have met him will surely remember his pleasant smile. He is survived by his wife Paramjit, a renowned plant molecular biologist, son: Sachin, and daughter: Ridhi.

Acknowledgments: We thank Dr. Aishverya Lakshmi, NIPGR, Delhi and Dr. N.K. Matta, Kurukshetra University, for providing photos and details about Late Prof. Jitendra P. Khurana's student days.

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Klaus-J. Appenroth[1], Rameshwar P. Sharma[2], K. Sowjanya Sree[3]

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

Update: Registration and Collections of duckweed clones / strains

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

S.No.	Director of the stock collection (Email ID)	Affiliation	Clone ID in private system	Available clones with Landolt ID system	Available clones with private ID system
1	Klaus J. Appenroth, Klaus.Appenroth@uni-jena.de	Matthias Schleiden Institute - Plant Physiology, University of Jena, Jena, Germany	KJAxxx	550 live, 36 herbarium specimens	50 (with KSS and BOG)
2	Manuela Bog, manuela.bog@uni-greifswald.de	Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany	BOGxxx	200	100 (with KJA)
3	Nikolai Borisjuk, nborisjuk@yahoo.com	Jiangsu Key Lab Ecoagr Biotechnol Hongze Lake, Huaian, China	NBxxxx	11	24
4	Hongwei Hou, houhw@ihb.ac.cn	Chinese Acad Sci, Inst Hydrobiology, Wuhan, Hubei, China		25	1
5	Marcel Jansen, M.Jansen@ucc.ie	University College Cork, Earth and Environmental Sciences, Cork, Ireland	MJxxx	3	22
7	Laura Morello, laura.morello@ibba.cnr.it	Landolt Stock Collection, Milano, Italy	only Landolt numbers	TBD	
8	Robert Laird, robert.laird@uleth.ca	Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada	RLxxx	5	70 (with RDSC and CCPCC)
9	Eric Lam, ericl89@hotmail.com	Rutgers Duckweed Stock Cooperative (RDSC), Rutgers University, NY, USA	DWCxxx ELxxx SGxxx et al.	691 in stock as of Jan. 15, 2022	138 in stock as of Jan. 15, 2022

10	Yubin Ma, mayubin@ouc.edu.cn	Ocean University of China, Qingdao, China	MYBxxxx	103	40
11	Viktor Olah, olahviktor@unideb.hu	Institute of Biology and Ecology University of Debrecen, Debrecen, Hungary	UDxxxx	2	35 (+ 8 additional clones from MJ collection)
12	Ingo Schubert, schubert@ipk-gatersleben.de	Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany	only Landolt numbers	50	
13	K. Sowjanya Sree, ksowsree@gmail.com; ksowsree9@cukerala.ac.in	Dept. of Environmental Science, Central University of Kerala, Periye, Kerala, India	KSSxxx JPKxx	45 live, 36 herbarium specimens	54
14	Shuqing Xu, shuqing.xu@uni-muenster.de	Institute for Evolution and Biodiversity, University of Münster, Münster, Germany	XUxxx	159	88
15	Jiaming Zhang, zhangjiaming@itbb.org.cn	Institute of Tropical Bioscience and Biotechnology, Hainan, Haiku, China	ZJxxxx	50	700
16	Hai Zhao, zhaohai@cib.ac.cn (c/o Yang Fang, fangyang@cib.ac.cn)	Chengdu Institute of Biology, Chinese Acad Sciences, Chengdu, China	ZHxxxx	640	174

TBD: to be determined

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Student Spotlight: Leone Ermes Romano

Università degli studi di Napoli Federico II, Italy
(email: leoneermes.romano@unina.it)

I'm Leone Ermes Romano, PhD student in Food Science at the University of Napoli Federico II. My background is plant science; specifically, I'm an Astro botanist. Since the beginning of my academic career, specifically during my Master's thesis, I have been involved in space-related research. I was a team member of the MULTITROP experiment that studied the interactions of multiple directional stimuli on the root of *Daucus carota*. The MULTITROP project saw us involved in designing, testing and handing over to the NASA staff the experiment to be performed onboard the International Space Station.

After this first approach with Astro botany, I have been involved in multiple other research projects that studied the effects of different gravity levels on the plant's growth. While deepening knowledge about the effect of different gravity levels on the plant's growth, I came across the incredible family of Lemnaceae, and it was love at first sight. Lemnaceae plants possess all the characteristics that make them great for space cultivation. The high biomass production rates, the high amount of edible biomass, the fact that they propagate mainly via vegetative reproduction make them ideal for space conditions.

During the first year of my PhD, I concentrated on studying the scientific literature on duckweeds. Results of this study were published in September 2021 in a scientific paper reviewing the traits that make the genus *Wolffia* one of the most suitable for space cultivation.

In this first year, I had the great opportunity to encounter Prof. Klaus J. Appenroth, whom I visited in Jena at the beginning of October 2021. Here I learned exceptional insights about Lemnaceae in general and specifically about the genus *Wolffia*. I was also trained with the sterilization protocols that help characterize these plants.

During this first year, I have planned the following year's experiments. I underlined the knowledge gap in the effects of light quality and quantity for the entire family Lemnaceae, but more specifically on the growth and protein content of *Wolffia globosa*. I have recently started testing the effect of light quality on ten different clones (provided by prof. Appenroth) of *W. globosa*.

Furthermore, I have developed a new protocol to monitor the growth of these tiny plants with machine learning software. This approach aims to supply researchers working with Duckweed with a new helpful instrument that can shift responsibility for monitoring relative growth rate from the human operator to a machine.

More recently, I took plants of *W. globosa* to study at the Elettra Synchrotron in Trieste, where I have conducted a morphology screening among clones of *W. globosa*. The investigation is ongoing, and I hope the results will be published later this year. In the future, I would like to experiment in space using plants of *W. globosa*.

If you find my work interesting, don't hesitate to contact me for more information and new collaborations.

From the Database

Highlights

Characterization of Frond and Flower Development and Identification of FT and FD Genes From Duckweed *Lemna aequinoctialis* Nd

Yoshida, A; Taoka, KI; Hosaka, A; Tanaka, K; Kobayashi, H; Muranaka, T; Toyooka, K; Oyama, T; Tsuji, H. (2021) Frontiers in Plant Science 12: 697206

Duckweeds (Araceae: Lemnoideae) are aquatic monocotyledonous plants that are characterized by their small size, rapid growth, and wide distribution. Developmental processes regulating the formation of their small leaf-like structures, called fronds, and tiny flowers are not well characterized. In many plant species, flowering is promoted by the florigen activation complex, whose major components are florigen FLOWERING LOCUS T (FT) protein and transcription factor FD protein. How this complex is regulated at the molecular level during duckweed flowering is also not well understood. In this study, we characterized the course of developmental changes during frond development and flower formation in *Lemna aequinoctialis* Nd, a short-day plant. Detailed observations of frond and flower development revealed that cell proliferation in the early stages of frond development is active as can be seen in the separate regions corresponding to two budding pouches in the proximal region of the mother frond. *L. aequinoctialis* produces two stamens of different lengths with the longer stamen growing more rapidly. Using high-throughput RNA sequencing (RNA-seq) and de novo assembly of transcripts from plants induced to flower, we identified the *L. aequinoctialis* FT and FD genes, whose products in other angiosperms form a transcriptional complex to promote flowering. We characterized the protein-protein interaction of duckweed FT and FD in yeast and examined the functions of the two gene products by overexpression in Arabidopsis. We found that *L. aequinoctialis* FTL1 promotes flowering, whereas FTL2 suppresses flowering.

The genome and preliminary single-nuclei transcriptome of *Lemna minuta* reveals mechanisms of invasiveness

Abramson, BW; Novotny, M; Hartwick, NT; Colt, K; Aevermann, BD; Scheuermann, R H; Michael, TP (2021) Plant Physiology DOI10.1093/plphys/kiab564

The ability to trace every cell in some model organisms has led to the fundamental understanding of development and cellular function. However, in plants the complexity of cell number, organ size and developmental time makes this a challenge even in the diminutive model plant Arabidopsis (*Arabidopsis thaliana*). Duckweed, basal non-grass aquatic monocots, provide an opportunity to follow every cell of an entire plant due to their small size, reduced body plan, and fast clonal growth habit. Here we present a chromosome-resolved genome for the highly invasive Lesser Duckweed (*Lemna minuta*) and generate a preliminary cell atlas leveraging low cell coverage single-nuclei sequencing. We resolved the 360 megabase genome into 21 chromosomes, revealing a core non-redundant gene set with only the ancient tau whole genome duplication shared with all monocots, and paralog expansion as a result of tandem duplications related to phytoremediation. Leveraging SMARTseq2 single-nuclei sequencing, which provided higher gene coverage yet lower cell count, we profiled 269 nuclei covering 36.9% (8,457) of the *L. minuta* transcriptome. Since molecular validation was not possible in this non-model plant, we leveraged gene orthology with model organism single cell expression datasets, gene ontology, and cell trajectory analysis to define putative cell types. We found that the tissue that we computationally defined as mesophyll expressed high levels of elemental transport genes consistent with this tissue playing a role in *L. minuta* wastewater detoxification. The *L. minuta* genome and preliminary cell map provide a paradigm to decipher developmental genes and pathways for an entire plant.

Agriculture

Biostimulant Effects of an Aqueous Extract of Duckweed (*Lemna minor* L.) on Physiological and Biochemical Traits in the Olive Tree

Regni, L; Del Buono, D; Miras-Moreno, B; Senizza, B; Lucini, L; Trevisan, M; Venturi, DM; Costantino, F; Proietti, P. (2021) Agriculture 11: 1299

Biostimulants are becoming increasingly popular in agriculture for their ability to induce beneficial effects in crops, paving the way towards the identification of new materials with biostimulant potential. This study evaluated the potential of different concentrations of an aqueous extract (0.25%, 0.50%, and 1.00%, dry weight/water volume, respectively) obtained from duckweed (*Lemna minor* L.) to stimulate olive plants. Leaf net photosynthesis (Pn), leaf transpiration rate (E), stomatal conductance (gs), sub-stomatal CO2 concentration (Ci), chlorophyll content and other plant growth parameters were investigated. As a result, the extract improved Pn, gs, Ci, chlorophyll content and plant biomass production (leaf fresh and dry weight). Furthermore, the duckweed extract generally increased the uptake of nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn), while it did not influence the content of sodium (Na), manganese (Mn) and copper (Cu). The untargeted metabolomic profiling of the extract revealed the presence of signalling compounds (including phytohormones), phenolics and glutathione. Such broad diversity of bioactives may support the stimulatory potential observed in olive. In summary, this study revealed for the first time that duckweed could be seen as a promising species to obtain extracts with biostimulant properties in olive trees.

Biochemistry

NDP-Sugar pathways overview of *Spirodela polyrhiza* and their relevance for bioenergy and biorefinery

Pagliuso, D; Navarro, BV; Grandis, A; Zerillo, MM; Lam, E; Buckeridge, MS (2022) Bioenergy Research DOI10.1007/s12155-021-10355-4

Duckweeds are fast-growing aquatic plants suitable for bioenergy due to fermentable-rich biomass with low lignin and unique cell wall. The plant cell wall is built from pathways of nucleotide sugar genes that culminate in cell wall synthesis and deposition. Therefore, understanding these pathways by mapping the genes involved and their expression would be necessary for developing tools to improve bioenergy production. In this work, the genes associated with the NDP-sugar pathway (de novo and salvage) were mapped and correlated to the chemical characterization of the giant duckweed (*Spirodela polyrhiza*) cell wall. This plant biomass has been characterized as having 3% starch, 49% soluble sugars, 40% cell wall, and 8% non-measured compounds. The cell walls are synthesized by the NDP-sugar pathway and represent a significant carbon sink. This sink results from the action of proteins encoded by 190 orthologs of the 38 targets of the NDP-sugar pathway, of which 49 are starch and sucrose-related, 54 pectins-related, 65 hemicellulose-related, and 23 cellulose-related. Chemical analysis of the cell wall revealed 49% pectins, 23% hemicellulose, and 10% cellulose. These carbohydrates can potentially provide biorefinery as adjuvants, cosmetics, food additives, stabilizers, gelling agents, and principally as biofuels.

Untargeted Analysis of *Lemna minor* Metabolites: Workflow and Prioritization Strategy Comparing Highly Confident Features between Different Mass Spectrometers

Wahman, R; Moser, S; Bieber, S; Cruzeiro, C; Schroeder, P; Gilg, A; Lesske, F; Letzel, T. (2021) Metabolites 11: 832

Metabolomics approaches provide a vast array of analytical datasets, which require a comprehensive analytical, statistical, and biochemical workflow to reveal changes in metabolic profiles. The biological interpretation of mass spectrometric metabolomics results is still obstructed by the reliable identification of the metabolites as well as annotation and/or classification. In this work, the whole *Lemna minor* (common duckweed) was extracted using various solvents and analyzed utilizing polarity-extended liquid chromatography (reversed-phase liquid chromatography (RPLC)-hydrophilic interaction liquid chromatography (HILIC)) connected to two time-of-flight (TOF) mass spectrometer types, individually. This study (introduces and) discusses three relevant topics for the untargeted workflow: (1) A comparison study of metabolome samples was performed with an untargeted data handling workflow in two different labs with two different mass spectrometers using the same plant material type. (2) A statistical procedure was observed prioritizing significant detected features (dependent and independent of the mass spectrometer using the predictive methodology Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA). (3) Relevant features were transferred to a prioritization tool (the FOR-IDENT platform (FI)) and were compared with the implemented compound database PLANT-IDENT (PI). This compound database is filled with relevant compounds of the Lemnaceae, Poaceae, Brassicaceae, and Nymphaceae families according to analytical criteria such as retention time (polarity and LogD (pH 7)) and accurate mass (empirical formula). Thus, an untargeted analysis was performed using the new tool as a prioritization and identification source for a hidden-target screening strategy. Consequently, forty-two compounds (amino acids, vitamins, flavonoids) could be recognized and subsequently validated in *Lemna* metabolic profile using reference standards. The class of flavonoids includes free aglycons and their glycosides. Further, according to our knowledge, the validated flavonoids robinetin and norwogonin were for the first time identified in the *Lemna minor* extracts.

Biotechnology

Production of bioethanol and biogas from *Spirodela polyrhiza* in a biorefinery concept and output energy analysis of the process

Rana, QU; Khan, MAN; Shiekh, Z; Parveen, S; Ahmed, S; Irfan, M; Gauttam, R; Shah, AA; Jamal, A; Khan, S. (2021) Biomass Conversion and Biorefinery DOI10.1007/s13399-021-02066-9

Spirodela polyrhiza is a fast-growing hydrophyte with the ability to attain high starch and high biomass yield which makes it an excellent alternative feedstock for the production of biofuels. In this study, *Spirodela* was given nutrient stress to enhance its starch content from 21 to 80%. The plant biomass with high starch was pretreated with dilute acid for conversion of starch to glucose which was fermented with indigenously isolated and optimized yeast strain and yielded 99.8% of theoretical ethanol yield. The fermentation vinasse was anaerobically digested, and 0.88 NL/g VS yield of biogas was obtained. Further, output energy analysis of bioethanol, biogas, and both bioethanol and biogas sequentially produced from *Spirodela* was carried out, which showed that highest amount of energy is produced in the process where high starch containing *Spirodela* plant biomass was used for the production of biomethane only. The results obtained showed that high starch *Spirodela* is best used in terms of energy when it is anaerobically digested for production of biogas only. To the best of our knowledge, this study is the first one which attempts to practically depict that this feedstock can be used in a sequential process to produce the two biofuel variants by using the same batch of feedstock. Further, this study for the first time evaluates the energy output potential of biofuels produced from *Spirodela*. It was interesting to see that in terms of energy output, duckweed has more potential for biogas production than that of ethanol, while many researchers have focused the production of ethanol only from *Spirodela*.

Accumulation of starch in duckweeds (Lemnaceae), potential energy plants

Appenroth, KJ; Ziegler, P; Sree, KS. (2021) Physiology and Molecular Biology of Plants 27: 2621-2633.

Special Issue

Starch can accumulate in both actively growing vegetative fronds and over-wintering propagules, or turions of duckweeds, small floating aquatic plants belonging to the family of the Lemnaceae. The starch synthesizing potential of 36 duckweed species varies enormously, and the starch contents actually occurring in the duckweed tissues are determined by growth conditions, various types of stress and the action of growth regulators. The present review examines the effects of phytohormones and growth retardants, heavy metals, nutrient deficiency and salinity on the accumulation of starch in duckweeds with a view to obtaining high yields of starch as a feedstock for biofuel production. Biotechnological approaches to degrading duckweed starch to its component sugars and the fermentation of these sugars to bio-alcohols are also discussed.

Duckweed biorefinery - Potential to remediate dairy wastewater in integration with microbial protein production

Hemalatha, M; Venkata Mohan, S. (2021) Biorecourse Technology 346: 126499

The phytoremediation potential of duckweed in treating dairy wastewater (DWW) was studied, focusing on its utilization as nutritional biomass. The process resulted in good treatment efficiency with removal of organic carbon of 74% (COD), nitrates of 66% and phosphates of 80%. The increase in duckweed fronds with time was observed (doubling time (DT) - 0.87) resulting in an overall dry weight of 3.73g. The lentils showed 58% of protein, 29.5% of carbohydrate (with 20% of starch), 15.6% of lipid (FAME-29.3%-saturated, 40.7%-mono- and 30%-poly-unsaturated fatty acids) and good amino acid content (34.04% essential and 65.92% non-essential). The biomass hydrolysate (mild acid pretreated) served as a substrate for microbial protein (MP) production using *Bacillus subtilis*, resulting in 60% of protein (0.57g protein/g COD consumed; 0.63g protein/g N consumed) and 21% of carbohydrate. The duckweed biomass offers multiple benefits including nutritional supplement in food/feed for livestock and poultry industries along with concurrent wastewater treatment as well serves as potential feedstock for biorefinery.

Developing a platform for production of the oxylipin KODA 9-hydroxy-10-oxo-octadecadienoic acid in plants

Ihara, Y; Wakamatsu, T; Yokoyama, M; Maezawa, D; Ohta, H; Shimojima, M. (2021) Journal of Experimental Botany DOI10.1093/jxb/erab557

KODA (9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid) is a plant oxylipin involved in recovery from stress. As an agrichemical, KODA helps maintain crop production under various environmental stresses. In plants, KODA is synthesized from alpha-linolenic acids via 9-lipoxygenase (9-LOX) and allene oxide synthase (AOS), although the amount is usually low except in the free-floating aquatic plant *Lemna paucicostata*. To improve KODA biosynthetic yield in other plants such as *Nicotiana benthamiana* and *Arabidopsis thaliana*, we developed a system to overproduce KODA *in vivo* via ectopic expression of *L. paucicostata* 9-LOX and AOS. The transient expression in *N. benthamiana* showed that the expression of these two genes is sufficient to produce KODA in leaves. However, stable expression of 9-LOX and AOS (with consequent KODA production) in *Arabidopsis* plants succeeded only when the two proteins were localized in plastids or the endoplasmic reticulum/lipid droplets. Although only small amounts of KODA could be detected in leaf extracts of transgenic *Nicotiana* or *Arabidopsis* plants, subsequent incubation of the extracts increased KODA abundance over time. Therefore, KODA production in transgenic plants stably expressing 9-LOX and AOS requires specific subcellular localization of these two enzymes and incubation of leaf crude extracts, which liberates alpha-linolenic acid via breakdown of endogenous lipids.

DF comment: *Lemna paucicostata* is a synonym for the accepted species *Lemna aequinoctialis* and is not recommended for continued usage in the field.

Duckweed growth model for large-scale applications: Optimizing harvesting regime and intrinsic growth rate via machine learning to maximize biomass yields

Calicioglu, O; Sengul, MY; Femeena, PV; Brennan, RA (2021) Journal of Cleaner Production 324: 129120

Duckweed has emerged as a potential feedstock for the environmentally sustainable and economically viable production of biofuels and protein. The aim of this study was to: (1) enhance an existing intrinsic duckweed growth model and use it to develop a general regression model that enables users to easily predict annual duckweed yield for large scale applications; and (2) determine the optimal parameter sets that produce the highest annual duckweed yield at a specific location for known values of daily temperature and photoperiod. Simulations performed using Stella Architect were used to compute annual duckweed yield and generate separate datasets for developing the regression model and optimization model. To improve duckweed yields for large-scale applications which incorporate regular harvesting, a harvesting regime was added to the intrinsic duckweed growth model. Two new parameters (harvest frequency and harvest ratio) and a control (harvest threshold) were used to describe the harvesting regime in the model. The general model was developed by fitting LASSO regression (R^2 = 0.95) with four variables: initial mat density, intrinsic growth rate, harvest ratio, and harvest frequency. This model offers a simple method for users to estimate annual duckweed yield in practical applications without the need for dynamic simulation runs. Optimum parameter values to maximize biomass production at a location in southwest Florida, USA, were determined using an optimization framework involving a deep neural network machine learning algorithm. Using an existing daylength model to predict daily photoperiod and inputting local temperature data, machine learning calculated a maximum yield of 70 dry tons per hectare per year for the Florida case study, under the following conditions: initial mat density = 169 g(dry) m^{-2} ; harvest threshold = 76 g(dry) m^{-2} ; nitrogen = 50.1 mg L^{-1}; phosphorus = 7.5 mg L^{-1} ; harvest ratio = 0.35; and harvest frequency = 1 day.

System management of *Lemna minor* in aquaponics

Camargo-Castellanos, JC; Flores-Garcia, L; Herrera-Diaz, IE; Alvarez-Gonzalez, CA; Albertos-Alpuche, PJ; Martinez-Yanez, R. (2021) Aquaculture Research DOI10.1111/are.15637

In farming, sustainability together with food safety is one of the main objectives to be achieved. Aquaponics is a technique that combines aquatic animals, such as fish, with the hydroponic production of plants that function as biological filters. The proper functioning of the system is based on the dynamic balance of the elements that make it up. Several studies confer aquatic macrophytes such as *L. minor*, great attributes, highlighting their use for feeding different species. Unfortunately, there is very little information on the system management of macrophytes in aquaponics. To determine the appropriate management parameters for the cultivation of *L. minor* integrated into the production of *Oreochromis niloticus*, in aquaponics, three experiments were carried out to evaluate the effect of the hydraulic retention time (HRT), the planting density, plant dissemination and the balance of nutrients in the systems. The results show that the highest biomass production and plant growth are achieved with high flow rates and short HRTs. Planting density has a direct effect on the behaviour of the plant. Regarding the balanced budget, between 7% and 8% of the nutrients (input in dry matter, N and P) are retained by *L. minor*, keeping the water quality within adequate limits for tilapia production.

Ecology

Are Rural and Small Community Aerated Wastewater Stabilization Ponds a Neglected Source of Microplastic Pollution?

Gao, ZQ; Cizdziel, JV; Wontor, K; Lu, HT (2021) Water 13: 2833

Wastewater treatment systems collect and treat sewage that includes microplastics (MPs). However, we are not aware of any studies on the occurrence and distribution of MPs in wastewater stabilization ponds (WSPs), which serve small communities worldwide. Here, we characterized MPs (~45 µm - 5 mm) in an aerated WSP serving ~500 houses and an adjacent lake. Putative MPs were most abundant in duckweed (*Lemna minor*) and sludge (75 ± 22 and 12.8 ± 3.1 particles/g, respectively: ± 1 standard deviation (SD), n = 6, dry weight). In the water, average concentrations (particles/L ± 1 SD, n = 6) were highest in the pond (4.1 ± 0.6), followed by effluent (3.9 ± 0.5) and the lake (2.6 ± 0.6). Over 20 types of MPs were identified in each different compartment, with the distribution varying somewhat between the water, sludge, and duckweed. Polyester and polyethylene were the predominant types, followed by polyethylene terephthalate, polyacrylate, polyvinyl chloride, polystyrene, and others. Morphologies consisted of fibers (62-71%), fragments (28-37%), and beads (1-6%). High-density polymers were more frequently found in sludge. Potential sources of the MPs include synthetic textiles from laundry and other plastics washed down household drains. Overall, with ~786,000 MPs/day released in the pond effluent and with duckweed a source of food for waterfowl, we demonstrate that WSPs can be point sources of MPs to both aquatic and terrestrial ecosystems and thus deserve further scrutiny.

Ecotoxicological Assessment and Environmental Risk of the Insecticide Chlorpyrifos for Aquatic Neotropical Indicators

Marques, MBL; Brunetti, IA; Faleiros, CA; da Cruz, C; Iqbal, HMN; Bilal, M; Americo-Pinheiro, JHP (2021) Water Air and Soil Pollution 232: 428

Chlorpyrifos (CPF) is an organophosphorus insecticide detected in aquatic environments considered harmful to living beings. The aim of this research was to evaluate the ecotoxicity of CPF for neotropical aquatic organisms of distinct trophic levels (*Lemna minor, Azolla caroliniana*, and *Wolffia brasiliensis* macrophytes; *Pomacea canaliculata* snail; *Macrobrachium acanthurus* shrimp; *Xiphophorus maculatus* and *Hyphessobrycon eques* fish), to verify the risk of environmental poisoning for each organism, and to determine the best bioindicator species of aquatic contamination by the insecticide. Ecotoxicological assays were carried out with different concentrations of CPF under controlled laboratory conditions standardized for each species. IC_{50};7d, LC_{50};7d, EC_{50};48h, and LC_{50};48h values were calculated using the Trimmed Spearman Karber software with 95% confidence limits. The toxicity data were used to classify the CPF according to the ecotoxicity categories for aquatic organisms. The risk of CPF environmental poisoning was determined by the quotient method considering different environmental scenarios. The sensitivity order of neotropical aquatic organisms to chlorpyrifos was *Macrobrachium acanthurus* (0.002 mg L^{-1}) > *Xiphophorus maculatus* (0.07 mg L^{-1}) > *Hyphessobrycon eques* (1.65 mg L^{-1}) > *Pomacea canaliculata* (30.66 mg L^{-1}) > *Azolla caroliniana* (849.72 mg L^{-1}) > *Wolffia brasiliensis* (1271.63 mg L^{-1}) = *Lemna minor* (1299.60 mg L^{-1}). The risk of poisoning by chlorpyriphos may vary according to the environmental concentration of the insecticide and the exposed trophic level. The best bioindicator and with the greatest risk of environmental poisoning was shrimp. The difference in CPF ecotoxicity for distinct aquatic trophic levels shows the relevance of evaluating the effects of contaminants considering food chains and highlights the importance of studying these levels in environmental monitoring programs.

Feed & Food

Duckweed (Lemnaceae) for potentially nutritious human food: A review

Xu, JW; Shen, YT; Zheng, Y ; Smith, G; Sun, XZSS; Wang, DH; Zhao, Y; Zhang, W; Li, YH. (2021) Food Reviews International DOI10.1080/87559129.2021.2012800

With continuous global population growth, the challenges of expanding demand for sufficient and nutritious foods need to be addressed by exploring new and supplemental agricultural and food systems. Duckweed

demonstrates a great potential for human food with many advantages. This review covers recent research on duckweed regarding plant cultivation, nutritional composition and quality, product development, and related safety issues and regulations for human food uses. Challenges and future research needs are discussed. Species and cultivation conditions have a significant effect on chemical and nutritional compositions of duckweed. Certain duckweed species contain high protein content (up to 45%) that provides all the essential amino acids meeting FAO references and can support human body growth and development. Duckweed also contains versatile carbohydrates including starch, cellulose, trace hemicellulose, and pectin that deliver functional properties and nutrients. In addition, duckweed is rich in minerals, vitamins, and phytochemicals, especially lutein and beta-carotene which are positively associated with reduced risk of many chronic diseases. Duckweed could be used either in conventional cereal-based foods by partial substitution of wheat flour or for extraction of proteins and phytochemicals as functional ingredients. Studies are warranted for developing a duckweed supply chain and establishing duckweed as a novel staple food.

A comparative study on physicochemical properties, pyrolytic behaviour and kinetic parameters of environmentally harmful aquatic weeds for sustainable shellfish aquaculture

Azwar, E; Chan, DJC; Kasan, NA; Rastegari, H; Yang, YF; Sonne, C; Tabatabaei, M; Aghbashlo, M; Lam, SS (2022) Journal of Hazardous Materials 424 (Part A): 127329

Aquatic weeds pose hazards to aquatic ecosystems and particularly the aquatic environment in shellfish aquaculture due to its excessive growth covering entire freshwater bodies, leading to environmental pollution particularly eutrophication intensification, water quality depletion and aquatic organism fatality. In this study, pyrolysis of six aquatic weed types (wild and cultured species of *Salvinia sp., Lemna* sp. and *Spirodela* sp.) were investigated to evaluate its potential to reduce and convert the weeds into value-added chemicals. The aquatic weeds demonstrated high fixed carbon (8.7-47.3 wt %), volatile matter content (39.0-76.9 wt %), H/C ratio (1.5-2.0) and higher heating value (6.6-18.8 MJ/kg), representing desirable physicochemical properties for conversion into biofuels. Kinetic analysis via Coats-Redfern integral method obtained different orders for chemical reaction mechanisms (n = 1, 1.5, 2, 3), activation energy (55.94-209.41 kJ/mol) and pre-exponential factor (4.08 x 104-4.20 x 1017 s-1) at different reaction zones (zone 1: 150-268°C, zone 2: 268-409°C, zone 3: 409-600°C). The results provide useful information for design and optimization of the pyrolysis reactor and establishment of the process condition to dispose this environmentally harmful species.

The effect of a high-polyphenol Mediterranean diet (GREEN-MED) combined with physical activity on age-related brain atrophy: the DIRECT PLUS randomized controlled trial

Kaplan, A; Zelicha, H; Meir, AY; Rinott, E; Tsaban, G; Levakov, G; Prager, O; Salti, M; Yovell, Y; Ofer, J; Huhn, S; Beyer, F; Witte, V; Villringer, A; Meiran, N; Emesh, TB; Kovacs, P; von Bergen, M; Ceglarek, U; Bluher, M; Stumvoll, M; Hu, FB; Stampfer, MJ; Friedman, A; Shelef, I; Avidan, G; Shai, I. (2022) The American Journal of Clinical Nutrition DOI10.1093/ajcn/nqac001

The effect of diet on age-related brain atrophy is largely unproven. To explore the effect of a Mediterranean diet higher in polyphenols and lower in red/processed meat (Green-MED diet) on age-related brain atrophy. This 18-month clinical trial longitudinally measured brain structure volumes by magnetic-resonance-imaging using hippocampal-occupancy (HOC) and lateral-ventricle-volume (LVV) expansion scores as neurodegeneration markers. Abdominally obese/dyslipidemic participants were randomly assigned to (1)-healthy dietary guidelines (HDG), (2)-Mediterranean (MED) diet, or (3)-Green-MED diet (MED diet higher in polyphenols and lower in red/processed meat). All subjects received free gym memberships and physical activity guidance. Both MED groups consumed 28g/day walnuts (+440 mg/d polyphenols). The Green-MED group consumed green-tea (3-4 cups/day) and Mankai (*Wolffia globosa* strain, 100g frozen-cubes/day) green shake (+800mg/day polyphenols). Among 284 participants (age = 51years; 88% men; BMI = 31.2kg/m2;

apolipoprotein E-epsilon4 genotype = 15.7%), 224 (79%) completed the trial with eligible whole-brain MRIs. The pallidum (-4.2%), third ventricle (+3.9%), and LVV (+2.2%) disclosed the largest volume changes. Compared to younger participants, atrophy was accelerated among those ≥ 50 years [HOC change = -1.0±1.4% vs. -0.06±1.1%; 95% confidence-interval (CI):0.6, 1.3; p<0.001; LVV change = 3.2±4.5% vs. 1.3±4.1%; 95%CI:-3.1, -0.8; p = 0.001]. In subjects ≥50years, HOC decline and LVV expansion were attenuated in both MED groups, with the best outcomes among Green-MED diet participants, as compared to HDG (HOC: -0.8±1.6% vs. -1.3±1.4%;95%CI: -1.5, -0.02; p = 0.042, LVV: 2.3±4.7% vs. 4.3±4.5;95%CI;0.3, 5.2; p = 0.021). Similar patterns were observed among younger subjects. Improved insulin sensitivity over the trial was the strongest parameter associated with brain atrophy attenuation (p<0.05). Greater Mankai, green-tea and walnuts intake and less red and processed meat were significantly and independently associated with reduced HOC decline (p<0.05). Elevated urinary levels of the Mankai-derived polyphenols: urolithin-A (r = 0.24;p = 0.013) and tyrosol (r = 0.26;p = 0.007) were significantly associated with lower HOC decline. A Green-MED, high-polyphenol diet, rich in Mankai, green tea and walnuts and low in red/processed meat is potentially neuroprotective for age-related brain atrophy.

Determining the nutritional value and antioxidant capacity of duckweed (*Wolffia arrhiza*) under artificial conditions

Hu, ZB; Fang, Y; Yi, ZL; Tian, XP; Li, JM; Jin, YL; He, KZ; Liu, PH; Du, AP; Huang, YH; Zhao H. (2022) LWT-Food Science and Technology 153: 112477

Duckweeds are traditionally used as a plant protein source in some Southeast Asian countries. In this study, we first obtained a high-protein strain (*Wolffia arrhiza* 7678a) under artificial conditions and determined its protein content in 50.89% of dry weight. More importantly, we further investigated its nutritional properties. The digestible indispensable amino acids score (DIAAS) reached 0.75, indicating the good protein quality of *W. arrhiza*. The proportion of unsaturated fatty acids in total fatty acids (TFA) was 70.85%, and three main fatty acids (palmitic acid, linoleic acid, alpha-linolenic acid) accounted for 89.7% of TFA. Compared with the conventional crops, it had much higher contents of total phenolics and flavonoids and showed higher antioxidant activities. Also, the low concentrations of antinutritional factors could make the nutrients of *W. arrhiza* easily absorbed. Therefore, *W. arrhiza* showed a great potential value for human food.

Safety of *Wolffia globosa* powder as a Novel food pursuant to Regulation (EU) 2015/2283

Turck, D; Bohn, T; Castenmiller, J; De Henauw, S; Hirsch-Ernst, KI; Maciuk, A; Mangelsdorf, I; McArdle, HJ; Naska, A; Pelaez, C. (2021) EFSA Journal 19: e06938. Doi: 10.2903/j.efsa.2021.6938

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on *Wolffia globosa* powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *Wolffia globosa* is an aquatic plant, one out of the 38 species of the water lentil family which is composed by five genera (i.e. *Spirodela, Landoltia, Lemna, Wolffiella* and *Wolffia*). The NF is produced by cultivation of *Wolffia globosa* plants under controlled conditions, washing with hot water and drying. The main constituents of the NF are protein, fibre and fat. The Panel notes that the concentration of trace elements and contaminants in the NF is highly dependent on the conditions of cultivation of the plant and the fertiliser composition. The NF is intended to be used as food ingredient in a variety of food categories and as food supplement. The target population is the general population except for food supplements which are intended to be consumed exclusively by adults. The Panel considers that with the exception of concerns related to the manganese intake, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The subchronic toxicity study provided with the NF revealed a number of significant findings and the Panel considers the middle dose (6.5 g/kg body weight (bw) per day) as the no observed adverse effect level (NOAEL). Based on the protein concentration, the Panel considers that the consumption of the NF may trigger allergic reactions. The Panel concluded that an

increase in manganese intake from the NF used as food ingredient or food supplements is of safety concern and the safety of the NF cannot be established.

DF Comment: There are presently 36 species of duckweeds

Safety of water lentil powder from Lemnaceae as a Novel Food pursuant to Regulation (EU) 2015/2283

Turck, D. et al. (2021) EFSA Journal 19: e6845. DOI 10.2903/j.efsa.2021.6845

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the safety of water lentil powder as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Water lentils refer to aquatic plants belonging to the Araceae family and represented by five genera (*Lemna, Wolffia, Wolffiella, Landoltia* and *Spirodela*). The NF is thermally washed and dried water lentils, which are produced as a polyculture crop consisting of species from the *Lemna* genus (70-100%) and the *Wolffia* genus (0-30%). The main constituents of the NF are protein, fibre and fat. The Panel notes that the concentration of trace elements and contaminants in the NF is highly dependent on the conditions of cultivation of the plant and the fertiliser composition. The NF is expected to be stable and to comply with the specifications during the suggested shelf life. The NF is intended for human consumption as a food ingredient in herbs, spices and seasonings, sauces, soups and broths, protein products, dietary food for weight control and as a food supplement. The target population is the general population, except for food supplements which are exclusively intended for consumption by adults. The Panel considers that based on the composition of the NF and the proposed intended uses, the NF is not nutritionally disadvantageous, except for the concerns regarding intake of manganese from the NF. No adverse effect was observed in the submitted 90-day subchronic study, at the highest dose, 1,000 mg/kg body weight (bw) per day of NF. The Panel considers that, based on the protein concentration, the consumption of the NF may trigger allergic reactions. The Panel concluded that an increase in manganese intake from the NF used as food ingredient or food supplements is of safety concern and the safety of the NF cannot be established.

Effect of green-Mediterranean diet on intrahepatic fat: the DIRECT PLUS randomised controlled trial

Meir, AY; Rinott, E; Tsaban, G; Zelicha, H; Kaplan, A; Rosen, P; Shelef, I; Youngster, I; Shalev, A; Bluher, M. (2021) Gut 70: 2085-2095

To examine the effectiveness of green-Mediterranean (MED) diet, further restricted in red/processed meat, and enriched with green plants and polyphenols on non-alcoholic fatty liver disease (NAFLD), reflected by intrahepatic fat (IHF) loss. For the DIRECT-PLUS 18-month randomized clinical trial, we assigned 294 participants with abdominal obesity/dyslipidaemia into healthy dietary guidelines (HDG), MED and green-MED weight-loss diet groups, all accompanied by physical activity. Both isocaloric MED groups consumed 28 g/day walnuts (+440 mg/day polyphenols provided). The green-MED group further consumed green tea (3-4 cups/day) and Mankai (a *Wolffia globosa* aquatic plant strain; 100 g/day frozen cubes) green shake (+1240 mg/day total polyphenols provided). IHF% 18-month changes were quantified continuously by proton magnetic resonance spectroscopy (MRS). Results Participants (age=51 years; 88% men; body mass index=31.3 kg/m^2; median IHF%=6.6%; mean=10.2%; 62% with NAFLD) had 89.8% 18-month retention-rate, and 78% had eligible follow-up MRS. Overall, NAFLD prevalence declined to: 54.8% (HDG), 47.9% (MED) and 31.5% (green-MED), p=0.012 between groups. Despite similar moderate weight-loss in both MED groups, green-MED group achieved almost double IHF% loss (-38.9% proportionally), as compared with MED (-19.6% proportionally; p=0.035 weight loss adjusted) and HDG (-12.2% proportionally; p<0.001). After 18 months, both MED groups had significantly higher total plasma polyphenol levels versus HDG, with higher detection of Naringenin and 2-5-dihydroxybenzoic-acid in green-MED. Greater IHF% loss was independently associated with increased Mankai and walnuts intake, decreased red/processed meat consumption, improved serum folate and adipokines/lipids biomarkers, changes in microbiome composition (beta-diversity) and specific bacteria

(p<0.05 for all). The new suggested strategy of green-Mediterranean diet, amplified with green plant-based proteins/polyphenols as Mankai, green tea, and walnuts, and restricted in red/processed meat can double IHF loss than other healthy nutritional strategies and reduce NAFLD in half.

Effect of inclusion of different levels of duckweed (*Lemna minor*) on the performance of broiler chicken

Zaffer, B; Sheikh, IU; Banday, MT; Adil, S; Ahmed, HA; Khan, AS; Nissa, SS; Mirza, U. (2021) Indian Journal of Animal Research 55: 1200-1205

Duckweed is a monocotyledon species of the family Lemnaceae. It is a small floating aquatic plant that grows very well on stagnant ponds and is commonly found throughout tropical countries in natural ponds, lakes and flooded rice fields. Duckweed has high crude protein content and a well-balanced amino acid profile and is also a good source of vitamins and minerals. Duckweed at different levels was utilized in the diet of broiler chicken to study their performance. One hundred and fifty day old broiler chicks of one week old were distributed randomly into five treatment groups viz T-1 : (Control), T-2: 5% Duckweed without enzyme, T-3: 5% Duckweed with enzyme, T-4: 10% Duckweed without enzyme and T-5: 10% Duckweed with enzyme having 30 chicks in each groups with three replicates of 10 chicks each. The Duckweed contains 20.33% crude protein, 3.10% ether extract, 18.06% crude fibre, 2.80% calcium, 1.10% phosphorous and 1660.77 ME (Kcal/Kg). Significantly (P ≤ 0.05) higher body weight was recorded in T-1 (1889.67 ± 13.28g) and T-3 (1878.65 ± 2.02g) groups followed by T-2 (1831.67 ± 3.51g), T-5 (1798.31 ± 1.76 g) and T-4 (1728.63 ± 2.60 g) groups, respectively. The average daily body weight gain was ranged between 37.87 to 41.66 g. The cumulative feed consumption was recorded to be highest in T-1 (3050.13 ± 14.01) and lowest in T-4 (2943.17 ± 8.54g) group. The Cumulative FCR was significantly (P ≤ 0.05) better (1.74 ± 0.01) in T-1 and T-3 groups in comparison to T-2 (1.78 ± 0.01), T-4 (1.85 ± 0.01) and T-5 (1.78 ± 0.04) groups. Total 3.3% mortality was recorded in all treatment groups except T-3 group in which there was no mortality during entire experimental period.

Interaction with other organisms

Draft genome sequence of Bryobacteraceae strain F-183

Yamamoto, KY; Yoneda,Y; Makino, A; et al. (2022) Microbiology Resource Announcements e0045321

Here, we report a draft genome sequence of a bacterial strain, F-183, isolated from a duckweed frond. Strain F-183 belongs to the family Bryobacteraceae of the phylum Acidobacteria, and its genomic information would contribute to understanding the ecophysiology of this abundant but rarely characterized phylum.

Linking plant-root exudate changes to micropollutant exposure in aquatic plants (*Lemna minor* and *Salvinia natans*). A prospective metabolomic study

Casas, ME; Matamoros, V. (2022) Chemosphere 287: 132056

Recent findings indicate that plant-root exudates can stimulate plant-associated microorganisms to enhance the biodegradation of contaminants in constructed wetlands. To understand this process, we studied the root exudation changes of two aquatic plants (*Lemna minor* and *Salvinia natans*) upon micro pollutants exposure (10, 100 and 1000 µg/L mixes containing naproxen, diclofenac, carbamazepine, and benzotriazole). After a 2-day exposure, plant exudates were collected, extracted and non-target analysis was performed with a gas chromatography-high resolution Orbitrap mass-spectrometer. Plants didn't show morphological or growth differences between the control and spiked reactors, but exudation changes were observed in both plants at all concentration levels. Partial least squares discriminant analysis showed that, for *Lemna minor*, the increase of micro pollutants exposure was linked to the reduction of sugar and fatty acid exudation. This may trigger

changes in the microbial community living on complex carbon forms. Instead, in *Salvinia natans*, micro pollutants exposure was linked to the release of long-chain compounds such as cuticular waxes and sesquiterpenoids, which might be related to stress signaling. These results demonstrate that plant micro pollutant-exposure at environmentally relevant concentration levels triggers changes in root exudates. This may help to design new strategies to enhance micro pollutants degradation in nature based solutions such as in constructed wetlands.

Growth Promotion of Giant Duckweed *Spirodela polyrhiza* (Lemnaceae) by Ensifer sp. SP4 Through Enhancement of Nitrogen Metabolism and Photosynthesis

Toyama, T; Mori, K; Tanaka, Y; Ike, M; Morikawa, M. (2021) Molecular Plant-Microbe Interactions 06210157R. DOI10.1094/MPMI-06-21-0157-R

Duckweeds (Lemnaceae) are representative producers in fresh aquatic ecosystems and also yield sustainable biomass for animal feeds, human foods, and biofuels, and contribute toward effective wastewater treatment; thus, enhancing duckweed productivity is a critical challenge. Plant-growth-promoting bacteria (PGPB) can improve the productivity of terrestrial plants; however, duckweed-PGPB interactions remain unclear and no previous study has investigated the molecular mechanisms underlying duckweed-PGPB interaction. Herein, a PGPB, Ensifer sp. strain SP4, was newly isolated from giant duckweed (*Spirodela polyrhiza*), and the interactions between *S. polyrhiza* and SP4 were investigated through physiological, biochemical, and metabolomic analyses. In *S. polyrhiza* and SP4 coculture, SP4 increased the nitrogen (N), chlorophyll, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) contents and the photosynthesis rate of *S. polyrhiza* by 2.5-, 2.5-, 2.7-, and 2.4-fold, respectively. Elevated photosynthesis increased the relative growth rate and biomass productivity of *S. polyrhiza* by 1.5- and 2.7-fold, respectively. Strain SP4 significantly altered the metabolomic profile of *S. polyrhiza*, especially its amino acid profile. N stable isotope analysis revealed that organic N compounds were transferred from SP4 to *S. polyrhiza*. These N compounds, particularly glutamic acid, possibly triggered the increase in photosynthetic and growth activities. Accordingly, we propose a new model for the molecular mechanism underlying *S. polyrhiza* growth promotion by its associated bacteria Ensifer sp. SP4, which occurs through enhanced N compound metabolism and photosynthesis. Our findings show that Ensifer sp. SP4 is a promising PGPB for increasing biomass yield, wastewater purification activity, and CO_2 capture of *S. polyrhiza*.

Antioxidative responses of duckweed (*Lemna minor* L.) to phenol and rhizosphere-associated bacterial strain *Hafnia paralvei* C32-106/3

Radulovic, O; Stankovic, S; Stanojevic, O; Vujcic, Z; Dojnov, B; Trifunovic-Momcilov, M; Markovic, M (2021) Antioxidants 10: 1719

Duckweed (*L. minor*) is a cosmopolitan aquatic plant of simplified morphology and rapid vegetative reproduction. In this study, an *H. paralvei* bacterial strain and its influence on the antioxidative response of the duckweeds to phenol, a recalcitrant environmental pollutant, were investigated. Sterile duckweed cultures were inoculated with *H. paralvei* in vitro and cultivated in the presence or absence of phenol (500 mg L^{-1}), in order to investigate bacterial effects on plant oxidative stress during 5 days. Total soluble proteins, guaiacol peroxidase expression, concentration of hydrogen peroxide and malondialdehyde as well as the total ascorbic acid of the plants were monitored. Moreover, bacterial production of indole-3-acetic acid (IAA) was measured in order to investigate *H. paralvei*'s influence on plant growth. In general, the addition of phenol elevated all biochemical parameters in *L. minor* except AsA and total soluble proteins. Phenol as well as bacteria influenced the expression of guaiacol peroxidase. Different isoforms were associated with phenol compared to isoforms expressed in phenol-free medium. Considering that duckweeds showed increased antioxidative parameters in the presence of phenol, it can be assumed that the measured parameters might be involved in the plant's defence system. *H. paralvei* is an IAA producer and its presence in the rhizosphere of duckweeds decreased

the oxidative stress of the plants, which can be taken as evidence that this bacterial strain acts protectively on the plants during phenol exposure.

Molecular Biology

Wolffia, a minimalist plant and synthetic biology chassis

Lam, Eric; Michael, TP (2021) Trends in Plant Science DOI10.1016/j.tplants.2021.11.014

A highly simplified species for genome engineering would facilitate rational design of a synthetic plant. A candidate species is the aquatic, non-grass monocot *Wolffia* (*Wolffia australiana*) in the Lemnaceae family. Commonly known as watermeal, *Wolffia* is a rootless ball of several thousand cells the size of a pinhead and the fastest growing plant known on Earth. Its extreme morphological reduction is coupled to transposon-mediated streamlining of its transcriptome, which represents a core set of nonredundant protein coding genes. Despite its body plan and transcriptome being highly specialized for continuous growth, *Wolffia* retains cell types relevant to higher plants. Systems level studies with this species could enable the creation of a defined biological chassis for synthetic plant construction.

The Dynamics of NO_3^- and NH_4^+ Uptake in Duckweed Are Coordinated with the Expression of Major Nitrogen Assimilation Genes

Zhou, Y; Kishchenko, O; Stepanenko, A; Chen, G; Wang, W; Zhou, J; Pan, C; Borisjuk, N. (2021) Plants 11: DOI10.3390/plants11010011

Duckweed plants play important roles in aquatic ecosystems worldwide. They rapidly accumulate biomass and have potential uses in bioremediation of water polluted by fertilizer runoff or other chemicals. Here we studied the assimilation of two major sources of inorganic nitrogen, nitrate (NO_3^-) and ammonium (NH_4^+), in six duckweed species: *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna aequinoctialis*, *Lemna turionifera, Lemna minor*, and *Wolffia globosa*. All six duckweed species preferred NH_4^+ over NO_3^- and started using NO_3^- only when NH_4^+ was depleted. Using the available genome sequence, we analyzed the molecular structure and expression of eight key nitrogen assimilation genes in *S. polyrhiza*. The expression of genes encoding nitrate reductase and nitrite reductase increased about 10-fold when NO_3^- was supplied and decreased when NH_4^+ was supplied. NO_3^- and NH_4^+ induced the glutamine synthetase (GS) genes GS1;2 and the GS2 by 2- to 5-fold, respectively, but repressed GS1;1 and GS1;3. NH_4^+ and NO_3^- upregulated the genes encoding ferredoxin- and NADH-dependent glutamate synthases (Fd-GOGAT and NADH-GOGAT). A survey of nitrogen assimilation gene promoters suggested complex regulation, with major roles for NRE-like and GAATC/GATTC cis-elements, TATA-based enhancers, GA/CTn repeats, and G-quadruplex structures. These results will inform efforts to improve bioremediation and nitrogen use efficiency.

Robust Agrobacterium-Mediated Transient Expression in Two Duckweed Species (Lemnaceae) Directed by Non-replicating, Replicating, and Cell-to-Cell Spreading Vectors

Peterson, A; Kishchenko, O; Zhou, YZ; Vasylenko, M; Giritch, A; Sun, J; Borisjuk, N; Kuchuk, M. (2021) FRONTIERS IN BIOENGINEERING AND BIOTECHNOLOGY 9: 5

Plant-based transient expression systems have recognized potential for use as rapid and cost-effective alternatives to expression systems based on bacteria, yeast, insect, or mammalian cells. The free-floating aquatic plants of the Lemnaceae family (duckweed) have compact architecture and can be vegetatively propagated on low-cost nutrient solutions in aseptic conditions. These features provide an economically feasible opportunity for duckweed-based production of high-value products via transient expression of recombinant products in fully contained, controlled, aseptic and bio-safe conditions in accordance with the

requirements for pharmaceutical manufacturing and environmental biosafety. Here, we demonstrated Agrobacterium-mediated high-yield transient expression of a reporter green fluorescent protein using deconstructed vectors based on potato virus X and sweet potato leaf curl virus, as well as conventional binary vectors, in two representatives of the Lemnaceae (*Spirodela polyrhiza* and *Landoltia punctata*). Aseptically cultivated duckweed populations yielded reporter protein accumulation of >1 mg/g fresh biomass, when the protein was expressed from a deconstructed potato virus X-based vector, which is capable of replication and cell-to-cell movement of the replicons in duckweed. The expression efficiency demonstrated here places duckweed among the most efficient host organisms for plant-based transient expression systems, with the additional benefits of easy scale-up and full containment.

Genomic analysis of the polyamine biosynthesis pathway in duckweed *Spirodela polyrhiza* L.: presence of the arginine decarboxylase pathway, absence of the ornithine decarboxylase pathway, and response to abiotic stresses

Upadhyay, RK; Shao, J; Mattoo, AK (2021) Planta 254: 108

Main conclusion Identification of the polyamine biosynthetic pathway genes in duckweed *S. polyrhiza* reveals presence of prokaryotic as well as land plant-type ADC pathway but absence of ODC encoding genes. Their differential gene expression and transcript abundance is shown modulated by exogenous methyl jasmonate, salinity, and acidic pH. Genetic components encoding for polyamine (PA) biosynthetic pathway are known in several land plant species; however, little is known about them in aquatic plants. We utilized recently sequenced three duckweed (*Spirodela polyrhiza*) genome assemblies to map PA biosynthetic pathway genes in *S. polyrhiza*. PA biosynthesis in most higher plants except for Arabidopsis involves two pathways, via arginine decarboxylase (ADC) and ornithine decarboxylase (ODC). ADC-mediated PA biosynthetic pathway genes, namely, one arginase (SpARG1), two arginine decarboxylases (SpADC1, SpADC2), one agmatine iminohydrolase/deiminase (SpAIH), one N-carbamoyl putrescine amidase (SpCPA), three S-adenosylmethionine decarboxylases (SpSAMDc1, 2, 3), one spermidine synthase (SpSPDS1) and one spermine synthase (SpSPMS1) in *S. polyrhiza* genome were identified here. However, no locus was found for ODC pathway genes in this duckweed. Hidden Markov Model protein domain analysis established that SpADC1 is a prokaryotic/biodegradative type ADC and its molecular phylogenic classification fell in a separate prokaryotic origin ADC clade with SpADC2 as a biosynthetic type of arginine decarboxylase. However, thermospermine synthase (t-SPMS)/Aculis5 genes were not found present. Instead, one of the annotated SPDS may also function as SPMS, since it was found associated with the SPMS phylogenetic clade along with known SPMS genes. Moreover, we demonstrate that *S. polyrhiza* PA biosynthetic gene transcripts are differentially expressed in response to unfavorable conditions, such as exogenously added salt, methyl jasmonate, or acidic pH environment as well as in extreme temperature regimes. Thus, *S. polyrhiza* genome encodes for complete polyamine biosynthesis pathway and the genes are transcriptionally active in response to changing environmental conditions suggesting an important role of polyamines in this aquatic plant.

Physiology & Stress

Physiological responses and transcriptome analysis of *Spirodela polyrhiza* under red, blue, and white light

Zhong, Y; Wang, L; Ma, ZM; Du, XL (2022) Planta 255: 11

Red light (RL) accelerated starch accumulation in *S. polyrhiza*, but higher protein content under blue light (BL) was associated with the upregulation of most DEGs enriched for specific GO terms and KEGG pathways. Red light (RL) and blue light (BL) greatly influence the growth and physiological processes of duckweed. Physiological and molecular mechanisms underlying the response of duckweed to different light qualities

remain unclear. This study employed physiological and transcriptomic analyses on duckweed, *Spirodela polyrhiza* "5510", to elucidate its differential response mechanisms under RL, BL, and white light conditions. Changes in growth indicators, ultrastructure alterations, metabolite accumulations, and differentially expressed genes (DEGs) were measured. The results showed that BL promoted both biomass and protein accumulations, while RL promoted starch accumulation. A total of 633, 518, and 985 DEGs were found in white-vs-red, white-vs-blue, and red-vs-blue comparison groups, respectively. In Gene Ontology (GO) enrichment analysis, the DEGs in all three comparison groups were significantly enriched in two GO terms, carboxylic acid metabolic process and lyase activity. In Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, the DEGs were greatly enriched in two pathways, histidine metabolism and isoquinoline alkaloid biosynthesis. Higher protein content under BL was associated with the upregulation of most DEGs enriched with the GO terms and KEGG pathways. Furthermore, the light qualities influenced the gene expression patterns of other metabolic pathways, like carotenoid biosynthesis, and the regulation of these genes may explain the level of photosynthetic pigment content. The results revealed the physiological changes and transcriptome-level responses of duckweed to three light qualities, thereby providing bases for further research studies on the ability of duckweed as a biomass energy source.

Regeneration of duckweed (*Lemna turionifera*) involves genetic molecular regulation and cyclohexane release

Yang, L; Sun, J; Yan, C; Wu, J; Wang, Y; Ren, Q; Wang, S; Ma, X; Zhao, L; Sun, J. (2022) Plos One 17: e0254265

Plant regeneration is important for vegetative propagation, detoxification and the obtain of transgenic plant. We found that duckweed regeneration could be enhanced by regenerating callus. However, very little is known about the molecular mechanism and the release of volatile organic compounds (VOCs). To gain a global view of genes differently expression profiles in callus and regenerating callus, genetic transcript regulation has been studied. Auxin related genes have been significantly down-regulated in regenerating callus. Cytokinin signal pathway genes have been up-regulated in regenerating callus. This result suggests the modify of auxin and cytokinin balance determines the regenerating callus. Volatile organic compounds release has been analysised by gas chromatography/ mass spectrum during the stage of plant regeneration, and 11 kinds of unique volatile organic compounds in the regenerating callus were increased. Cyclohexane treatment enhanced duckweed regeneration by initiating root. Moreover, Auxin signal pathway genes were down-regulated in callus treated by cyclohexane. All together, these results indicated that cyclohexane released by regenerating callus promoted duckweed regeneration. Our results provide novel mechanistic insights into how regenerating callus promotes regeneration.

An endogenous basis for synchronisation characteristics of the circadian rhythm in proliferating *Lemna minor* plants

Ueno, K; Ito, S; Oyama, T (2021)) New Phytologist DOI10.1111/nph.17925

The circadian clock is a cell-autonomous system that functions through the coordination of time information in the plant body. Synchronisation of cellular clocks is based on coordination mechanisms; the synchronisation characteristics of proliferating plants remain unclear. The bioluminescence circadian rhythms of fronds (leaf-like plant units) of proliferating *Lemna minor* plants carrying a circadian bioluminescence reporter, AtCCA1:LUC, were spatiotemporally analysed at a cell-level resolution. We focused on spontaneous circadian organisation under constant light conditions for plants with light : dark treatment (LD grown) or without it (LL grown). Fronds developing even from an LL-grown parental frond showed coherent circadian rhythms among them. This allowed the maintenance of circadian rhythmicity in proliferating plants. Inside a frond, a centrifugal phase/period pattern was observed in LD-grown plants, whereas various phase patterns with travelling waves were formed in LL-grown plants. These patterns were model simulated by local coupling of heterogeneous cellular circadian oscillators with different initial synchronous states in fronds. Spatiotemporal analysis of the

circadian rhythms in proliferating plants reveals spontaneous synchronisation manners that are associated with local cell-cell coupling, spatial phase patterns and developmental stages.

Light intensity drives different growth strategies in two duckweed species: *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleiden

Strzalek, M; Kufel, L (2021) Peer J 9: 12698

Duckweed species *Lemna minor* and *Spirodela polyrhiza* are clonal plants with vegetative organs reduced to a frond and a root in *L. minor* or a frond and several roots in *S. polyrhiza*. They reproduce vegetatively by relatively rapid multiplication of their fronds. The habit of *S. polyrhiza* (large fronds with up to 21 roots) makes it a strong competitor among representatives of the family Lemnaceae, probably due to different resource-use strategies compared to small duckweed. In our study, light was the resource that affected the plants before and during the laboratory experiment. We sampled the plants from natural habitats differing in light conditions (open and shady) and grew them for 16 days in a thermostatic growth room at 22°C under a 16:8 photoperiod and three light intensities (125, 236, 459 µmol photons m^{-2} s^{-1}) to investigate the trade-off between frond enlargement and multiplication. Both species from the open habitat had higher growth rates based on the frond numbers and on surface area of fronds compared to plants from the shady habitat. They adopted different species-specific strategies in response to the experimental light conditions. The species size affected the growth rates in *L. minor* and *S. polyrhiza*. Spirodela polyrhiza grew slower than *L. minor*, but both species grew fastest at medium light intensity (236 µmol m^{-2} s^{-1}). *Lemna minor* maintained the growth rates at high light intensity, while *S. polyrhiza* slowed down. *Spirodela polyrhiza* responded to deteriorating light conditions by increasing its frond surface area, thus optimising light capture. *Lemna minor* from the shady habitat enhanced light harvest by increasing chlorophyll a concentration, but did not invest more in frond enlargement than *L. minor* from the open habitat. Under shady conditions, *S. polyrhiza* is likely to achieve an advantage over *L. minor* due to the larger frond size of the former. Our findings suggest the existence of a trade-off between size and number in duckweed.

Duckweeds: their utilization, metabolites and cultivation

Baek, G; Saeed, M; Choi, HK. (2021) Applied Biological Chemistry 64: 73

Duckweeds are floating plants of the family Lemnaceae, comprising 5 genera and 36 species. They typically live in ponds or lakes and are found worldwide, except the polar regions. There are two duckweed subfamilies-namely *Lemnoideae* and *Wolffioideae*, with 15 and 21 species, respectively. Additionally, they have characteristic reproduction methods. Several metabolites have also been reported in various duckweeds. Duckweeds have a wide range of adaptive capabilities and are particularly suitable for experiments requiring high productivity because of their speedy growth and reproduction rates. Duckweeds have been studied for their use as food/feed resources and pharmaceuticals, as well as for phytoremediation and industrial applications. Because there are numerous duckweed species, culture conditions should be optimized for industrial applications. Here, we review and summarize studies on duckweed species and their utilization, metabolites, and cultivation methods to support the extended application of duckweeds in future.

Physiological and Biochemical Parameters of Common Duckweed *Lemna minor* after the Exposure to Tetracycline and the Recovery from This Stress

Krupka, M; Michalczyk, DJ; Zaltauskaite, J; Sujetoviene, G; Glowacka, K; Grajek, H; Wierzbicka, M; Piotrowicz-Cieslak, AI (2021) Molecules 26: DOI10.3390/molecules26226765

In this study, the ability of *Lemna minor* L. to recover to normal growth, after being degraded in a tetracycline-containing medium, was extensively investigated. The plants were exposed to tetracycline (TC) at concentrations of 1, 2.5, and 10 mM. Subsequently, their physiological status was analysed against the

following criteria: rate of plant growth; free radical accumulation; antioxidant enzyme activity; chlorophyll content; HSP70 protein content; cell membrane permeability, and mitochondrial activity. The study showed that duckweed can considerably recover from the damage caused by antibiotics, within a week of cessation of stress. Of the plant properties analysed, mitochondrial activity was the most sensitive to antibiotic-induced disturbances. After transferring the plants to a tetracycline-free medium, all plant parameters improved significantly, except for the mitochondrial activity in the plants grown on the medium containing the highest dose of tetracycline. In the plants treated with this antibiotic at the concentration of 10 mM, the proportion of dead mitochondria increased and was as high as 93% after one week from the beginning of the recovery phase, even after the transfer to the tetracycline-free medium.

Estimation of mass, chlorophylls, and anthocyanins of *Spirodela polyrhiza* with smartphone acquired images

Tan, WH; Ibrahim, H; Chan, DJC. (2021) Computers and Electronics in Agriculture 190: 106449

Premium cameras may provide excellent quality images for analysis, but not everyone can afford to own one compared to a smartphone camera. Therefore, this has given rise to several attempts to incorporate smartphone and image analysis in analytical procedures. This study intended to seek the possibilities of using a smartphone to capture images and subsequent analysis to estimate mass, chlorophylls, and anthocyanin simultaneously in *Spirodela polyrhiza* by correlation models. This work serves as the substitution for conventional protocols in which determination of mass and phytochemicals in plants require the destruction of samples, usage of reagents, long processing time, and dependent on the availability of high-end equipment. In this study, the image taken with a smartphone camera was processed, and necessary information was extracted using ImageJ software. The area of plantlets was measured, and the relationship between area and mass was studied. Color parameters values extracted from the image were transformed into different combinations to explore the strength of the relationship between color parameters and combinations with chlorophyll and anthocyanin content of *S. polyrhiza*. Saturation channel from HSI color space was found to predict the mass of plantlets slightly more accurately than a* channel from L*a*b* color space. The mean green (G) value of the image was a robust parameter to predict chlorophyll contents in *S. polyrhiza* with a high r^2 of 0.9693 and the lowest error compared to other color parameters and their combinations. Compared with other color parameters and combinations, the 2G - B value presented the most robust relationship with anthocyanin contents in *S. polyrhiza*, having r^2 = 0.8638 and the lowest percentage of errors. The mean G value predicts chlorophylls content in *S. polyrhiza* with 9.5 ± 7.3% of errors, while 2G-B estimates anthocyanins content with 10.42 ± 6.82% errors. This research demonstrated that the images captured with smartphones could be a ground-breaking strategy to predict mass, chlorophylls, and anthocyanins content in *S. polyrhiza* sufficiently accurate, rapid, and cost-effective compared with the traditional chemical method.

Assessing the bioavailability of dissolved rare earths and other trace elements: Digestion experiments with aquatic plant species *Lemna minor* ("duckweed" reference standard BCR-670)

Zocher, AL; Klimpel, F; Kraemer, D; Bau, M. (2021) Applied Geochemistry 134: 105025

Reference material BCR-670 (*Lemna minor*, "duckweed") is one of the very few plant reference materials for which certified data for all rare earths and yttrium (REY) are available. However, biological samples like plants or fungi often include detrital inorganic material because substrate particles (e.g., soil or atmospheric dust) may be closely associated with the organic component. Hence, the digestion approach used may strongly affect element yields. We here present concentration data for major, minor and trace elements with a focus on REY (with and without off-line preconcentration and matrix separation) following high-pressure high-temperature HNO_3-HCl-HF decomposition and low-pressure low-temperature HNO_3 extraction, respectively. Rare earths and yttrium concentrations after HNO_3-HCl-HF decomposition are in good agreement with certified data but corroborate that preconcentration and matrix separation prior to quadrupole ICP-MS analyses result

in improved data quality compared to data obtained without preconcentration and matrix separation. The results of both the decomposition and the extraction protocol show excellent reproducibilities, respectively, but the REY concentrations of the HNO_3 extractions are lower than the certified bulk data and are accompanied by considerably lower concentrations of Al, Zr and Th. This suggests that the HNO_3 extraction does not dissolve trace elements from detrital (alumino)silicate particles associated with the plant material. Our results expand the existing data set for BCR-670 not only for REY but also for a range of other elements (e.g., Li, Co, Rb, Sr, Ba, Mg, Mn, Na, P) including elements for which no or hardly any information had hitherto been available. Therefore, our dataset helps to improve the overall characterisation of BCR-670. The new data obtained from HNO_3 extraction may be used to assess analytical quality in studies focusing on the chemical composition of the biological component of plants and fungi, which is a prerequisite to quantify the bioavailability and bioaccumulation of trace elements such as the REY in plants and fungi.

Phytoremediation

Synthesis of activated carbon from *Lemna minor* plant and magnetized with iron (III) oxide magnetic nanoparticles and its application in removal of Ciprofloxacin

Yilmaz, M; Al-Musawi, TJ; Saloot, MK; Khatibi, AD; Baniasadi, M; Balarak, D. (2022) Biomass Conversion and Biorefinery DOI10.1007/s13399-021-02279-y

The current study was done by preparing activated carbon from the common duckweed, *Lemna minor*, after magnetization using Fe3O4 nanoparticles. The resultant product (Fe3O4-ACLM) was employed to adsorb ciprofloxacin (CIP) from the contaminated water, in the batch adsorption mode. The characteristic distinctive features or parameters of the materials utilized were ascertained with the aid of scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM); the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) analysis, point of zero charge (pH(pzc)), and vibrating sample magnetometry (VSM) were also used. From the results, it was clear that when the initial CIP concentration was 25 mg/L, and pH was 3, in the presence of Fe3O4-ACLM in a 0.75 g/L dosage, and contact time of 75 min, 100% removal percentage was achieved. However, the adsorbent recycling and reuse tests demonstrated that in just six periods of adsorbent use a marginal 8.5% decrease was noted in the adsorbent efficiency. The Fe3O4-ACLM was observed to show super-paramagnetic behavior with 37.6 emu/g saturation magnetization. Four models namely the Langmuir, Freundlich, Dubinin-Radushkevich (D-R), and Temkin isotherms were used for the adsorption isotherm studies. From the results of the goodness-of-fit parameters, the Langmuir isotherm revealed greater consistency with the equilibrium data, demonstrating maximum adsorption capacities of 134.2, 149.5, 161.4, and 178.7 mg/g at temperatures of 20, 30, 40, and 50 degrees C, respectively. Further, the CIP adsorption onto the Fe3O4-ACLM surface was, by nature, endothermic and spontaneous, according to the thermodynamic study. In conclusion, the Fe3O4-ACLM was proven to be efficient, recyclable, and excellent as an alternative adsorbent capable of CIP antibiotic removal from contaminated water.

Silver modified hydrophytes for heavy metal removal from different water resources

El Awady, FR; Abbas, MA; Abdelghany, AM; El-Amir, YA. (2021) BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY 11: 14555-14563

Phytoremediation of three different aquatic plants powders *Lemna minor* L., *Azolla filiculoides* Lam. and *Pistia stratiotes* L. studied against different heavy metals (HM) and after modifications with interfacial layer synthesized silver nanoparticles. Prepared samples tested for the selective absorbance of chromium, cadmium, lead, and zinc. *L. minor* and *P. stratiotes* show selective absorption against lead, while *Azolla*

filiculoides show higher absorption against chromium. Absorption of all heavy metal concentrations was found to be enhanced after interfacial modification with green synthesized silver nanoparticles.

Potential of *Lemna minor* for removal of methylene blue in aqueous solution: Kinetics, adsorption mechanism, and degradation pathway

Imron, MF; Ananta, AR; Ramadhani, IS; Kurniawan, SB; Abdullah, SRS. (2021) ENVIRONMENTAL TECHNOLOGY & INNOVATION 24: 101921

Dye wastewater produced from textile industry effluent contains non-biodegradable complex chemical compounds that harmful to living organism. Thus, treating dye wastewater before releasing it into surface water is extremely important. *Lemna minor* is one of ideal aquatic plants that can be used as phytoremediator of various pollutants. The objective of this research is to determine the potential and mechanism of *Lemna minor* to remove methylene blue (MB). This research was conducted in batch reactor. The effect of plant weight (1, 2, 3 g), contact time (0-4 days), and initial concentration (25, 50, 75 mg/L), as well as reaction kinetics and adsorption isotherms of MB removal were also determined. Results showed that equilibrium plant weight, contact time, and concentration were achieved at 2 g of plant weight for 2 days with 82.48 ± 1.09% of removal efficiency in 50 mg/L of MB concentration. For kinetics, all initial plant weights and concentration were well fitted with pseudo-second-order model, while the adsorption isotherm is Freundlich isotherm models with qmax of 1.14 mg/g. Based on those, the adsorption process of MB by *L. minor* was determined as chemisorption. Our results also found that the removal mechanisms of MB by *L. minor* are phytosorption (hydrogen bonding and electrostatic interaction) and biodegradation (desulfurization and denitrification). Our findings showed that the removal processes of MB by *L. minor* were influenced by plant weight, initial concentration, and contact time. Therefore, it can be concluded that *L. minor* can be used as a phytoremediation agent to remove pollutants.

Accumulation of Iron, Zinc and Lead by *Azolla pinnata and Lemna minor* and activity in contaminated water

Hafez, SM; Hassan, AM; El-Shahat, RM; Kassem, MA. (2021) Egyptian Journal of Chemistry 64: 5017-5030

In this study, two aquatic macrophytes namely, *Azolla* pinnata and *Lemna minor* are floating plants were obtained from Agric. Microbial Dept., Soils, Water and Environment Research Institute (SWERI), Agric. Res. Center (ARC), Giza, Egypt and used to some heavy metal such as Iron, Zinc and lead This study reported the ability of two aquatic plants (*A. pinnata* and *L. minor*) to remove Iron, Zinc and lead from aqueous solutions FeSO4 * $7H_2O$, $ZnSO_4$ * $7H_2O$ and $C_4H_6O_4Pb$ * 3H2O of four different initial concentrations (0-100 ppm) for 20 days under greenhouse conditions. The results indicated that *A. pinnata* gave higher growth density than that recorded for *L. minor* during all the tested incubation periods from zero time up to 20 days. Results obtained in this study showed a maximum removal of Fe, Zn (88.18, 84.63 %) by *L. minor* at 100 ppm initial metal concentration however the maximum removal by A. pinnata at the same concentration was (86.97, 81.14%) after 20 day of incubation. These *A. pinnata* appeared to be more efficient than *L. minor* for removing Pb .On the other hand *A. pinnata* was better than *L. minor* in biomass for each of the elements used in the experiment during the incubation period.

Phytotoxicity

Metabolomic and transcriptomic investigation of the mechanism involved in enantioselective toxicity of imazamox in *Lemna minor*

Li, R; Luo, CX; Qiu, JS; Li, YF; Zhang, H; Tan, HH (2022) Journal of Hazardous Materials 425: 127818

Imazamox (IM) is a chiral pesticide that has been widely used in agriculture. Currently, few studies have investigated the toxicity mechanisms of imazamox to aquatic macrophyte from the enantiomer level. In this study, the enantioselective effects of IM on the toxicity and physiological and biochemical system of aquatic macrophyte *Lemna minor* were systematically investigated. Metabolomic and transcriptomic for *Lemna minor* were used to identify potential mechanisms of toxicity. 7 d EC_{50}s for racemic-, R-, and S-IM were 0.036, 0.035, and 0.203 mg/L, respectively, showing enantioselective toxicity. In addition, IM caused *Lemna minor* lipid peroxidation and antioxidant damage, and inhibited the activities of the target enzymes. Metabolomic and transcriptomic data indicated that R-IM interferenced differentially expressed genes and metabolites of *Lemna minor* which were enriched in carbon fixation during photosynthesis, glutathione metabolic pathway, pentose phosphate pathway, zeatin biosynthesis, and porphyrin and chlorophyll metabolism. S-IM affected phenylalanine metabolism, phenylpropanoid biosynthesis, zeatin biosynthesis and secondary metabolite biosynthesis. Racemic IM influenced carbon fixation during operation, glutathione metabolic pathway, zeatin biosynthesis and pentose phosphate pathway. The results provide new insights into the enantioselective toxicity mechanisms of IM to *Lemna minor*, and lay the foundation for conducting environmental risk assessments.

Ecotoxicological and genotoxic effects of dimethyl phthalate (DMP) on *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleid. plants under a short-term laboratory assay

Pietrini, F; Iannilli, V; Passatore, L; Carloni, S; Sciacca, G; Cerasa, M; Zacchini, M (2022) Science of the Total Environment 806 (Part 4): 150972

The environmental occurrence of phthalates (PAE) is of great concern for the ecosystem and human health. Despite of their recognized toxicity on biota, a lack of knowledge is still present about the effects of PAE on plants. In this scenario, the effects of dimethyl phthalate (DMP) on duckweed plants (*Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleid.), two model plant species for ecotoxicological and trophic studies, were investigated. Under a 7-day lab assay, morphological (biometric indicators), physiological (pigment content and photosynthetic performance) and molecular (DNA damage) parameters were studied. No effects were observed at growth and physiological level in both plants at 3 and 30 mg/L DMP. On the contrary, at 600 mg/L DMP, a concentration used for plant acute toxicity studies, a remarkable growth inhibition and pigment content and photosynthetic parameters reduction compared to control were observed in both plants species, particularly in *Spirodela*. Alkaline Comet assay in 24 h-treated plants revealed a genotoxic damage induced by DMP, particularly relevant in *Spirodela*. These results described for the first time the adverse effects exerted by DMP on aquatic plants, contributing to highlight the environmental risk associated to the presence of this compound in the aquatic ecosystem.

Role of Nramp transporter genes of *Spirodela polyrhiza* in cadmium accumulation

Chen, Y; Li, GJ; Yang, JJ; Zhao, XY; Sun, ZL; Hou, HW. (2021) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 227: 112907

As a pollutant, Cd causes severe impact to the environment and damages living organisms. It can be uptaken from the environment by the natural resistance-associated macrophage protein (Nramp) in plants. However, the ion absorption function of Nramp transporter genes in *Spirodela polyrhiza* has not been reported. In this study, SpNramp1, SpNramp2, and SpNramp3 from *S. polyrhiza* were cloned and their functions were analyzed in *S. polyrhiza* and yeast. Growth parameters and physicochemical indices of wild-type and transgenic lines were measured under Cd stress. Results revealed that SpNramp1, SpNramp2, and SpNramp3 were identified as plasma membrane-localized transporters, and their roles in transporting Cd were verified in yeast. In *S. polyrhiza*, SpNramp1 overexpression significantly increased the content of Cd, Fe, Mn, and fresh weight. SpNramp2 overexpression increased Mn and Cd. SpNramp3 overexpression increased Fe and Mn

concentrations. These results indicate that SpNramp1, SpNramp2, and SpNramp3 had a different preference for ion absorption. Two *S. polyrhiza* transgenic lines (OE1 and OE3) were obtained. One of them (OE1) showed a stronger accumulation ability, and the other one (OE3) exhibited tolerance capacity to Cd. This study provides new insight into the functions of SpNramp1, SpNramp2, and SpNramp3 and obtains important enrichment lines (OE1) for manipulating Cd accumulation, phytoremediation, and ecological safety.

Mixture toxicity of six pharmaceuticals towards *Aliivibrio fischeri*, *Daphnia magna*, and *Lemna minor*

Bialk-Bielinska, A; Grabarczyk, L; Mulkiewicz, E; Puckowski, A; Stolte, S; Stepnowski, P. (2021) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH DOI10.1007/s11356-021-17928-y

As the knowledge on the joint effects of pharmaceuticals towards different non-target organisms is still limited, the aim of our study was to evaluate the toxicity of mixtures of pharmaceuticals, as well as their baseline toxicity towards three selected organisms, namely the bioluminescent bacteria *Aliivibrio fischeri*, the crustacean *Daphnia magna*, and the duckweed *Lemna minor*. Different mixtures composed of three up to five pharmaceuticals having the same or different mechanisms of action in terms of their therapeutic activity (non-steroidal anti-inflammatory drugs, opioid analgesic, antibacterial and anti-epileptic drugs) were investigated. The observed EC_{50}s were compared with those predicted using the concentration addition (CA) and independent action (IA) models. In general, the EC_{50} values for mixtures predicted with the CA model were lower than those obtained with the IA model, although, in some cases, test predictions of these two models were almost identical. Most of the experimentally determined EC_{50} values for the specific mixtures were slightly higher than those predicted with the CA model; hence, a less than additive effect was noted. Based on the obtained results, it might be concluded that the CA model assumes the worst-case scenario and gives overall closer predictions; therefore, it should be recommended also for modelling the mixture toxicity of pharmaceuticals with different modes of action.

Chlorophyll fluorescence imaging-based duckweed phenotyping to assess acute phytotoxic effects

Olah, V; Hepp, A; Irfan, M; Meszaros, I. (2021) Plants 10: 2763

Duckweeds (Lemnaceae species) are extensively used models in ecotoxicology, and chlorophyll fluorescence imaging offers a sensitive and high throughput platform for phytotoxicity assays with these tiny plants. However, the vast number of potentially applicable chlorophyll fluorescence-based test endpoints makes comparison and generalization of results hard among different studies. The present study aimed to jointly measure and compare the sensitivity of various chlorophyll fluorescence parameters in *Spirodela polyrhiza* (giant duckweed) plants exposed to nickel, chromate (hexavalent chromium) and sodium chloride for 72 h, respectively. The photochemistry of Photosystem II in both dark- and light-adapted states of plants was assessed via in vivo chlorophyll fluorescence imaging method. Our results indicated that the studied parameters responded with very divergent sensitivity, highlighting the importance of parallelly assessing several chlorophyll fluorescence parameters. Generally, the light-adapted parameters were more sensitive than the dark-adapted ones. Thus, the former ones might be the preferred endpoints in phytotoxicity assays. Fv/Fm, i.e., the most extensively reported parameter literature-wise, proved to be the least sensitive endpoint; therefore, future studies might also consider reporting Fv/Fo, as its more responsive analogue. The tested toxicants induced different trends in the basic chlorophyll fluorescence parameters and, at least partly, in relative proportions of different quenching processes, suggesting that a basic distinction of water pollutants with different modes of action might be achievable by this method. We found definite hormetic patterns in responses to several endpoints. Hormesis occurred in the concentration ranges where the applied toxicants resulted in strong growth inhibition in longer-term exposures of the same duckweed clone in previous studies. These findings indicate that changes in the photochemical efficiency of plants do not necessarily go hand in

hand with growth responses, and care should be taken when one exclusively interprets chlorophyll fluorescence-based endpoints as general proxies for phytotoxic effects.

Enantioselective bioactivity, toxicity, and degradation in vegetables and soil of chiral fungicide Mandipropamid

Zhang, J; Wu, Q; Zhong, Y; Wang, Z; He, Z; Zhang, Y; Wang, M. (2021) Journal of Agriculture and Food Chemistry 69: 13416-13424.

Mandipropamid (MDP) is a widely used chiral fungicide to control oomycete pathogens with two enantiomers. In this study, the enantioselective bioactivity, toxicity, and degradation of MDP were investigated for the first time. The bioactivity of S-MDP was 118-592 times higher than that of R-MDP and 1.14-1.67 times higher than that of Rac-MDP against six phytopathogens. Molecular docking found that S-MDP formed a strong halogen bond with HIS 693 of cellulose synthase and possessed a lower binding energy, which validated the results of the bioactivity assay. S-MDP showed lower toxicity toward *Spirodela polyrhiza*, while it exhibited higher toxicity in *Danio rerio* embryo and larva. S-MDP preferentially degraded in cowpea and pepper, while R-MDP preferentially degraded in soil. There is no significant difference between the two enantiomers in the toxicity of adult *D. rerio* and in cucumber degradation. Therefore, the development of the S-enantiomer was considered as a better option to exhibit high efficiency, which could reduce the residual risk of the pesticide and ensure environmental safety.

Aquatic Toxicity Effects and Risk Assessment of 'Form Specific' Product-Released Engineered Nanomaterials

Lehutso, RF; Wesley-Smith, J; Thwala, M. (2021) INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 22: 12468

The study investigated the toxicity effects of 'form specific' engineered nanomaterials (ENMs) and ions released from nano-enabled products (NEPs), namely sunscreens, sanitisers, body creams and socks on *Pseudokirchneriella subcapitata*, *Spirodela polyrhiza*, and *Daphnia magna*. Additionally, risk estimation emanating from the exposures was undertaken. The ENMs and the ions released from the products both contributed to the effects to varying extents, with neither being a uniform principal toxicity agent across the exposures; however, the effects were either synergistic or antagonistic. *D. magna* and *S. polyrhiza* were the most sensitive and least sensitive test organisms, respectively. The most toxic effects were from ENMs and ions released from sanitisers and sunscreens, whereas body creams and sock counterparts caused negligible effects. The internalisation of the ENMs from the sunscreens could not be established; only adsorption on the biota was evident. It was established that ENMs and ions released from products pose no imminent risk to ecosystems; instead, small to significant adverse effects are expected in the worst-case exposure scenario. The study demonstrates that while ENMs from products may not be considered to pose an imminent risk, increasing nanotechnology commercialization may increase their environmental exposure and risk potential; therefore, priority exposure cases need to be examined.

Graphene oxide interaction with *Lemna minor*: Root barrier strong enough to prevent nanoblade-morphology-induced toxicity

Malina, T; Lamaczova, A; Marsalkova, E; Zboril, R; Marsalek, B. (2021) Chemopsphere 132739. DOI10.1016/j.chemosphere.2021.132739

The production of graphene oxide (GO) along with its applications in various aquatic environments is vastly increasing thanks to its rapidly expanding range of new GO-based environmental technologies. Therefore, the fate of GO in aquatic environments is an important issue, as it could become an environmental challenge if its potential toxic mechanism is not addressed properly. Number of studies reporting the toxicity of GO to various aquatic organisms is still increasing. However, research data on the possible toxic mechanism of GO towards

aquatic plants have yet to be collected, especially regarding GO's surface chemistry. Here, we studied the interaction of three differently oxidized GO systems with model aquatic plant *Lemna minor*. We found that although none of the three GOs caused lethal phytotoxicity to Lemna after 7 days, the mechanism of action was dependent on the GO's surface oxidation. Based on the amount of functional surface groups, the GO was able to directly interact with the *Lemna*'s root through its edges. However, in this case in contrast to algae and crustaceans, the interaction did not lead to a mechanical damage. Therefore, our results showed that GO is not hazardous to *Lemna minor* even at very high concentrations (up to 25mg/L), because the root barrier proved to be strong enough to prevent GO's penetration and its consequent toxicity.

Utilization of eutrophicated *Lemna minor* for biosorption of acid blue dye

Prakash, P; Kumar, JA; Dhandapani, B; Vishnu, D; Sree, SH; Madhumeena, S; Lavanya, Y; Inbathamizh, L. (2021) BIOMASS CONVERSION AND BIOREFINERY DOI10.1007/s13399-

Dyes and pigments utilized for colouring purposes by textile industries play a pivotal role in the accumulation of numerous toxic substances in an aquatic environment. This provokes a keen interest in the environmentalist in developing a novel sorbent material that could remove the organic pollutants from the waste effluent. In the current study, *Lemna minor*, a duckweed plant, acts as the low cost, eco-friendlier sorbent to remove the toxic acid blue 113 dye from the effluents. The characteristic traits of the dried biosorbent were analysed by scanning electron microscope to analyse the porous and rough exterior surface, Fourier transform infrared spectroscopy reveal the presence of specific entities such as amine, hydroxyl, carboxyl and alkyl groups and gas chromatography and mass spectroscopy for the presence of numerous polyphenolic compounds. Optimization was done by response surface methodology to study their potential in the adsorption capacity of the adsorbent. The removal efficiency of 98.5% was attained in the batch study at optimized parameters of contact time (40 min), pH (4.0), temperature (40 °C) and concentration of sorbent (1 g). The reaction mechanism was evaluated with the kinetics and isotherm studies. Kinetics study includes pseudo-first-order, pseudo-second-order, Elovich and intra-particle diffusion, and the isotherm study includes Langmuir, Freundlich, Langmuir-Freundlich and Temkin isotherm models. The system follows the pseudo-second-order kinetics model, Langmuir and Langmuir-Freundlich isotherm model with the maximum sorption capacity of 395.7 mg/g. Thermodynamic studies were evaluated to determine the spontaneous and endothermic nature of the reaction.

Taxonomy & Geobotany

Lemnaceae and Orontiaceae are phylogenetically and morphologically distinct from Araceae

Tippery, NP; Les, DH; Appenroth, KJ; Sree, KS; Crawford, DJ; Bog, M. (2021) Plants 10: 2639

Duckweeds comprise a distinctive clade of pleustophytic monocots that traditionally has been classified as the family Lemnaceae. However, molecular evidence has called into question their phylogenetic independence, with some authors asserting instead that duckweeds should be reclassified as subfamily Lemnoideae of an expanded family Araceae. Although a close phylogenetic relationship of duckweeds with traditional Araceae has been supported by multiple studies, the taxonomic disposition of duckweeds must be evaluated more critically to promote nomenclatural stability and utility. Subsuming duckweeds as a morphologically incongruent lineage of Araceae effectively eliminates the family category of Lemnaceae that has been widely used for many years. Instead, we suggest that Araceae subfamily Orontioideae should be restored to family status as Orontiaceae, which thereby would enable the recognition of three morphologically and phylogenetically distinct lineages: Araceae, Lemnaceae, and Orontiaceae.

New Insights into Interspecific Hybridization in *Lemna* L. Sect. *Lemna* (Lemnaceae Martinov)

Braglia, L; Breviario, D, Giani, S; Gavazzi, F; De Gregori, J; Morello, L. (2021) Plants 10: 2767

Duckweeds have been increasingly studied in recent years, both as model plants and in view of their potential applications as a new crop in a circular bioeconomy perspective. In order to select species and clones with the desired attributes, the correct identification of the species is fundamental. Molecular methods have recently provided a more solid base for taxonomy and yielded a consensus phylogenetic tree, although some points remain to be elucidated. The duckweed genus *Lemna* L. comprises twelve species, grouped in four sections, which include very similar sister species. The least taxonomically resolved is sect. *Lemna*, presenting difficulties in species delimitation using morphological and even barcoding molecular markers. Ambiguous species boundaries between *Lemna minor* L. and *Lemna japonica* Landolt have been clarified by Tubulin Based Polymorphism (TBP), with the discovery of interspecific hybrids. In the present work, we extended TBP profiling to a larger number of clones in sect. *Lemna*, previously classified using only morphological features, in order to test that classification, and to investigate the possible existence of other hybrids in this section. The analysis revealed several misidentifications of clones, in particular among the species *L. minor, L. japonica* and *Lemna gibba* L., and identified six putative *'L. gibba'* clones as interspecific hybrids between *L. minor* and *L. gibba*.

Hide and seek: molecular barcoding clarifies the distribution of two cryptic duckweed species across Alberta

Senevirathna, KM; Crisfield, VE; Burg, TM; Laird, RA. (2021) Botany 12: 795-801

Regional and global biodiversity may be underestimated due to the presence of cryptic species: species that are morphologically similar, but genetically distinct. Here, we focus on two cryptic duckweed species, *Lemna minor* L. and *Lemna turionifera* Landolt, which have overlapping geographic ranges and are easily mistaken for one another. We developed species-specific primers based on DNA barcoding sequences to facilitate the rapid identification of these two monomorphic duckweeds, allowing us to investigate their presence and distribution in Alberta, Canada. While current reports indicate the presence of *L. turionifera* (and the morphologically distinct *Lemna trisulca* L.) in Alberta, our data indicate that *L. minor* is also present, predominantly in the southern part of the province. Thus, this paper (i) contributes to the accuracy and completeness of a regional flora, and (ii) provides useful and flexible tools for the rapid molecular identification of cryptic *Lemna* species, which are of wide interest in diverse fields such as biotechnology, toxicology, bioremediation, and ecology.

Morphological characterization and DNA barcoding of duckweed species in Saudi Arabia

Al-Dakhil, M; Alghamdi, S; Migdadi, H; Afzal, M; Ali, AA (2021) Plants 10: 2438

Duckweeds, or Lemnaceae, are widespread aquatic plants. Morphology-based identification of duckweed species is difficult because of their structural complexity. Hence, molecular tools provide significant advantages for characterizing and selecting species or clones for sustainable commercial use. In this study, we collected and characterized ten duckweed isolates from nine different regions in Saudi Arabia (SA). Based on the morphological characterization and phylogenetic analysis of intergenic spacer sequences of chloroplast DNA using six barcoding markers, the clones were classified into three genera, represented by seven species: *Lemna gibba* L., *Lemna minor* L., *Lemna japonica* Landolt, *Lemna aequinoctialis* Welw., *Lemna perpusilla* Torr., *Spirodela polyrhiza* (L.) Schleid., and *Landoltia punctate* G. Mey. *Lemna gibba* was revealed to be a distinct dominant duckweed species in many regions of SA. Five barcoding markers showed *that L. gibba*, *L. minor*, and *L. punctata* were the most widely distributed species in the country. However, *L. punctata*, *L. perpusilla*, and *S. polyrhiza* were the dominant species in the Al-Qassim, Madinah-1, and Madinah-2 regions, respectively. Moreover, the morphological traits revealed variations for these clones, relative to other studied duckweed clones. According to the results obtained in this study, three out of six plastid markers (trnH-psbA, matK, and atpF-atpH) helped to identify the dominant duckweed species in Saudi Arabia. Further evaluation based on adaptability, molecular genetic studies, and functional genomics is needed for these species to be used at the commercial level in Saudi Arabia.

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). 1,000 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

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