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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

Volume 9 (4), issue 35, pages 105 - 143 (2021)

Tenth anniversary of the 1st ICDRA

Cover page

Commemorating the 10th anniversary of the first ICDRA

Participants of the 1st International Conference of Duckweed Research & Application (ICDRA-2011).

From the left in the first row: M M Almudena, L Landesman, X Xiao, Z Yuan, K-J Appenroth, H Zhao, J J Cheng, N Wu, E Lam, L Ma, R E Bell, G Zhang, Y Zhang, T Oyama, M Gu; From the left in the second row: A A Salmean, -, -, -, -, Y Fang, S Bao, L Chen, H Wang, A Bell, X Ma, Y Pen, W Zhang, Y Huang, F Long, Y Xiao; From the left in the third row: -, X Tao, G Wang, -, Ming Zhu, J Farrell, K He, Z Wang, M Wang, Y Zaho, -, H Zhang, X Yan, Y Liu; From the left in the fourth row: G Zhang, H Su, Q Zhang, Q Chen, X Gao

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: *Lemna japonica* Landolt

Lemna japonica has interesting morphological features: Fronds are often reddish on the lower surface and shiny on the upper surface; papulae near the tip are larger than the proximal ones; 4th and 5th nerve originate from the lower parts of the inner nerves, but not from the node. Originally it was found growing in East Asia, i.e. China, Japan, Korea which is the reason of its name but is now introduced in many countries like USA, Scandinavia and Germany. It was shown very recently, that *L. japonica* is likely a hybrid between *L. minor* (as mother) and *L. turionifera* (L. Morello et al. (2021) *Lemna* species identification made easy. Duckweed Forum 9: 30-33) using the TBP method, thus supporting the suggestion by Elias Landolt decades ago based on morphological criteria. As a taxonomic consequence, molecular plastidic markers cannot distinguish between *L. minor* and *L. japonica* (Borisjuk et al. (2015) Plant Biology 17: 42-49). Further, delineation of *L. minor* and *L. japonica* based on morphological markers is also sometimes not possible (Personal communication from Laura Morello, CNR-IBBA, Italy) as they share most of the morphological characters (Landolt, 1986). Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India; Legend by Dr. Klaus-J. Appenroth, Friedrich Schiller University, Jena, Germany.

ISCDRA
International Steering Committee on Duckweed Research and Applications

Letter from the Editor:

Oct. 28th, 2021

Dear Readers of the *Duckweed Forum*,

I hope this greeting finds you in health and happiness, wherever you may be. In the Northeast of the United States, the brilliant foliage outside of my window is a reminder that Fall is in full swing and the end of another year is fast upon us.

While the pandemic in the U.S. appears at this time to be abating, thanks in a large part to increased rate of vaccination, the threat of resurgence remains significant. It is sobering to reflect on the fact that millions have perished globally in a short time of 2 years since the beginning of the first outbreak, despite all the advanced technologies that are at our disposal. The importance of decision making and coordinated responses in the global community for effectively containing the spread of the virus cannot be overstated. In a way, this pandemic could very much serve as a foreshadow of a greater catastrophe to confront humanity in the form of Climate Change. While this challenge builds up slower in time scale compared to a disease outbreak, it is a force of Nature that will be much harder to reverse and its impact will be felt by billions for generations to come. With world leaders meeting next week to take stock of the dire warnings from the scientific communities around the world, I sincerely hope serious actions will finally be implemented to mitigate this existential threat to life on our planet.

As many of our devoted readers have seen in these pages of the *Duckweed Forum* over the past years, our field's importance and endeavors are being recognized as reflected in the ever-increasing number and quality of publications in the Database section. For the Cover Picture of the present issue, it is with gratitude for me to look back a decade ago to note that this month was the 10th Anniversary of the 1st ICDRA conference which I co-organized with Prof. Zhao Hai in Chengdu, China. This biennial meeting has continued to grow and has served as an important community-building event to help foster understanding and networking among researchers in our field. I am glad to have contributed to this effort and see the fruits from all the collaborative work that has emerged. I hope the collective effort from research and application specialists working on duckweed will produce true societal impact in the near future and will provide an effective tool to help humanity confront critical challenges in this century. The new generation of students and researchers that we can attract and train, such as those that we highlight in our Spotlight section, will be our best hope to fully realize the potential of duckweeds in the years to come.

With these positive thoughts on the state of our duckweed research community, I like to wish all of our readers health, success and happiness in 2022. As always, I like to thank my colleagues in the ISCDRA, as well as ad hoc contributors, for their time and effort to bring information and knowledge to share with everyone in our community.

Sincerely,

Eric Lam

Chair, ISCDRA

Commemorating the 10th anniversary of the first ICDRA

Yang Fang and Hai Zhao

Chinese Academy of Sciences (CAS) Key Laboratory of Environmental and Applied Microbiology, Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chengdu 610041, China

Time flies. It has been 10 years since the 1st International Conference of Duckweed Research & Application (ICDRA-2011), which brought duckweed researchers around the world together in Chengdu, China from October 7-10. As the initiator, organizer and witness of the first conference, we are glad to note that the ICDRA has been successfully held for five times (if it were not for the epidemic COVID-19 sweeping the world, the sixth conference would have been held in Germany this year). The scale of the conference is getting bigger and bigger, with more and more participants and more commercial attention, which also greatly promotes the research and cooperation in the duckweed field.

Looking back 10 years ago, our research team just entered the field of duckweed research. It was urgent for us to have academic exchanges and cooperation with international teams who are specialized in duckweed research. We obtained financial support from the Ministry of Science and Technology of China, and contacted Prof. Jay J. Cheng of NC State University and Prof. Eric Lam of Rutgers, The State University of New Jersey. They all responded positively to our consultation and strongly supported us in convening the conference. Furthermore, Prof. Eric Lam decided to co-organize the first duckweed conference with us. Therefore, the first conference was held in Chengdu, the city where our institute is located. A post-conference excursion to a field demonstration site in Kunming, Yunnan province, was also arranged by our team.

Prof. Jay J. Cheng and Prof. Eric Lam provided us a list of experts in duckweed research, and we made contact with them one by one, including Prof. Elias Landolt. Unfortunately, he was unable to travel long distances due to physical reasons at that time, but he expressed in his email that he was very happy that we would hold such an international conference on duckweed, and also wished us a successful conference. Although Prof. Elias Landolt was unable to attend it, we still have more than 60 participants from 6 countries to attend the conference (Cover Photo of this issue). Although many researchers had never been to China before, they all responded positively and participated in ICDRA-2011.

After three months of preparation, all the logistics for hosting the conference was ready. Special thanks should be given to Mr. Gu Mingxuan, who was in charge of the foreign affairs of our institute at that time (at the right first position in the first row of the Cover Photo). He guided us in organizing the conference such as the protocols for booking the hotel, arranging for transportation and meals, and also participated in parts of preparatory work. Meanwhile, we also need to thank our colleagues and students who worked in Kunming, Yunnan, for building our demonstration site there with the highest efficiency, and enabled us to obtain a lot of important data there very quickly. Their efforts made the field investigation tour of this conference a complete success (Fig. 1).

The conference organized 5 sessions with a total of 18 reports from 6 countries including China, the United States, Germany, Japan, Denmark and Australia. Although the number of reports is much smaller than that of the subsequent conferences, it is an important first step. This conference brought duckweed researchers from all over the world together and re-formed a cohesive circle, which is of great significance to the revival of duckweed research. At last, the closing session of this conference passed the resolution that duckweed conference should be held every two years, and also decided to duplicate and stock duckweed culture collection in Chengdu, with sister collections at Rutgers (USA), Zurich (Switzerland) and Jena (Germany). The conference also decided to maintain the 4-digit code system started by Landolt in order to provide community standard and minimize confusion. Prof. Elias Landolt also agreed with these ideas and extended his support in the identification and numbering of duckweed clones in use. In a word, we believe the sharing of this information with the broad Plant Biology community has been an important step toward the renaissance of this excellent plant model that will have important impact on our quest for sustainable development of the world (Zhao et al., 2012).

On the occasion of the 10th anniversary of ICDRA-2011, this dedication commemorates the convening of the first international duckweed conference.

Zhao H, Appenroth K, Landesman L, Salmeán AA, Lam E. (2012) Duckweed rising at Chengdu: summary of the 1st International Conference on Duckweed Application and Research. Plant Mol Biol. 78(6): 627-32. doi: 10.1007/s11103-012-9889-y.

Figure 1: The field demonstration tour in Kunming, Yunnan. In order to enable meeting participants to have a more hands-on understanding of our team's research progress in a region where light and heat conditions are most suitable for duckweed growth to promote wastewater treatment in China, we organized this field trip. In the middle of the photo is the meeting organizer Hai Zhao.

Homage to avid duckweed researchers

Jitendra P. Khurana (30. 10. 1954 – 27. 10. 2021)

Professor Dr. Jitendra P. Khurana passed away a few days before his 67th birthday, on 27th October 2021. He served as a Professor at the Department of Plant Molecular Biology, University of Delhi South Campus, India. Prof. Khurana was an eminent academician, a distinguished scientist and an efficient administrator. From 2016, until his retirement in 2019, he served as Director of the University of Delhi South Campus, and as Pro-Vice Chancellor (interim) of the University of Delhi. Already during his early carrier working with Late Prof. S. C. Maheshwari, he investigated duckweeds focusing on flower induction in *Wolffia microscopica*, *Wolffiella hyalina, Lemna aequinoctialis* and *Spirodela polyrhiza*. Most recently, last month, Prof. Khurana published the following paper with a cover photo contribution:

Sree, KS; Maheshwari, SC; Appenroth, KJ; Khurana, JP (2021) History of discovery of the fastest growing angiosperm, *Wolffia microscopica* (Griff.) Kurz entwined with British India. - *Current Science* 121: 724-726.

In the next issue of the "Duckweed Forum" we will present a detailed obituary of Prof. Dr. Jitendra P. Khurana.

Manfred Eichhorn (16. 8. 1934 – 12. 8. 2021)

The author of the contribution "Historical Accounts: Matthias Jacob Schleiden (1804-1881) and the beginning of Lemnaceae research" to Duckweed Forum 6 (4): 127-129 (2018) has recently passed away. PD Dr. sc. nat. Manfred Eichhorn had served as a member of the Department of Plant Physiology at the Friedrich Schiller University of Jena, Germany, during his scientific life where he also earned all his scientific degrees. He worked most of his time with duckweeds, concentrating on *Wolffia arrhiza*.

One of his publications is: "Changes in the polyadenylated RNA from *Wolffia arrhiza* continuously illuminated with white, blue or red light", Biochem. Physiol. Pflanzen 178: 183-192 (1983). DOI: 10.1016/S0015-3796(83)80031-6. We wish to acknowledge Prof. Eichhorn's lifelong dedication to the study of duckweed and their biology.

Announcing call for 5th ISCDRA Nominees

Procedure for the election of members to the 5th International Steering Committee on Duckweed Research and Applications

The International Steering Committee on Duckweed Research and Applications (ISCDRA) was founded during the 2nd International Conference on Duckweed Research and Applications (ICDRA) at Rutgers, the State University of New Jersey, New Brunswick, NJ in 2013.

Members of the committee cooperate with each other in order to steer and promote duckweed research and applications for the benefit of our community. Assisting in the publication of the community newsletter, the *Duckweed Forum*, is one of the obligations, among others, that are expected of the committee members.

1) The ISCDRA should consist of 5 members who will be elected before the biennial ICDRA in a secret poll.

2) Anyone who has previously attended any of the ICDRA or will be attending it this year, or receives the ISCDRA Newsletter can suggest potential candidates including themselves. Candidates should have attended at least one of the three previous ICDRA meetings. Suggestions may be sent to the present Chair of the ISCDRA - Prof. Dr. Eric Lam, Email: ericL89@hotmail.com. The deadline for submission of candidate names is 22nd January, 2022.

3) The voting procedure will be announced in the next *Duckweed Forum* issue, scheduled to be available in January, 2022.

4) The five newly elected members will be notified by email and they will elect the head of the committee before the 6th ICDRA-2022 in Gatersleben, Germany.

5) In case that by chance all elected members are either from the applied field or from the research field, the elected Chair will appoint one additional member from the missing field.

6) At the end of the ISCDRA meeting (General Assembly) the previous Committee will report shortly about the activities since the previous election and the duty will be transferred to the newly elected ISCDRA.

Request for applications to host ICDRA- 2024

In order to identify the best venue possible for the next meeting of the ICDRA, applications from interested organizations are requested to be sent to one or more members of the ISCDRA by April 10th, 2022.

The applications should briefly introduce the proposed venue, its benefit/attractions, relevance to duckweed research and/or applications, and the responsible organizer's credentials as well as experience. The list of all applications will be send out to the community in April, 2022 issue of "*Duckweed Forum*" before the 6th ICDRA and decision by popular vote of the attendees will be made during the "General Assembly" at the end of the conference in Gatersleben, Germany, on 01st June, 2022.

Update: 6th ICDRA- 2022

IPK Gatersleben

Originally, the next "International Conference on Duckweed Research and Applications" was planned to be held on May 30 – June 02, 2021 in Gatersleben, Germany (photo). Because of the restrictions caused by the CoVID-19 pandemic we were obliged to postpone the conference to 2022, held as

ICDRA 2022: 6th INTERNATIONAL CONFERENCE ON DUCKWEED RESEARCH AND APPLICATIONS,
29 May to 01 June 2022
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany

We invite you cordially to participate in this conference, the first in Europe as well as the first time in Germany. Further information can be found on the homepage:

https://icdra-2022.ipk-gatersleben.de/

For formal invitations or answer to any questions, do not hesitate to contact us.

Best regards,
Organizers of 6th ICDRA

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Announcement: Important Deadlines for attendees

Duckweed Research and Applications Workshop in the

Plant and Animal Genome (PAG29) Conference 2022

January 8-12, 2022

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



After a year off, PAG, a premier genomics-centric meeting is back on in five months !

Organizers Eric Lam (Rutgers U.) and Todd Michael (Salk Institute) are organizing the Duckweed Research and Applications Workshop to take place on January 11th during the PAGXXIX in San Diego (January 8-12, 2022) and would encourage everyone in the community to attend. We expect to have a good lineup of exciting speakers with new results from diverse studies with duckweed.

Please register at this link: https://www.intlpag.org/2022/attend/registration-and-fees and note these deadlines:

Deadline for workshop abstract submission: December 14th, 2021
Deadline for poster abstract submission: October 29, 2021
Deadline for speaker selection by organizers: November 19, 2021

Email Eric Lam (ericL89@hotmail.com) or Todd Michael (toddpmichael@gmail.com) to inquire if you are interested to attend as a speaker as soon as possible. We look forward to hearing from you.

Photo: *Wolffiella neotropica* / E. Lam

Student Spotlight: Alexandra Mireya Chávez Argandoña

Institute for Evolution and Biodiversity, Westefälische Wilhems-Universität Münster, Germany
(email: achaveza@uni-muenster.de)

My interest for understanding living creatures started since I was a child, and at high school, I already knew I wanted to become a Biologist. During my bachelor at the Universidad Peruana Cayetano Heredia (Peru) I studied how behaviour and diet of species change due to differences in the environment. However, later I realized that, to better understand variation within species, I also needed to get into the molecular and genomic areas. My knowledge of evolution using genomic tools was established during my master (M.Sc.) studies at the Universidad del Bio-Bio (Chile), at the same time when research in non-genetic inheritance was getting stronger and it was being introduced into the modern theory of evolution.

The great opportunity to study the role of non-genetic traits in adaptation came during my PhD, which was offered by Dr. Meret Huber and Prof. Dr. Shuqing Xu from the Westefälische Wilhems-Universität Münster (Germany). They have access to the perfect model to understand the impact that non-genetic traits have in the evolution of species: The Giant Duckweed (*Spirodela polyrhiza*). Due to its fast-vegetative propagation (2-3 days), medium frond size (~6 mm), small genome (~150 Mb) and the possibility to sterilize it, it becomes possible to study the effect that stress has in 20 generations in only 2 months, without interference from the molecular resetting of meiosis. Also, *S. polyrhiza* provides an opportunity to separate the effect of non-genetic variance from those of genetic variance, which until now, is a highly confounding factor in non-genetic inheritance studies.

S. polyrhiza, a worldwide distributed species, is affected by different biotic and abiotic fluctuating oxidative factors, such as predation by aphids and snails, herbicides like diquat, and other agents like copper and salinity. These factors produce reactive oxygen species (ROS) that damage the cell walls and plant metabolism. However, this first exposure to stress may also produce, rather than a priming effect, a non-genetic transgenerational inheritance (passed for more than 3 generations). The factors involved in non-genetic transgenerational inheritance could go from those clustered in transgenerational plasticity (passing of bacteria, nutrients, hormones and/or other ions) to epigenetic mechanisms (histone modification, DNA methylation, small RNAs and/or prions).

My work with *S. polyrhiza* approaches the effect of non-genetic transgenerational inheritance from the ecological side, with aims such as identifying its effects on fitness of the species, characterizing the factors involved in non-genetic inheritance, and to identify the genetic basis behind the non-genetic inheritance. To do so, it is important to be able to follow one generation after the other, for which our working team applies the treatment called “single descendant propagation”. As its name implies, we only pass one offspring of each generation to continue the lineage and we mark each parental generation to identify it from the offspring (Fig. 1). My experiments using this treatment have shown that the fitness of different *S. polyrhiza* genotypes increases under the recurrent presence of oxidative stress after a recovery phase in the absence of stress. Thus, *S. polyrhiza* shows a positive resistance after such treatment in comparison to those plants kept in the absence

Figure1: Single descendant propagation of *Spirodela polyrhiza* under aseptic conditions. The older generation carries a mark that allows an investigator to identify it and thus, separate the new generation into a new propagation tube.

Figure 2: Post-propagation of recently sterilized *Spirodela polyrhiza* genotypes into new Erlenmeyer flasks for cultivation, a step needed to begin with the experiments.

of oxidative stress. Although most of our experiments are done under sterile conditions in the lab (Fig. 2), they are not limited to this environment. Thus, we have also performed experiments in outdoor conditions, considering fluctuations in temperature, radiation stress and stress induced by algae. These experiments have allowed us to work with thousands of individuals within which we could continue with the single-descendant treatment (Fig. 3). Such experiments have permitted us to assess the effect of oxidative stress in longer time frames and whether non-genetic transgenerational traits could be under natural selection.

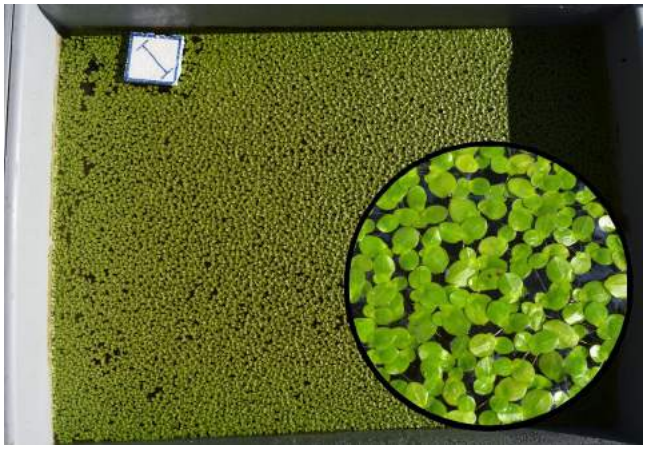

Figure 3: *Spirodela polyrhiza* population and single-descendants in outdoor conditions. Within the plant population, single descendants are marked (in the inset it is decorated with two red dots) to follow them through each generation. Reference floating stone on the upper left corner measures 5 cm.

Both, the transgenerational inheritance as well as the effect of natural selection in non-genetic traits, would produce an increase in the species fitness; while the first one would be a gradual acquisition of the resistance under recurrent stress, selection would act on the individuals carrying the acquired resistant traits. Thus, to disentangle the effects of selection from the transgenerational inheritance, we assessed the fitness of plants coming from the single descendant treatment (transgenerational inheritance) and of plants coming from the population subjected to constant bottleneck events (natural selection). Surprisingly, we have found that natural selection acts on non-genetic traits in clonal plants, showing that this non-genetic inheritance may play a crucial role in the evolution of species.

To identify the factors causing the non-genetic inheritance, I am assessing the effect that the acquired bacteria in *S. polyrhiza* can have on the fitness of plants under recurrent oxidative stress, as well as the effect of molecular mechanism by using inhibitors for the cytosine methylation maintenance machinery and the histone de-acetylation machinery. Furthermore, to identify the genetic basis of this transgenerational inheritance, I am assessing the variation among genotypes

with the aim to find candidate genes causing the acquired resistance under recurrent oxidative stress. Although our previous experiments have already shown differences among genotypes coming from the four genetic clusters in *S. polyrhiza* (America, Europe, Asia and India), the assessment of 205 genotypes present in our group's collection will allow a strong whole genome association study (GWAS) to identify the candidate genes that are associated with variability of the non-genetic transgenerational inheritance in *S. polyrhiza*.

Even though this is just the beginning of a broad path to find the role of non-genetic inheritance in the evolutions of clonal species, *S. poyrhiza* has shown itself to be an ideal model for us to identify potential factors in a relatively short time.

From the Database

Highlights

Coordination of leaf economics traits within the family of the world's fastest growing plants (Lemnaceae)

Ishizawa, H; Onoda, Y; Kitajima, K; Kuroda, M; Inoue, D; Ike, M (2021) Journal of Ecology 109: 2950-2962

The duckweed family (Lemnaceae) is a group of free-floating aquatic plants with bodies consisting of single floating fronds that multiply clonally. Although they are known to have the fastest relative growth rate (RGR) among higher plants, their functional trait coordination in relation to within-family variation of RGR is poorly understood. We tested how duckweed species fit within the trait covariation patterns known as the world-wide leaf economics spectrum (LES). To this end, several functional traits were evaluated for 15 duckweed species, and their covariation patterns were compared with those in the global database of plant functional traits. As a group, duckweeds exhibited the most acquisitive suite of traits, with extremely small leaf mass per area (LMA), short life span and high mass-based photosynthetic rate (A(mass)). These LES traits showed a tight correlation with RGR, corroborating our hypothesis that acquisitive leaf resource economics underpins their extremely high RGR. However, unlike other higher plants, LMA showed weak association with leaf life span and A(mass) within duckweed family. We also found a unique positive correlation between duckweed LMA and area-based photosynthetic rates, an indication that their LMA represents different functional significance compared to typical higher plants. Duckweeds, the world's fastest growing plants, mostly follow the world-wide LES and locate at its extreme end. The slight deviation from the LES highlights that duckweeds experience some physical and chemical constraints not faced by other higher plants.

Transgenerational non-genetic inheritance has fitness costs and benefits under recurring stress in the clonal duckweed *Spirodela polyrhiza*

Huber, M; Gablenz, S; Hofer, M (2021) Proceedings of the Royal Society B-Biological Sciences 288: 20211269

Although non-genetic inheritance is thought to play an important role in plant ecology and evolution, evidence for adaptive transgenerational plasticity is scarce. Here, we investigated the consequences of copper excess on offspring defences and fitness under recurring stress in the duckweed *Spirodela polyrhiza* across multiple asexual generations. Growing large monoclonal populations (greater than 10000 individuals) for 30 generations under copper excess had negative fitness effects after short and no fitness effect after prolonged growth under recurring stress. These time-dependent growth rates were likely influenced by environment-induced transgenerational responses, as propagating plants as single descendants for 2 to 10 generations under copper excess had positive, negative or neutral effects on offspring fitness depending on the interval between initial and recurring stress (5 to 15 generations). Fitness benefits under recurring stress were independent of flavonoid accumulations, which in turn were associated with altered plant copper concentrations. Copper excess modified offspring fitness under recurring stress in a genotype-specific manner, and increasing the interval between initial and recurring stress reversed these genotype-specific fitness effects. Taken together, these data demonstrate time- and genotype-dependent adaptive and non-adaptive transgenerational responses under recurring stress, which suggests that non-genetic inheritance alters the evolutionary trajectory of clonal plant lineages in fluctuating environments.

Agriculture

Physiological and biochemical effects of an aqueous extract of *Lemna minor* L. as a potential biostimulant for maize

Del Buono, D; Bartucca, ML; Ballerini, E; Senizza, B; Lucini, L; Trevisan, M (2021) Journal of Plant Growth regulation DOI: 10.1007/s00344-021-10491-3

Biostimulants are receiving increasing attention for their beneficial effects on crops, driving interest in identifying new plant extracts that could exert such stimulatory effects. This work aimed to evaluate the potential of an aqueous extract obtained from duckweed (*Lemna minor* L.), a freshwater species, to act as a biostimulant in maize. For this purpose, duckweed plants were collected from a natural basin and then transferred, stabilized, and grown under controlled conditions. The duckweed extract was first characterized through untargeted profiling, which revealed an abundance of bioactive phytochemicals. A relatively high amount of low-molecular-weight secondary metabolites such as phenolics (6714.99 mg kg^{-1}) and glucosinolates (4563.74 mg kg^{-1}) were present in the plant extract. Maize seeds were primed with different concentrations of this extract (0.01%, 0.05%, 0.50%, and 1.00%, dry weight/water volume), and some physiological and biochemical traits of the crop were recorded. The duckweed extract improved maize germination, biomass, leaf area, pigment content, and vigor index. The most effective treatment was the 0.50% concentration, which improved the majority of the measured growth traits. The extract at concentrations of 0.05%, 0.50%, and 1.00% stimulated the assimilation of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), and copper (Cu). In summary, this study revealed that duckweed is a promising species that can be cultured and grown under controlled conditions for obtaining extracts with biostimulant properties.

Biochemistry

Determination of the content of biologically active substances from some aquatic higher plants

Ramazanova, AA; Yernazarova, GI; Turasheva, SK; Ablaikhanova, NT (2021) Pakistan Journal of Botany 53: 1893-1899

The article identifies the following biologically active substances in the surface and the underground parts of *Eichhornia crassipes*, *Pistia Stratiotes*, and *Lemna minor* aquatic plants (roots, stems, leaves): alkaloids, anthraquinones, proteins, tannins, flavonoids, phenolic compounds, polysaccharides, anthraquinones, and coumarins. A brief overview of scientific works has been given for certain types of biologically active substances and their biological activity, importance, biosynthesis, and genetic transfer. The results of this research show that high amount of biologically active substances (BAS) was found in *Eichhornia crassipes* aquatic plant in terms of tannins, which in the roots amounted to 7.476%, and in the above-ground part - to 6.73%. The content of polysaccharides was 5.907%, and in the roots - 2.642%. By the amount of BAS detected in the composition of *Pistia stratiotes* aquatic plant, polysaccharides content in the aerial part was 3.073%, and in the roots - 4.881%, the content of flavonoids in the aerial part was 4.833%, and in the roots - 3.716%. Among BAS in *Lemna minor* water plant, the content of flavonoids was 5.463%.

Protocols for isolating and characterizing polysaccharides from plant cell walls: a case study using rhamnogalacturonan-II

Barnes, WJ; Koj, S; Black, IM; Archer-Hartmann, SA; Azadi, P; Urbanowicz, BR; Pena, MJ; O'Neill, MA (2021) Biotechnology for Biofuels 14: 142

In plants, a large diversity of polysaccharides comprise the cell wall. Each major type of plant cell wall polysaccharide, including cellulose, hemicellulose, and pectin, has distinct structures and functions that

contribute to wall mechanics and influence plant morphogenesis. In recent years, pectin valorization has attracted much attention due to its expanding roles in biomass deconstruction, food and material science, and environmental remediation. However, pectin utilization has been limited by our incomplete knowledge of its structure. Herein, we present a workflow of principles relevant for the characterization of polysaccharide primary structure using nature's most complex polysaccharide, rhamnogalacturonan-II (RG-II), as a model. We outline how to isolate RG-II from celery and duckweed cell walls and from red wine using chemical or enzymatic treatments coupled with size-exclusion chromatography. From there, we applied mass spectrometry (MS)-based techniques to determine the glycosyl residue and linkage compositions of the intact RG-II and derived oligosaccharides including special considerations for labile monosaccharides. In doing so, we demonstrated that in the duckweed *Wolffiella repanda* the arabinopyranosyl (Arap) residue of side chain B is substituted at O-2 with rhamnose. We used electrospray-MS techniques to identify non-glycosyl modifications including methyl-ethers, methyl-esters, and acetyl-esters on RG-II-derived oligosaccharides. We then showed the utility of proton nuclear magnetic resonance spectroscopy (H^1-NMR) to investigate the structure of intact RG-II and to complement the RG-II dimerization studies performed using size-exclusion chromatography. The complexity of pectic polysaccharide structures has hampered efforts aimed at their valorization. In this work, we used RG-II as a model to demonstrate the steps necessary to isolate and characterize polysaccharides using chromatographic, MS, and NMR techniques. The principles can be applied to the characterization of other saccharide structures and will help inform researchers on how saccharide structure relates to functional properties in the future.

Biotechnology

The world smallest plants (*Wolffia* sp.) as potential species for bioregenerative life support systems in space

Romano, LE; Aronne, G (2021) Plants 10: 1896

To colonise other planets, self-sufficiency of space missions is mandatory. To date, the most promising technology to support long-duration missions is the bioregenerative life support system (BLSS), in which plants as autotrophs play a crucial role in recycling wastes and producing food and oxygen. We reviewed the scientific literature on duckweed (Lemnaceae) and reported available information on plant biological traits, nutritional features, biomass production, and space applications, especially of the genus *Wolffia*. Results confirmed that the smallest existing higher plants are the best candidate for space BLSS. We discussed needs for further research before criticalities to be addressed to finalise the adoption of *Wolffia* species for space missions.

Influence of the Nitrate-N to Ammonium-N ratio on relative growth rate and crude protein content in the duckweeds *Lemna minor* and *Wolffiella hyalina*

Petersen, F; Demann, J; Restemeyer, D; Ulbrich, A; Olfs, HW; Westendarp, H; Appenroth, KJ (2020) Plants 10: 1741

In order to produce protein-rich duckweed for human and animal consumption, a stable cultivation process, including an optimal nutrient supply for each species, must be implemented. Modified nutrient media, based on the N-medium for duckweed cultivation, were tested on the relative growth rate (RGR) and crude protein content (CPC) of *Lemna minor* and *Wolffiella hyalina*, as well as the decrease of nitrate-N and ammonium-N in the media. Five different nitrate-N to ammonium-N molar ratios were diluted to 10% and 50% of the original N-medium concentration. The media mainly consisted of agricultural fertilizers. A ratio of 75% nitrate-N and 25% ammonium-N, with a dilution of 50%, yielded the best results for both species. Based on the dry weight (DW), *L. minor* achieved a RGR of 0.23±0.009 d^{-1} and a CPC of 37.8±0.42%, while *W. hyalina*'s maximum RGR was 0.22±.017 d^{-1}, with a CPC of 43.9±0.34%. The relative protein yield per week and m^2 was highest at this ratio

and dilution, as well as the ammonium-N decrease in the corresponding medium. These results could be implemented in duckweed research and applications if a high protein content or protein yield is the aim.

Deep eutectic solvent-based microwave-assisted extraction for the chromatographic analysis of bioactive flavonoids in *Spirodela polyrrhiza*

Hao, H; Lin, L; Liu, S; Kang, Y; Wang, Y; Huang, JM; Weng, WY (2021) Journal of Chromatographic Science DOI:10.1093/chromsci/bmab092

Deep eutectic solvents (DESs) are regarded as promising solvents to extract chemicals from plant materials. In this study, a DES-based microwave-assisted extraction (MAE) method was developed for the chromatographic analysis of four bioactive flavonoids in *Spirodela polyrrhiza*. Chromatographic separation was achieved on a Promosil C18-column. Prior to the HPLC analysis, the flavonoids were rapidly extracted by a DES-MAE process using choline chloride/levulinic acid (1:2, mol/mol) as the solvent. The extraction parameters were optimized using response surface methodology, and the optimal DES-MAE was fast and efficient compared with conventional solvent-based MAE and ultrasonic-assisted extraction using DES. The recoveries of optimized DES-MAE for the four flavonoids ranged from 97.80 to 103.29%. This study demonstrates that the validated DES-MAE-HPLC method is efficient, accurate and applicable to the determination of flavonoids in *S. polyrrhiza*.

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

New insights into moisture sorption characteristics, nutritional compositions, antioxidant and morphological properties of dried duckweed (*Wolffia arrhiza* (L.) Wimm).

Masavang, S; Winckler, P; Tira-Umphon, A; Phahom, T (2021) Journal of the Science of Food and Agriculture DOI:10.1002/jsfa.11555

Duckweed has been considered as alternative future food materials due to its high nutritional values but it contains high moisture content resulting in short shelf life. Moisture sorption isotherms are used to design dehydration and storage conditions in order to prolong food products shelf life. Nowadays, the information of sorption isotherm of duckweed has not been reported then it was investigated in this study. Scanning electron microscopy (SEM) is frequently used to study food microstructure. However, this technique has to perform under high-vacuum condition and take long period of time. Two-photon imaging microscopy was then selected to study the microstructure of dried duckweed instead of the SEM. Among five sorption isotherm models, Peleg model gave the highest goodness of fit. The monolayer moisture content (M0) of duckweed was in the range from 7.43 to 7.92 % d.b. and 8.87 to 8.86 % d.b. for GAB and BET models, respectively. The moisture changing behaviors at each relative humidity step could be described by two exponential and reaction order kinetics. A clear cell structure (hexagonal shape's like) and stomata as well as the structural images (both 2D and 3D) were obtained from two-photon microscopy technique. The Peleg model was the best-describing moisture sorption behaviors of dried duckweed and the shape of sorption isotherms were classified as type III isotherm. The M0 of dried duckweed ranged from 7.43 - 8.86% d.b. Two-photon microscopy was a potent tool for studying microstructure and compositing 3D image of dried duckweed.

DF: The exact botanical name is *Wolffia arrhiza (L.) Horkel ex Wimm*

Recovery of waste nutrients by duckweed for reuse in sustainable agriculture: Second-year results of a field pilot study with sorghum

Pulido, CRF; Caballero, J; Bruns, MA; Brennan, RA (2021) Ecological Engineering 168: 106273

Between 62% and 92% of industrial and municipal wastewater in upper-middle, low-middle, and low income countries is discharged to the environment untreated, releasing valuable nutrients such as nitrogen (N) and phosphorus (P) into rivers, lakes, and oceans (Lipponen and Nikiforova, 2017). This, in addition to excess nutrients often present in agricultural runoff due to overuse and misuse of fertilizers, can lead to eutrophication, often causing irreparable damage to aquatic ecosystems. For these reasons, new techniques

must be found to effectively recover waste nutrients and upcycle them into natural soil amendments that can be used to enrich soil quality and grow food for future generations while minimizing agricultural runoff. Duckweed is a small floating aquatic plant that can hyperaccumulate nutrients present in wastewater and agricultural runoff and then be harvested and reused to replace or supplement commercial soil fertilizers. As part of a two-year field trial, duckweed was tested for the second consecutive year in this study as a soil amendment in comparison to, and in combination with, commercial fertilizer for the growth of sorghum, a drought-resistant grain. Relative to fertilizer in all cases, soils amended with duckweed generated less ammonia and nitrate in surficial runoff. No differences in P in cumulative runoff were found among the different treatments ($p = 0.509$). Additionally, duckweed application produced sorghum grains with greater N and P content than other treatments (1.63±0.03% N ($p = 0.001$) and 0.35±0.0% P ($p = 0.016$)). Duckweed treatments also showed increased soil residue carbon and P after harvesting the crop. When normalized by germination rate, sorghum yield was similar across treatments. In agreement with first-year findings, the results indicated that duckweed may be a viable alternative to commercial fertilizer from an environmental and agricultural perspective, providing acceptable yields and contributing to the buildup of beneficial nutrients in the soil profile. Additional testing is needed to further evaluate potential germination inhibitors, greenhouse gas emissions (ex., N_2O), and efficacy when applied to different crops and soil types.

Gel-forming properties of pectins from callus culture of *Lemna minor*

Gyunter, EA; Popeiko, OV (2021) Chemistry of Natural Compounds 57: 799-802

A pectin fraction (LMC-I) with the best gel-forming properties as compared to LMC was obtained from *Lemna minor* L. callus culture and characterized by separation of starting pectin LMC through ultrafiltration membranes. Hydrogels based on pectin LMC-I of molecular mass > 300 kDa with a lower degree of methylation (11.9%) and a greater content of galacturonic acid (68%) were stronger regardless of the pectin and $CaCl_2$ contents. The hydrogels became stronger if the concentration of pectin or cross-linking agent $CaCl_2$ was increased.

Techno-economic analysis and life cycle assessment of an integrated wastewater-derived duckweed Biorefinery

Calicioglu, O; Femeena, PV; Mutel, CL; Sills, DL; Richard, TL; Brennan, RA (2021) ACS Sustainable Chemistry and Engineering 9: 9395-9408

Duckweeds are efficient aquatic plants for wastewater treatment due to their high nutrient uptake capabilities, growth rates, and resilience to severe environmental conditions. The high starch and cellulose contents of duckweed species make them an attractive feedstock for biofuels and biochemicals. Experimental studies have shown that sequential anaerobic bioprocessing of duckweed into ethanol, carboxylates, methane, and soil amendment in a biorefinery system is technically feasible. This study aims to identify challenges and opportunities for large-scale wastewater-derived duckweed biorefineries as a way to promote a circular bioeconomy. The most suitable end products from wastewater-derived duckweed biomass, determined in a series of previously reported laboratory batch experiments, were used to estimate the bioproduct yields during the hypothetical operation of a large-scale biorefinery. Techno-economic analysis (TEA) revealed a minimum duckweed selling price of \$7.69 Mg^{-1} dry matter and a minimum ethanol selling price of \$2.17/L or \$8.23 gal^{-1}. Duckweed pond construction and duckweed harvesting accounted for the largest share of capital (55.6%) and operating expenses (90.4%), respectively. A cradle-to-gate life cycle assessment (LCA) revealed that duckweed pond construction led to increased land use change impacts, but water-quality and eutrophication impacts could be significantly reduced with this integrated system through efficient nutrient upcycling.

Biogenic ZnO nanoparticles synthesized using a novel plant extract: Application to enhance physiological and biochemical traits in maize

Del Buono, D; Di Michele, A; Costantino, F; Trevisan, M; Lucini, L (2021) Nanomaterials 11: 1270

The need to increase crop productivity and resistance directs interest in nanotechnology. Indeed, biogenic metal oxide nanoparticles can promote beneficial effects in plants, while their synthesis avoids the environmental impacts of conventional synthetic procedures. In this context, this research aimed to synthesize biogenic zinc oxide nanoparticles (ZnO-NPs) using, for the first time, an extract of a wild and spontaneous aquatic species, *Lemna minor* (duckweed). The effectiveness of this biogenic synthesis was evidenced for comparison with non-biogenic ZnO-NPs (obtained without using the plant extract), which have been synthesized in this research. XRD (X-ray diffraction), FE-SEM (field emission gun electron scanning microscopy), EDX (energy dispersive x-ray spectroscopy), TEM (transmission electron microscope) and UV-vis (ultraviolet-visible spectrophotometry) showed the biogenic approach effectiveness. The duckweed extract was subjected to UHPLC-ESI/QTOF-MS (ultra high-pressure liquid chromatography quadrupole time of flight mass spectrometry) phenolic profiling. This untargeted characterization highlighted a high and chemically diverse content in the duckweed extract of compounds potentially implicated in nanoparticulation. From an application standpoint, the effect of biogenic nanoparticles was investigated on some traits of maize subjected to seed priming with a wide range of biogenic ZnO-NPs concentrations. Inductive effects on the shoot and root biomass development were ascertained concerning the applied dosage. Furthermore, the biogenic ZnO-NPs stimulated the content of chlorophylls, carotenoids, and anthocyanin. Finally, the study of malondialdehyde content (MDA) as a marker of the oxidative status further highlighted the beneficial and positive action of the biogenic ZnO-NPs on maize.

Ecology

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, Y; Sonne, C; Tabatabaei, M; Aghbashlo, M; Lam, SS (2021) Journal of Hazardous Materials 424:127329

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*104-4.20*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.

Feed & Food

Measuring the effect of Mankai(R) (*Wolffia globosa*) on the gut microbiota and its metabolic output using an in vitro colon model

Diotallevi, C; Gaudioso, G; Fava, F; Angeli, A; Lotti, C; Vrhovsek, U; Rinott, E; Shai, I; Gobbetti, M; Tuohy, K (2021) Journal of Functional Food 84: 104597

Mankai(R) is a cultivated strain of *Wolffia globosa* an aquatic plant of the family Lemnaceae commonly known as Duckweeds. Recent studies suggest that consumption of a Mankai(R) enriched diet may provide positive health effects by decreasing body weight and improving glucose homeostasis and plasma lipid profiles.

However, the effects of Mankai(R) alone on the composition and metabolic output of the human gut microbiota has not been fully investigated. Here, Mankai(R) was digested and fermented in vitro using a batch culture model of the proximal colon. Inulin and cellulose were used as readily and poorly fermentable control fibers respectively. Mankai(R) significantly stimulated the production of phenolic metabolites and short chain fatty acids by the gut microbiota ($p<0.05$). Three major microbial metabolites, 3-4-hydroxyphenyl propionic acid, 3-3-hydroxyphenyl propanoic acid and protocatechuic acid were significantly increased after 24 h fermentation. Moreover, Mankai(R) treatment lowered the overall microbial diversity ($p<0.05$), in line with a selective microbiome modulation.

A controlled human intervention trial to study protein quality by amino acid uptake kinetics with the novel *Lemna* protein concentrate as case study

Mes, JJ; Esser, D; Oosterink, E; van den Dool, RTM; Engel, J; de Jong, GAH; Wehrens, R; van der Meer, IM (2021) International Journal of Food Science and Nutrition DOI: 10.1080/09637486.2021.1960958

A human intervention trial was conducted to study amino acid uptake of the novel *Lemna* protein concentrate (LPC) in comparison to whey (WPC). The study was a cross-over, double-blind, controlled trial in which 12 healthy participants received 20 g of LPC and WPC in randomised order. The LPC consumption resulted in a significant lower postprandial increase in almost all individual amino acids, total amino acid (TAA) and total essential amino acids (TEAA) compared to WPC based on area under the curve (AUC) calculations. When the AUC after WPC consumption was set at 100%, LPC showed a relative AUC of 60.4% for TAA and 66.3% for the TEAA. Interindividual variation for LPC was high with an uptake of TEAA of LPC compared to WPC ranging from 18.2 to 94.2%. Human intervention trials can partly replace animal trials as they fully reflect the human situation and provide estimates on individual variations.

Sustainability analysis of fish feed derived from aquatic plant and insect

Goyal, S; Ott, D; Liebscher, J; Hofling, D; Muller, A; Dautz, J; Gutzeit, HO; Schmidt, D; Reuss, R (2021) Sustainability 13: 7371

Fish and meat production and processing will grow drastically in the coming decades. In aquacultural systems, insects are gaining interest as feed to provide a sustainable alternative to the fishmeal paradox, whose production leads to high consumption of resources and negative environmental impacts. Within the scope of this study, the production of fish feed from *Hermetia illucens* larvae and *Lemna minor* in an inline recirculating aquaponics model for urban sites was developed and optimized, which efficiently combines waste and environmental service concepts in one production system. At the same time, the value chain produces high-quality, market-accessible raw materials for the fish feed industry. All investigations were accompanied by a comparative Life Cycle Assessment (LCA) to measure and compare ecological effects to finally result in sustainable alternatives. The results achieved in this research show that fish feed based on *H. illucens* and *L. minor* can have the potential to be ecologically competitive or more sustainable than standard feed. It should be noted that the comparison here represents the results of the project on a pilot scale. Various optimization potentials were shown, which are essential for the large-scale implementation of the breeding of both species as well as their processing up to the fish feed pellets.

Does ingestion of duckweed (*Lemna minor*) improve the growth and productive performance of juvenile Nile tilapia (Oreochromis niloticus) given formulated feeds in a recirculation system?

Cipriani, LA; Ha, N; de Oliveira, NS; Fabregat, TEP (2021) Aquaculture International 29: 2197-2205

The present study evaluated the effect of voluntary ingestion of duckweed (*Lemna minor*) on the growth and productive performance of Nile tilapia (*Oreochromis niloticus*) reared in a recirculation system (RAS). The constant availability of fresh *Lemna* was compared with a control treatment without supplementation. In both

treatments, the fish were fed a commercial feed for 28 days. The experimental design was completely randomized with two treatments and seven replications. Nile tilapia juveniles (21.95±0.22 g) were acclimated in 14 tanks (70 l) with a density of 10 fish per tank. All fish were fed daily with an extruded commercial diet (32% CP), three times a day, until apparent satiety. An amount of *Lemna* was manually collected daily, washed, drained, and treated with absorbent paper to remove excess surface water, and then weighed and supplied to the tilapia tanks. Daily consumptions of feed and *Lemna* were quantified. At the beginning of the experiments and after 28 days, all fish were fasted for 24 h, then anesthetized with eugenol (50 mg l^{-1}), and weighed individually. Nile tilapia juveniles grown in a RAS can voluntarily ingest up to 0.5% of live weight in fresh *Lemna* with no effects on growth and productive performance. The amount of *Lemna* consumed decreased over time, from 0.5 to 0.2% of body weight. The fish that were exposed to *Lemna* consumed less feed in the first week of the experiment, but this reduction was compensated over time with no impact on the total feed consumption (478.59 ±21.74 g).

History

History of discovery of the fastest growing angiosperm, *Wolffia microscopica* (Griff.) Kurz entwined with British India

Sree, KS; Maheshwari, SC; Appenroth, KJ; Khurana, JP (2021) Current Science 121: 724-726

Wolffia microscopica (Griff.) Kurz, the fastest multiplying angiosperm, belongs to the family Lemnaceae (the duckweed family) and characteristically exhibits frequent flowering. Interestingly, the discovery of this plant species originally designated as *Grantia microscopica* by William Griffith (1810-45) is interlinked with the ascent of the British in India. In this note, a historic account of the discovery, nomenclature and uniqueness of this species of duckweed endemic to the Indian subcontinent is presented in view of its gaining attention as a potential bioresource when there is resurgence in duckweed research globally for its utility as a model plant for both basic and applied studies.

Interaction with other organisms

Microalgae biofilm formation and antioxidant responses to stress induce by *Lemna minor* L., *Chlorella vulgaris*, and *Aphanizomenon flos-aquae*

Ugya, AY; Ari, HA; Hua, XY (2021) Ecotoxicology and Environmental Safety 221: 112468

The study shows how microalgae biofilm formation and antioxidant responses to the production of reactive oxygen species (ROS) is alter by the presences of *Lemna minor* L., *Chlorella vulgaris*, and *Aphanizomenon flos-aquae*. The study involves the cultivation of the biofilm of *Chlorella vulgaris* and *Aphanizomenon flos-aquae* in three bioreactors. The condition of growth for the biofilm formation was varied across the three bioreactors to enable the dominance *Chlorella vulgaris* and *Aphanizomenon flos-aquae* in one of the bioreactors. *Lemna minor* L. was also introduce into one of the bioreactors to determine its effect on the biofilm formation. The result obtained shows that *C. vulgaris* and *A. flos-aquae* dominate the biofilm, resulting in a high level of H_2O_2 and O_2^- (H_2O_2 was 0.122±0.052 and 0.183±0.108 mmol/L in *C. vulgaris* and *A. flos-aquae*, respectively, and O_2^- was 0.261±0.039 and 0.251±0.148 mmol/L in *C. vulgaris* and *A. flos-aquae*, respectively). The study also revealed that the presence of *L. minor* L. tend to reduce the oxidative stress to the biofilm leading to low production of ROS (H_2O_2 was 0.086±0.027 and 0.089±0.045 mmol/L in *C. vulgaris* and *A. flos-aquae* respectively, and O_2^- was 0.185±0.044 and 0.161±0.065 mmol/L in *C. vulgaris* and *A. flos-aquae* respectively). The variation in the ability of the biofilm of *C. vulgaris* and *A. flos-aquae* to respond via chlorophyll, carotenoid, flavonoid, anthocyanin, superoxide dismutase, peroxidase, catalase, glutathione reductase activities, antioxidant reducing power, phosphomolybdate activity, DPPH reduction activity, H_2O_2 scavenging activity, lipid content and organic carbon also supports the fact that the presence of biomass of microalgae and aquatic macrophytes tend to affect the process of microalgae biofilm formation and the ability of the biofilm to

produce antioxidant. This high nutrient utilization leads to the production of biomass which can be used for biofuel production and other biotechnological products.

Microplastics shift impacts of climate change on a plant-microbe mutualism: Temperature, CO_2, and tire wear particles

O'Brien, AM; Lins, TF; Yang, Y; Frederickson, ME; Sinton, D; Rochman, CM (2021) Environmental Research 203:111727

Anthropogenic stressors can affect individual species and alter species interactions. Moreover, species interactions or the presence of multiple stressors can modify the stressor effects, yet most work focuses on single stressors and single species. Plant-microbe interactions are a class of species interactions on which ecosystems and agricultural systems depend, yet may be affected by multiple global change stressors. Here, we use duckweed and microbes from its microbiome to model responses of interacting plants and microbes to multiple stressors: climate change and tire wear particles. Climate change is occurring globally, and microplastic tire wear particles from roads now reach many ecosystems. We paired perpendicular gradients of temperature and carbon dioxide (CO_2) treatments with factorial manipulation of leachate from tire wear particles and duckweed microbiomes. We found that tire leachate and warmer temperatures enhanced duckweed and microbial growth, but caused effects of microbes on duckweed to become negative. However, induced negative effects of microbes were less than additive with warming and leachate. Without tire leachate, we observed that higher CO_2 and temperature induced positive correlations between duckweed and microbial growth, which can strengthen mutualisms. In contrast, with tire leachate, growth correlations were never positive, and shifted negative at lower CO_2, again suggesting leachate disrupts this plant-microbiome mutualism. In summary, our results demonstrate that multiple interacting stressors can affect multiple interacting species, and that leachate from tire wear particles could potentially disrupt plant-microbe mutualisms.

Antifungal and antioxidant potential of methanolic extracts from *Acorus calamus* L., *Chlorella vulgaris* Beijerinck, *Lemna minuta* Kunth and *Scenedesmus dimorphus* (Turpin) Kutzing

Dinev, T; Tzanova, M; Velichkova, K; Dermendzhieva, D; Beev, G (2021) Applied Sciences 11: 4745

Plant extracts are an important alternative to antibiotics, which are ever more restricted because of their developing microbial resistance and some adverse effects that have been observed following frequent application. The aim of the present study was to determine the antifungal and antioxidant activity of the methanolic extracts of *Acorus calamus*, *Chlorella vulgaris*, *Lemna minuta* and *Scenedesmus dimorphus*. The antifungal activity of the extracts against strains of *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus carbonarius*, *Fusarium graminearum*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Alternaria alternata* was evaluated via the agar well diffusion method. The antioxidant activity of the extracts was measured through the determination of three parameters-total phenolic content, total flavonoid content and radical scavenging potential (determined through UV/Vis analysis). *A. calamus* extracts had the highest antimicrobial activity against eight fungal strains, followed by the *C. vulgaris*, *L. minuta* and *S. dimorphus* extracts, which were inhibitory against two to three strains. Among the extracts from the species studied, the extract from *S. dimorphus* showed the highest antioxidant potential, as determined via the DPPH (1,1'-diphenyl-2-picrylhydrazil-radical) method. This correlated to its high total phenolic and flavonoid content. From *A. calamus* and *L. minuta*, methanolic extracts were obtained that exhibited similar values of the aforementioned parameters, followed by *C. vulgaris* extracts, which showed the lowest antioxidant activity. Based on the Pearson correlation coefficients, the impacts of the total phenolic content and the total flavonoid content on radical scavenging capacity are similar, and flavonoids were a significant part of the total phenolic compounds extracted from the plant materials studied.

Eco-evolutionary interaction between microbiome presence and rapid biofilm evolution determines plant host fitness

Tan, JQ; Kerstetter, JE; Turcotte, MM (2021) Nature Ecology and Evolution 5: 670

The authors demonstrate eco-evolutionary dynamics between the microbiome and a constituent member, which jointly affect fitness in the host plant. Microbiomes are important to the survival and reproduction of their hosts. Although ecological and evolutionary processes can happen simultaneously in microbiomes, little is known about how microbiome eco-evolutionary dynamics determine host fitness. Here we show, using experimental evolution, that fitness of the aquatic plant *Lemna minor* is modified by interactions between the microbiome and the evolution of one member, *Pseudomonas fluorescens*. Microbiome presence promotes *P. fluorescens*' rapid evolution to form biofilm, which reciprocally alters the microbiome's species composition. These eco-evolutionary dynamics modify the host's multigenerational fitness. The microbiome and non-evolving *P. fluorescens* together promote host fitness, whereas the microbiome with *P. fluorescens* that evolves biofilm reduces the beneficial impact on host fitness. Additional experiments suggest that the microbial effect on host fitness may occur through changes in microbiome production of auxin, a plant growth hormone. Our study, therefore, experimentally demonstrates the importance of the eco-evolutionary dynamics in microbiomes for host-microbiome interactions.

Molecular Biology

Genome of the world's smallest flowering plant, *Wolffia australiana*, helps explain its specialized physiology and unique morphology

Park, H; Park, JH; Lee, Y; Woo, DU; Jeon, HH; Sung, YW; Shim, S; Kim, SH; Lee, KO; Kim, JY, Kim, CK; Bhattacharya, D; Yoon, HS; Kang, YJ (2021) Communications Biology 4: 900

Watermeal, *Wolffia australiana*, is the smallest known flowering monocot and is rich in protein. Despite its great potential as a biotech crop, basic research on *Wolffia* is in its infancy. Here, we generated the reference genome of a species of watermeal, *W. australiana*, and identified the genome-wide features that may contribute to its atypical anatomy and physiology, including the absence of roots, adaxial stomata development, and anaerobic life as a turion. In addition, we found evidence of extensive genome rearrangements that may underpin the specialized aquatic lifestyle of watermeal. Analysis of the gene inventory of this intriguing species helps explain the distinct characteristics of *W. australiana* and its unique evolutionary trajectory. Halim Park and Jin Hwa Park et al. report the nuclear genome sequence of the duckweed *Wolffia australiana*, the smallest known flowering plant. The genome assembly represents an improvement over a recently published genome and highlights genome rearrangements that may be linked to its specialized aquatic adaptations.
DF: For high quality genome draft see Michael, TP et al. Genome Research 31: 1-14 (2021)

Mosaic arrangement of the 5S rDNA in the aquatic plant *Landoltia punctata* (Lemnaceae)

Chen, GM; Stepanenko, A; Borisjuk, N (2021) Frontiers in Plant Science 12: 678689

Duckweeds are a group of monocotyledonous aquatic plants in the Araceae superfamily, represented by 37 species divided into five genera. Duckweeds are the fastest growing flowering plants and are distributed around the globe; moreover, these plants have multiple applications, including biomass production, wastewater remediation, and making pharmaceutical proteins. Dotted duckweed (*Landoltia punctata*), the sole species in genus Landoltia, is one of the most resilient duckweed species. The ribosomal DNA (rDNA) encodes the RNA components of ribosomes and represents a significant part of plant genomes but has not been comprehensively studied in duckweeds. Here, we characterized the 5S rDNA genes in *L. punctata* by cloning and sequencing 25 PCR fragments containing the 5S rDNA repeats. No length variation was detected in the 5S rDNA gene sequence, whereas the nontranscribed spacer (NTS) varied from 151 to 524 bp. The NTS variants

were grouped into two major classes, which differed both in nucleotide sequence and the type and arrangement of the spacer subrepeats. The dominant class I NTS, with a characteristic 12-bp TC-rich sequence present in 3-18 copies, was classified into four subclasses, whereas the minor class II NTS, with shorter, 9-bp nucleotide repeats, was represented by two identical sequences. In addition to these diverse subrepeats, class I and class II NTSs differed in their representation of cis-elements and the patterns of predicted G-quadruplex structures, which may influence the transcription of the 5S rDNA. Similar to related duckweed species in the genus *Spirodela*, *L. punctata* has a relatively low rDNA copy number, but in contrast to *Spirodela* and the majority of other plants, the arrangement of the 5S rDNA units demonstrated an unusual, heterogeneous pattern in *L. punctata*, as revealed by analyzing clones containing double 5S rDNA neighboring units. Our findings may further stimulate the research on the evolution of the plant rDNA and discussion of the molecular forces driving homogenization of rDNA repeats in concerted evolution.

Identification, structure analysis, and transcript profiling of phosphate transporters under Pi deficiency in duckweeds

Zhao, XY; Li, GJ; Sun, ZL; Chen, Y; Guo, WJ; Li, YX; Chen, YM; Yang, JJ; Hou, HW (2021) International Journal of Biological Macromolecules 188: 595-608

Phosphate transporters (PHTs) mediate the uptake and translocation of phosphate in plants. A comprehensive analysis of the PHT family in aquatic plant is still lacking. In this study, we identified 73 PHT members of six major PHT families from four duckweed species. The phylogenetic analysis, gene structure and protein characteristics analysis revealed that PHT genes are highly conserved among duckweeds. Interaction network and miRNA target prediction showed that SpPHTs could interact with the important components of the nitrate/ phosphate signaling pathway, and spo-miR399 might be a central regulator that mediates phosphate signal network in giant duckweed (*Spirodela polyrhiza*). The modeled 3D structure of SpPHT proteins shared a high level of homology with template structures, which provide information to understand their functions at proteomic level. The expression profiles derived from transcriptome data and quantitative real-time PCR revealed that SpPHT genes are respond to exogenous stimuli and remarkably induced by phosphate starvation, phosphate is absorbed from aquatic environment by the whole duckweed plant. This study lays the foundation for further functional studies on PHT genes for genetic improvement and the promotion of phosphate uptake efficiency in duckweeds.

Agrobacterium-mediated genetic transformation of Taiwanese Isolates of *Lemna aequinoctialis*

Wang, KT; Hong, MC; Wu, YS; Wu, TM (2021) Plants 10: 1576

Duckweed (*Lemna aequinoctialis*) is one of the smallest flowering plants in the world. Due to its high reproduction rate and biomass, duckweeds are used as biofactors and feedstuff additives for livestock. It is also an ideal system for basic biological research and various practical applications. In this study, we attempt to establish a micropropagation technique and Agrobacterium-mediated transformation in *L. aequinoctialis*. The plant-growth regulator type and concentration and Agrobacterium-mediated transformation were evaluated for their effects on duckweed callus induction, proliferation, regeneration, and gene transformation efficiency. Calli were successfully induced from 100% of explants on Murashige and Skoog (MS) medium containing 25.0 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 μM thidiazuron (TDZ). MS medium containing 4.5 μM 2,4-D and 2.0 μM TDZ supported the long-lasting growth of calli. Fronds regenerated from 100% of calli on Schenk and Hildebrandt (SH) medium containing 1.0 μM 6-benzyladenine (6-BA). We also determined that 200 μM acetosyringone in the cocultivation medium for 1 day in the dark was crucial for transformation efficiency (up to 3±1%). Additionally, we propose that both techniques will facilitate efficient high-throughput genetic manipulation in Lemnaceae.

Cloning and expression pattern of phosphate transporter 1;1 cDNA sequence from *Spirodela polyrhiza* (in Chinese)

Deng, Z; Peng, W; Lu, Z; Fu, M (2021) Sheng wu gong cheng xue bao = Chinese Journal of Biotechnology 37:2474-2482. DOI:10.13345/j.cjb.200567

Spirodela polyrrhiza is a floating plant widely used in biomass utilization and eutrophication phytoremediation. It becomes a common aquatic plant everywhere with the increasingly serious eutrophication. It has been reported that *S. polyrrhiza* has a good effect on the remediation of eutrophication water. In order to study the absorption and transportation of phosphorus in *S. polyrrhiza*, we extracted RNA from *S. polyrrhiza* and then reverse transcribed it into cDNA, which was used as a template to amplify a specific fragment. The full-length sequence of the open reading frame (ORF) was 1 620 bp, encoding 539 amino acids, named SpPHT1;1, and the accession number in GenBank was MN720003. Bioinformatical analysis showed that SpPHT1;1 had no intron. The protein it encoded was a stable, hydrophobic protein with 11 transmembrane domains. SpPHT1;1 structure was similar to that of major facilitator superfamily (MFS) superfamily members. The cluster analysis showed that SpPHT1;1 was closely related to ZMPHT2 in maize and SBPHT1-8 in sorghum. So, it might belong to plant PHT1 family. The expression of SpPHT1;1 in leaf was significantly more than that of root under normal phosphorus condition. Low phosphorus condition could promote gene expression, and the relative expression level of SpPHT1;1 arrived at the peak at 48 h both in root and leaf. High phosphorus condition could inhibit gene expression. These results indicated that SpPHT1;1 expression would be affected by external phosphorus concentration. The results of this study are helpful for further research on the function of phosphate transporter. It also can provide theoretical basis for further development and utilization of *S. polyrrhiza*.

DF: The correct spelling of the duckweed species is Spirodela polyrhiza

Genome-wide identification and comparative analysis of the WRKY gene family in aquatic plants and their response to abiotic stresses in giant duckweed (*Spirodela polyrhiza*)

Zhao, XY; Yang, JJ; Li, GJ; Sun, ZL; Hu, SQ; Chen, Y; Guo, WJ; Hou, HW (2021) Genomics 113: 1761-1777

WRKY is one of the largest transcription factor families across higher plant species and is involved in important biological processes and plant responses to various biotic/abiotic stresses. However, only a few investigations on WRKYs have been conducted in aquatic plants. This study first systematically analyzed the gene structure, protein properties, and phylogenetic relationship of 693 WRKYs in nine aquatic and two wetland plants at the genome-wide level. The pattern of WRKY groups in two aquatic ferns provided new evidence for the origin and evolution of WRKY genes. ARE cis-regulatory elements show an unusual high frequency in the promoter region of WRKY genes, indicating the adaptation to the aquatic habitat in aquatic plants. The WRKY gene family experienced a series of gene loss events in aquatic plants, especially group III. Further studies were conducted on the interaction network of SpWRKYs, their target genes, and non-coding RNAs. The expression profile of SpWRKYs under phosphate starvation, cold, and submergence conditions revealed that most SpWRKYs are involved in the response to abiotic stresses. Our investigations lay the foundation for further study on the mechanism of WRKYs responding to abiotic stresses in aquatic plants.

Morphology

Frond architecture of the rootless duckweed *Wolffia globosa*

Yang, JJ; Zhao, XY; Li, GJ; Hu, SQ; Hou, HW (2021) BMC Plant Biology 21: 387

The plant body in duckweed species has undergone reduction and simplification from the ancient *Spirodela* species towards more derived *Wolffia* species. Among the five duckweed genera, *Wolffia* members are rootless and represent the smallest and most reduced species. A better understanding of *Wolffia* frond architecture is necessary to fully explore duckweed evolution. We conducted a comprehensive study of the morphology and

anatomy of *Wolffia globosa*, the only Wolffia species in China. We first used X-ray microtomography imaging to reveal the three-dimensional and internal structure of the *W. globosa* frond. This showed that new fronds rapidly budded from the hollow reproductive pocket of the mother fronds and that several generations at various developmental stages could coexist in a single *W. globosa* frond. Using light microscopy, we observed that the meristem area of the *W. globosa* frond was located at the base of the reproductive pocket and composed of undifferentiated cells that continued to produce new buds. A single epidermal layer surrounded the *W. globosa* frond, and the mesophyll cells varied from small and dense palisade-like parenchyma cells to large, vacuolated cells from the ventral to the dorsal part. Furthermore, *W. globosa* fronds contained all the same organelles as other angiosperms; the most prominent organelles were chloroplasts with abundant starch grains. Our study revealed that the reproductive strategy of *W. globosa* plants enables the rapid accumulation of biomass and the wide distribution of this species in various habitats. The reduced body plan and size of *Wolffia* are consistent with our observation that relatively few cell types are present in these plants. We also propose that *W. globosa* plants are not only suitable for the study of structural reduction in higher plants, but also an ideal system to explore fundamental developmental processes of higher plants that cannot be addressed using other model plants.

Physiology & Stress

Features of the duckweed *Lemna* that support rapid growth under extremes of light Intensity

Stewart, JJ; Adams, WW; Lopez-Pozo, M; Garcia, ND; McNamara, M; Escobar, CM; Demmig-Adams, B (2021) Cells 10: 1481

This study addresses the unique functional features of duckweed via comparison of *Lemna gibba* grown under controlled conditions of 50 versus 1000 µmol photons m^{-2} s^{-1} and of a *L. minor* population in a local pond with a nearby population of the biennial weed *Malva neglecta*. Principal component analysis of foliar pigment composition revealed that *Malva* was similar to fast-growing annuals, while *Lemna* was similar to slow-growing evergreens. Overall, *Lemna* exhibited traits reminiscent of those of its close relatives in the family Araceae, with a remarkable ability to acclimate to both deep shade and full sunlight. Specific features contributing to duckweed's shade tolerance included a foliar pigment composition indicative of large peripheral light-harvesting complexes. Conversely, features contributing to duckweed's tolerance of high light included the ability to convert a large fraction of the xanthophyll cycle pool to zeaxanthin and dissipate a large fraction of absorbed light non-photochemically. Overall, duckweed exhibited a combination of traits of fast-growing annuals and slow-growing evergreens with foliar pigment features that represented an exaggerated version of that of terrestrial perennials combined with an unusually high growth rate. Duckweed's ability to thrive under a wide range of light intensities can support success in a dynamic light environment with periodic cycles of rapid expansion.

Effect of salt stress on proximate composition of duckweed (*Lemna minor* L.)

Ullah, H; Gul, B; Khan, H; Zeb, U (2021) Heliyon 7: e07399

The shortage of conventional feedstuff is one of the rising issues faced by the developing countries of the world. To bridge the gap between supply and demand of the major feedstuff it is desirable to practice the use of non-conventional feed resources. Duckweeds are the aquatic macrophytes growing in stagnant water bodies that offer a choice to be used as an alternate feed. Before the use of any alternate feed, it is vital to know the nutritional composition of the feed under diverse environmental conditions. The objective of this study was to investigate the influence of salinity, abiotic stress, on the proximate composition of duckweed (*Lemna minor* L.). The experiment was laid out in Completely Randomized Design (CRD) with 3 repeats. Data was collected on protein, lipid, carbohydrate, and mineral contents. In the laboratory trial plants were grown under the saline condition of different concentrations ranging from 2 g NaCl L^{-1} to 12 g NaCl L^{-1} for a growing

period of 20 days. The biomasses obtained were tested for proximate composition. ANOVA of the result exhibited a significant effect of salinity on the proximate composition of the plant. Protein residues of the plant started declining above the concentration of 4 g NaCl L^{-1} until the lowest value was obtained at 12 g NaCl L^{-1}. Lipid composition showed more sensitivity to the stress with a sharp decline above 2 g NaCl L^{-1} having a minimum value at 12 g NaCl L^{-1}. Carbohydrate contents increased with increasing salinity up to 6 g NaCl L^{-1} above which a decrease was observed. The highest accumulation of the macronutrients i.e., Ca, Mg, took place in the lower range of concentration of the salt. The percentage compositions of micronutrients such as Fe, Mn, and Zn percentage were reduced at a higher range of salinity while the optimum level was recorded in plants treated with 2 g NaCl L^{-1}, followed by control. The total accumulation of both macro and micronutrients was higher in the plant material treated with a lower level of salt concentration, concluding a significant effect of salinity on proximate composition. As for the Indus water salinity level, the plant has the capacity of tolerance and can be grown without affecting its proximate composition.

Genome-wide identification of the Nramp gene family in *Spirodela polyrhiza* and expression analysis under cadmium stress

Chen, Y; Zhao, XY; Li, GJ; Kumar, S; Sun, ZL; Li, YX; Guo, WJ; Yang, JJ; Hou, HW (2021) International Journal of Molecular Sciences 22: 6414

Natural resistance-associated macrophage proteins (Nramps) are specific metal transporters in plants with different functions among various species. The evolutionary and functional information of the Nramp gene family in *Spirodela polyrhiza* has not been previously reported in detail. To identify the Nramp genes in *S. polyrhiza*, we performed genome-wide identification, characterization, classification, and cis-elements analysis among 22 species with 138 amino acid sequences. We also conducted chromosomal localization and analyzed the synteny relationship, promoter, subcellular localization, and expression patterns in *S. polyrhiza*. beta-Glucuronidase staining indicated that SpNramp1 and SpNramp3 mainly accumulated in the root and joint between mother and daughter frond. Moreover, SpNramp1 was also widely displayed in the frond. SpNramp2 was intensively distributed in the root and frond. Quantitative real-time PCR results proved that the SpNramp gene expression level was influenced by Cd stress, especially in response to Fe or Mn deficiency. The study provides detailed information on the SpNramp gene family and their distribution and expression, laying a beneficial foundation for functional research.

In silico analysis of glycosyltransferase 2 family genes in duckweed (*Spirodela polyrhiza*) and its role in salt stress tolerance

Jiang, ML; Wang, P; Xu, LG; Ye, XX; Fan, HX; Cheng, JX; Chen, JT (2021) Open Life Sciences 16: 583-593

Plant glycosyltransferase 2 (GT2) family genes are involved in plant abiotic stress tolerance. However, the roles of GT2 genes in the abiotic resistance in freshwater plants are largely unknown. We identified seven GT2 genes in duckweed, remarkably more than those in the genomes of *Arabidopsis thaliana*, *Oryza sativa*, *Amborella trichopoda*, *Nymphaea tetragona*, *Persea americana*, *Zostera marina*, and *Ginkgo biloba*, suggesting a significant expansion of this family in the duckweed genome. Phylogeny resolved the GT2 family into two major clades. Six duckweed genes formed an independent subclade in Clade I, and the other was clustered in Clade II. Gene structure and protein domain analysis showed that the lengths of the seven duckweed GT2 genes were varied, and the majority of GT2 genes harbored two conserved domains, PF04722.12 and PF00535.25. The expression of all Clade I duckweed GT2 genes was elevated at 0 h after salt treatment, suggesting a common role of these genes in rapid response to salt stress. The gene Sp01g00794 was highly expressed at 12 and 24 h after salt treatment, indicating its association with salt stress resilience. Overall, these results are essential for studies on the molecular mechanisms in stress response and resistance in aquatic plants.

Effects of environmental parameters on *Lemna minor* growth: An integrated experimental and modelling approach

Van Dyck, I; Vanhoudt, N; Vives I Batlle, J; Horemans, N; Nauts, R; Van Gompel, A; Claesen, J; Vangronsveld, J (2021) Journal of Environmental management 300:113705

Pollution of surface waters is a worldwide problem for people and wildlife. Remediation and phytoremediation approaches can offer a solution to deal with specific scenarios. *Lemna minor*, commonly known as duckweed, can absorb and accumulate pollutants in its biomass. To evaluate if *L. minor* could be applied for phytoremediation purposes, it is necessary to further investigate its remediation capability and to identify which parameters affect the remediation process. Such a model must include both plant growth and pollutant exchange. A remediation model based on a robust experimental study can help to evaluate *L. minor* as a proper remediation strategy and to predict the outcome of a *L. minor* based remediation system. To set up this model, this paper focusses on a detailed experimental study and a comprehensive mathematical modelling approach to represent *L. minor* growth as a function of biomass, temperature, light irradiation and variable nutrient concentrations. The influence of environmental conditions on *L. minor* growth was studied, by composing 7 days growth curves. Plants were grown under predefined environmental conditions (25°C, 14h photoperiod, 220 µmol m^{-2} s^{-1} light intensity and a modified Hoagland solution with 23.94 mgN L^{-1} and 3.10 mgP L^{-1} (N:P ratio of 7.73)) as standard for all experiments. The influence of different temperatures (6, 10, 15, 20, 25, 30 and 35°C), light intensities (63, 118, 170, 220 and 262 µmol m^{-2} s^{-1}), photoperiods (12h and 14h) and N:P ratios (1.18, 3.36, 7.73 and 29.57) were tested in the model. As a result, a growth model was optimised using separate data sets for temperature, light intensity, photoperiod and nutrients and validated by further integrated testing. The growth model is a stable platform for application in phytoremediation of radionuclides in contaminated water, to be extended in future studies with information of pollutant uptake, pollutant-nutrient interactions and transfer to the biomass.

Effects of light source and photoperiod on growth of duckweed *Landoltia punctata* and its water quality

Gallego, LM; Chien, YH; Angeles, IP (2021) Aquaculture Research DOI: 10.1111/are.15581

This study investigated the effects of light source [LED white (LW), fluorescent white (T5) and LED blue (LB)] and photoperiod (12:12, 16:08, 24:00 light/dark) on growth of duckweed *Landoltia punctata* and the resulting effects on its water quality for 16 days. The average daily relative growth rate (RGR) reached about 0.519 g d^{-1}. Both light source and photoperiod had no significant difference on the mean RGR; however, their interaction had significant effects on duckweed's growth ($p \leq 0.05$). Moreover, except T5 (24:00) > [T5 (12:12) ≥ T5 (16:08) ≥ LB (24:00)], LW (12:12) > [T5 (16:08) ≥ LB (24:00)], and LB (12:12) > LB (24:00), there were no differences in RGR in all pair-comparisons of treatment ($p \leq 0.05$). Nitrate (NO_3^-) mostly influenced weight increment (WI), 70%. For light source and photoperiod effects on water quality, no total ammonia nitrogen (TAN) was detected in all treatments after 16 days, while NO_3^- increased gradually. In addition, results show that most of the total nitrogen (TN) was contributed from NO_3^- (R^2 = 0.9999). Overall, our findings could contribute on producing duckweed in a controlled and programmed condition for maximum production and quality. Constructed models and practical application contribute in predicting nutrients sensitivity and proven useful in water management or water quality assessments.

Green light attenuates blue-light-induced chloroplast avoidance movement in *Arabidopsis* and *Landoltia punctata*

Schmalstig, JG; Jainandan, K (2021) American Journal of Botany 108: 1525-1539

Premise Chloroplast movement to the anticlinal walls in excess light, referred to as chloroplast avoidance movement, is one strategy to prevent high light damage. Chloroplast avoidance movement is mediated by the blue-light photoreceptor phototropin. Since some blue-light effects are reversed by green light, we investigated the effect of green wavelengths on chloroplast avoidance. Methods Chloroplast position was visualized via microscopy and by transmission of red light through the leaves of *Arabidopsis thaliana* and *Landoltia punctata*

(duckweed). Results Green light reduced blue-light-induced chloroplast avoidance movement but only when green light was presented simultaneously with blue light. Green light alone had no effect on chloroplast position. An action spectrum for green-light attenuation of chloroplast avoidance in duckweed revealed peaks at 510, 550, and 590 nm. Blue-light-induced chloroplast avoidance movement in three Arabidopsis mutants with reduced nonphotochemical quenching, npq1, npq4, and npq7 was not affected by green light. Conclusions The action spectrum does not conform to any known photoreceptor. The lack of a green-light response in the npq mutants of Arabidopsis suggests a possible role for the xanthophyll cycle or a signal from the chloroplast in control of chloroplast avoidance movement.

Brassinolide enhances the level of brassinosteroids, protein, pigments, and monosaccharides in *Wolffia arrhiza* treated with brassinazole

Chmur, M; Bajguz, A (2021) Plants 10: 1311

Brassinolide (BL) represents brassinosteroids (BRs)-a group of phytohormones that are essential for plant growth and development. Brassinazole (Brz) is as a synthetic inhibitor of BRs' biosynthesis. In the present study, the responses of *Wolffia arrhiza* to the treatment with BL, Brz, and the combination of BL with Brz were analyzed. The analysis of BRs and Brz was performed using LC-MS/MS. The photosynthetic pigments (chlorophylls, carotenes, and xanthophylls) levels were determined using HPLC, but protein and monosaccharides level using spectrophotometric methods. The obtained results indicated that BL and Brz influence *W. arrhiza* cultures in a concentration-dependent manner. The most stimulatory effects on the growth, level of BRs (BL, 24-epibrassinolide, 28-homobrassinolide, 28-norbrassinolide, catasterone, castasterone, 24-epicastasterone, typhasterol, and 6-deoxytyphasterol), and the content of pigments, protein, and monosaccharides, were observed in plants treated with 0.1 mu MBL. Whereas the application of 1 μM and 10 μM Brz caused a significant decrease in duckweed weight and level of targeted compounds. Application of BL caused the mitigation of the Brz inhibitory effect and enhanced the BR level in duckweed treated with Brz. The level of BRs was reported for the first time in duckweed treated with BL and/or Brz.

High starch accumulation mechanism and phosphorus utilization efficiency of duckweed (*Landoltia punctata*) under phosphate starvation

Li, JM; Du, AP; Liu, PH; Tian, XP; Jin, YL; Yi, ZL; He, KZ; Fang, Y; Zhao, H (2021) Industrial Crops and Products 167: 113529

Phosphorus is an essential element for plant growth and reproduction. This study aimed to investigate the metabolic response, growth, and starch accumulation mechanisms in duckweed strain, Landoltia punctata 0202, under phosphate starvation. The results revealed that, under phosphate starvation, the total phosphorus in L. punctata 0202 decreased, while total carbon increased. After 15 days of phosphate starvation, biomass yield increased from 12.64 to123.87 g m^{-2}, while phosphorus utilization efficiency increased to 761.78 g g^{-1}. The starch content accumulated from 2.14 to 38.05 % by day 15, and the starch yield reached 47.14 g m^{-2} on day 15. Furthermore, biochemical and transcriptome analysis results showed a sharp increase in ADP-glucose pyrophosphorylase activity and the expression of genes encoding granule starch synthase and starch branching enzyme under phosphate starvation supported starch accumulation in *L. punctata* 0202. Additionally, large amounts of high-affinity phosphate transporters, vacuolar phosphate efflux transporters, and purple acid phosphatases were expressed. In summary, based on multi-level physiological and biochemical results, transcriptomic analyses, and preliminary analysis of the phosphate efflux transporter protein function, this study revealed the mechanism of starch accumulation induced by phosphate starvation and high-efficiency phosphate recycling in *L. punctata*. These findings offer an important foundation and insight into the molecular mechanisms influencing the uptake and utilization of nutrients. The screened functional genes exhibited potential for crop improvements under stressful environmental conditions.

Phytoremediation

Efficacy of *Lemna minor* and *Typha latifolia* for the treatment of textile industry wastewater in a constructed wetland under citric acid amendment: A lab scale study

Ishaq, HK; Farid, M; Zubair, M; Alharby, HF; Asam, ZU; Farid, S; Bamagoos, AA; Alharbi, BM; Shakoor, MB; Ahmad, SR; Rizwan, M; Ali, S (2021) Chemosphere 283: 131107

Lead (Pb), copper (Cu) and chromium (Cr) are one of the most harmful heavy metals (HMs), entering into the food chain through the irrigation of crops with an industrial effluent. The present study was performed to evaluate the toxic effects of textile effluents and performance of citric acid (CA) on phytoextraction potential *of Lemna minor* L. and *Typha latifolia* L. in an artificially designed wetland. Different doses of textile wastewater (0, 25, 50, 75, and 100%) and CA (10 mM) were applied alone and in combination. Plants were harvested and the data was collected regarding agronomic traits, photosynthetic pigments, antioxidant enzymes, reactive oxygen species (ROS), electrolytic leakage (EL) and HMs uptake and accumulation. The results depicted that the concentration and accumulation of Cu, Pb and Cr in different parts of *T. latifolia* plant was increased with and without CA addition. The maximum concentration of Pb, Cu and Cr increased in leaves by 279, 240 & 171%, in stem by 192, 172 & 154%, and in roots by 224, 183 & 168%, respectively. Similarly, the accumulation of Pb, Cu and Cr increased in leaves by 91, 71 & 36%, in stem by 57, 46 & 36% and in roots by 76, 53 & 45%, respectively in plants treated with 100% textile effluent as compared to the 25% textile effluent treated plants under CA amendment. In *L. minor*, the concentration of Pb, Cu & Cr increased by 542, 411 and 397% while accumulation increased by 101, 59 & 55% respectively in overall plant biomass.

Stigmasterol root exudation arising from Pseudomonas inoculation of the duckweed rhizosphere enhances nitrogen removal from polluted waters

Lu, YF; Kronzucker, HJ; Shi, WM (2021) Environmental Pollution 287: 117587

Rhizospheric microorganisms such as denitrifying bacteria are able to affect 'rhizobioaugmention' in aquatic plants and can help boost wastewater purification by benefiting plant growth, but little is known about their effects on the production of plant root exudates, and how such exudates may affect microorganismal nitrogen removal. Here, we assess the effects of the rhizospheric Pseudomonas inoculant strain RWX31 on the root exudate profile of the duckweed *Spirodela polyrhiza*, using gas chromatography/mass spectrometry. Compared to untreated plants, inoculation with RWX31 specifically induced the exudation of two sterols, stigmasterol and p-sitosterol. An authentic standard assay revealed that stigmasterol significantly promoted nitrogen removal and biofilm formation by the denitrifying bacterial strain RWX31, whereas beta-sitosterol had no effect. Assays for denitrifying enzyme activity were conducted to show that stigmasterol stimulated nitrogen removal by targeting nitrite reductase in bacteria. Enhanced N removal from water by stigmasterol, and a synergistic stimulatory effect with RWX31, was observed in open duckweed cultivation systems. We suggest that this is linked to a modulation of community composition of nirS- and nirK-type denitrifying bacteria in the rhizosphere, with a higher abundance of Bosea, Rhizobium, and Brucella, and a lower abundance of Rubrivivax. Our findings provide important new insights into the interaction of duckweed with the rhizospheric bacterial strain RWX31 and their involvement in the aquatic N cycle and offer a new path toward more effective bio-formulations for the purification of N-polluted waters.

The effect of chelating agents on iron plaques and arsenic accumulation in duckweed (*Lemna minor*)

Yang, GL; Yang, MX; Lv, SM; Tan, AJ (2021) Journal of Hazardous Materials 419: 126410

Iron plaques have been found to limit the phytoremediation efficiency by reducing iron solubility, while chelating agents can increase the bioavailability of iron from Fe plaques to numerous terrestrial plants. However, the effects of chelating agents on Fe plaques along the As accumulation in aquatic plants remain unknown. In this study, the effects of five chelating agents (EDTA, DTPA, NTA, GLDA, and CA) on the As (As(III) or As(V)), phosphate, and iron uptake by iron plaques and duckweed (*Lemna minor*) were examined. The results showed that the chelating agents increased the As accumulation in *L. minor* plants by desorbing and mobilizing As from Fe plaques. The desorption rates of As(V) (As(III)) from the Fe plaques by the chelating agents were 5.26-8.77% (8.70-15.02%), and the plants/DCB extract ratios of As(V) (As(III)) increased from 2.63±0.13 (1.97±0.06) to the peak value of 3.38±0.21 (2.70±0.14) upon adding chelating agents. Besides, the addition of chelating agents increased the uptake of P and Fe by *L. minor* plants. This work provides a theoretical basis for the remediation of As-contaminated waters by duckweed with the help of chelating agents.

Magnetic and electric field accelerate Phytoextraction of copper *Lemna minor* duckweed

Politaeva, N; Badenko, V (2021) PLOS ONE 16: e0255512

In accordance with the opinion of the World Health Organization and the World Water Council the development of effective technologies for the treatment of wastewater from heavy metals for their discharge into water bodies or reuse is an urgent task nowadays. Phytoremediation biotechnologies is the most environmentally friendly and cheapest way of the treatment of wastewater, suitable for sustainable development principals. The main disadvantage of the phytoremediation is the slow speed of the process. A method for accelerating the process of phytoremediation by the combined effect of magnetic and weak electric fields is proposed. The purpose of this study is to determine the values of the parameters of the magnetic and weak electric fields that are most suitable for extracting cuprum ions from wastewater using the higher aqua plants (*Lemna minor*). A corresponding technological process based on the results of the study is proposed. The results have shown that the removal of copper cations from sulfate solutions effectively occurs in the initial period of time (1-5 hours) under the influence of a magnetic field with an intensity of H = 2 kA/m. Under the combined influence of an electrical current with density j = 240 mu A/cm^2 and a magnetic field (H = 2 kA/m) the highest rate of copper extraction by duckweed leaves is achieved. Under these conditions, the greatest growth and development of plant leaves occurs. The paper presents the results of determining of the parameters of the electrochemical release from the eluate of the spent phytomass of duckweed. It has been determined that the release of metal occurs at E = 0.32 V. An original scheme for wastewater treatment from copper with subsequent separation of copper from the spent phytomass of duckweed is proposed. In general, the presented results are a scientific justification of wastewater treatment technologies and a contribution to resolving the crisis in the field of fresh water supply. An important contribution in the circular economy is a technology recommendation proposed for recovering copper from duckweed after wastewater treatment.

Density dependence influences the efficacy of wastewater remediation by *Lemna minor*

Walsh, E; Coughlan, NE; O'Brien, S; Jansen, MAK; Kuehnhold, H (2021) Plants 10: 1366

As part of a circular economy (CE) approach to food production systems, Lemnaceae, i.e., duckweed species, can be used to remediate wastewater due to rapid nutrient assimilation and tolerance of non-optimal growing conditions. Further, given rapid growth rates and high protein content, duckweed species are a valuable biomass. An important consideration for duckweed-mediated remediation is the density at which the plants grow on the surface of the wastewater, i.e., how much of the surface of the medium they cover. Higher duckweed density is known to have a negative effect on duckweed growth, which has implications for the development of duckweed-based remediation systems. In the present study, the effects of density (10-80% plant surface coverage) on *Lemna minor* growth, chlorophyll fluorescence and nutrient remediation of synthetic dairy processing wastewater were assessed in stationary (100 mL) and re-circulating non-axenic (11.7 L) remediation systems. Overall, *L. minor* growth, and TN and TP removal rates decreased as density

increased. However, in the stationary system, absolute TN and TP removal were greater at higher densities (50-80% coverage). The exact cause of density related growth reduction in duckweed is unclear, especially at densities well below 100% surface coverage. A further experiment comparing duckweed grown at 'low' and 'high' density conditions with the same biomass and media volume conditions, showed that photosynthetic yield, Y(II), is reduced at high density despite the same nutrient availability at both densities, and arguably similar shading. The results demonstrate a negative effect of high density on duckweed growth and nutrient uptake, and point towards signals from neighbouring duckweed colonies as the possible cause.

Investigation of hybrid methods for elimination of Brilliant Blue Dye from water phase using various nanomaterials combined with activated sludge and duckweed

Zarzycki, PK; Lewandowska, L; Fenert, B; Piaskowski, K; Kobaka, J (2021) Nanomaterials 11: 1747

The main goal of this experimental work is screening of different natural and synthetic nanomaterials and biopolymers that may improve elimination of stable micropollutants from water phase. In this work, as a target chemical acting as the micropollutant molecule, the Brilliant Blue (BB) dye was selected. We tested different active matrices dispersed in water phase including activated carbon (AC), lyophilized graphene oxide (GO), beta-cyclodextrin (CD), raw dandelion pappus (DP), microcrystalline cellulose(MC), and raw pine pollen (PP), as well as two types of Egyptian Blue mineral pigments (EB1 and EB2). Graphene oxide and Egyptian Blue nanomaterials were synthesized in our laboratory. We investigated potential application of such nanoparticles and biopolymer conglomerates as additives that may tune the activated sludge (AS) microorganisms or duckweed water plant (DW) and increase efficiency of micropollutants removal from wastewater. Studied nanomaterials/biopolymers were used in two different experimental modes involving real activated sludge microorganisms (24 h experiment) as well as duckweed plant (16 day experiment). Quantitative data of BB were obtained using microfluidic type device based on micro-TLC plate. This approach enabled direct determination of target component without sample pre-treatment like pre-concentration or pre-purification. Within single analytical run calibration line, retention standard spots (methyl red) and multiple samples were analyzed simultaneously. Due to the multivariate nature of these experiments, quantitative data were explored with chemometric tools including AHC (agglomerative hierarchical clustering), PCA (principal component analysis), and FA (factor analysis). Experimental data and multivariate calculations revealed that BB is strongly resistant on biodegradation, however, inclusion complexes formation with beta-cyclodextrinmay induce degradation of this dye in the presence of duckweed. It is hoped that results of our experimental work can be used for designing of future experiments for fast screening of different additives and improvement of technological processes, focusing on purification of sewage and water from micropollutants.

Estimation of the potential of *Lemna minor* for effluent remediation in integrated multi-trophic aquaculture using newly developed synthetic aquaculture wastewater

Paolacci, S; Stejskal, V; Jansen, MAK (2021) Aquaculture International 29:: 2101-2118

Aquaculture is an important source of animal protein and a key contributor to global food security. However, aquaculture can exert a negative effect on the aquatic environment due to the release of effluents containing high nutrient levels. In integrated multi-trophic aquaculture (IMTA), the waste produced by one species is the input for another, referred to as extractive species (ES). Potential ES include plants. In the present study, it was explored whether *Lemna minor* can be used to remove nitrogen and phosphorus from aquaculture wastewater. A representative synthetic wastewater was designed based on the composition of aquaculture effluents found in the literature. Synthetic wastewater was found to be a suitable medium for growth *of L. minor*, and plants readily took up NH_4^+-N, NO_3^-N and $PO4^{3-}$-P. In particular, NH_4^+-N concentrations rapidly decreased. The highest removal rates per square meter of water surface, calculated for NH_4^+-N, NO_3^-N and PO_4^{3-}-P, were, respectively, 158, 206 and 32 mg m^{-2} day^{-1}, and these rates were achieved at a plant surface density of 80%. As removal of nutrients is essentially a surface area-related process, the effect of plant density on nutrient uptake was

determined. Uptake of nutrients per square meter of surface area was highest at the highest plant density. Yet, when uptake rates were calculated per square meter of water area covered by *Lemna* fronds, the highest removal rates were found at the lowest plant density, and this is likely to be associated with a reduced intraspecific competition. The present work enables the calculation of potential nutrient uptake by Lemna minor and lays the foundation for a more scientific approach to the design of duckweed-based aquaculture wastewater treatment systems.

Lemna minor cultivation for treating swine manure and providing micronutrients for animal feed

Devlamynck, R; de Souza, MF; Leenknegt, J; Jacxsens, L; Eeckhout, M; Meers, E (2021) Plants 10: 1124

The potential of *Lemna minor* to valorise agricultural wastewater into a protein-rich feed component to meet the growing demand for animal feed protein and reduce the excess of nutrients in certain European regions was investigated. Three pilot-scale systems were monitored for nine weeks under outdoor conditions in Flanders. The systems were fed with a mixture of the liquid fraction and the biological effluent of a swine manure treatment system diluted with rainwater in order that the weekly N and P addition was equal to the N and P removal by the system. The design tested the accumulation of elements in a continuous recirculation system. Potassium, Cl, S, Ca, and Mg were abundantly available in the swine manure wastewaters and tended to accumulate, being a possible cause of concern for long-operating recirculation systems. The harvested duckweed was characterised for its mineral composition and protein content. In animal husbandry, trace elements are specifically added to animal feed as micronutrients and, thus, feedstuffs biofortified with essential trace elements can provide added value. Duckweed grown on the tested mixture of swine manure waste streams could be considered as a source of Mn, Zn, and Fe for swine feed, while it is not a source of Cu for swine feed. Moreover, it was observed that As, Cd, and Pb content were below the limits of the feed Directive 2002/32/EC in the duckweed grown on the tested medium. Overall, these results demonstrate that duckweed can effectively remove nutrients from agriculture wastewaters in a recirculated system while producing a feed source with a protein content of 35% DM.

Phytotoxicity

The changes in *Lemna minor* metabolomic profile: A response to diclofenac incubation

Wahman, R; Cruzeiro, C; Grassmann, J; Schroder, P; Letzel, T (2021) Chemosphere 287:132078

Metabolomics is an emerging approach that investigates the changes in the metabolome profile. In the present study, *Lemna minor* -considered as an experimental aquatic plant model- was incubated with 10 and 100 µM diclofenac (DCF) for 96 h, respectively. Knowing that DCF is internationally often problematic in wastewater effluents and that it might affect particularly the metabolic profiles in aquatic plants, mainly the oxidoreductase, dehydrogenase, peroxidase, and glutathione reductase activities, here it was hypothesized (H) that in the common duckweed, DCF might increase the phenolic and flavonoids pathways, as an antioxidant response to this stress (H1). Also, it was expected DCF to alternate the physiological characteristics, especially the molecular interaction and biochemical properties, of *Lemna* (H2). Metabolic changes were investigated with target and untargeted screening analysis using RPLC-HILIC-ESI-TOF-MS. Twelve amino acids were identified in all treatments, together with three organic acids (p-coumaric, cinnamic, and sinapic acids). In untargeted screening, the important metabolites to discriminate between different treatments were assigned to *Lemna* such as organic acids, lignin, sugars, amino acids, dipeptides, flavonoids, biflavonoids, fatty acids, among others. In resume, *Lemna* responded to both DCF concentrations, showing different stress patterns. A similar metabolic response had already been identified in other studies in exposing *Lemna* to other anthropogenic stressors (like pesticides).

Bisphenol A effects in aqueous environment on *Lemna minor*

Pop, CE; Draga, S; Maciuca, R; Nita, R; Craciun, N; Wolff, R (2021) Processes 9: 1512

The link between different plastic waste pollutants and their impact on the natural aquatic environment and food chain remains a constant and growing issue. Bisphenol A (BPA), a known endocrine disruptor produced in large quantities primarily in the industry of polycarbonate plastics, can accumulate in vegetal and animal tissue, thus magnifying through trophic levels. In this study we exposed viable specimens of the aquatic plant *Lemna minor* under controlled conditions to 50, 100 and 200 ppm BPA levels in order to partially observe the toxic effects of BPA. Colonies ceased to form during the exposure and chlorosis was present especially in the 100 ppm group. Interestingly enough, a high density formation of non-fermenting bacteria as well as coliforms was also observed in the BPA exposed cultures but not in the control groups. The levels of Malondialdehyde (MDA) in the vegetal tissue indicated cellular insults and severe damage, results that were correlated with the HPLC BPA determined concentrations of 0.1%, 0.2% and 0.4%.

Toxic effects of 2,4,4 ′-trichlorobiphenyl (PCB-28) on growth, photosynthesis characteristics and antioxidant defense system of *Lemna minor* L.

Wang, CT; Sun, Y; Ruan, HH; Yang, J (2021)Plant Physiology and Biochemistry 166: 505-511

Polychlorinated biphenyls (PCBs) are a common category of persistent man-made organic pollutants that are widespread in the ambient environment. Although *Lemna minor* L. is an extensively applied plant for aquatic remediation in ecotoxicology research worldwide, little is known regarding its responses to the potentially toxic effects of PCBs. For this study, a 14-day dissolved exposure was conducted to explore the effects of 2,4,4'-trichlorobiphenyl (PCB-28) on the growth, photosynthesis characteristics and antioxidant defense system of L. minor plants. We found that 100 and 200 μg/L of PCB-28 decreased the fresh weight, chlorophyll and protein content, and activities of superoxide dismutase, peroxidase, glutathione S-transferase, and nitroreductase, whereas plasma membrane permeability, and the malondialdehyde and reactive oxygen species concentrations were increased. However, it was observed that 5 and 20 μg/L of PCB-28 had no significant effects on these physiological indices. The ultra-structure of chloroplast demonstrated that 100 and 200 μg/L PCB-28 severely damaged the chloroplast structures. Moreover, correlation analysis revealed that the content of reactive oxygen species had negative correlations with the fresh weight, chlorophyll and protein content, as well as the activities of superoxide dismutase, peroxidase, glutathione S-transferase, and nitroreductase, but had positive correlations with the malondialdehyde content and plasma membrane permeability. This work provides valuable data toward elucidating the physiology and biochemistry of PCBs induced phytotoxicity.

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

Escola Casas, M; Matamoros, V (2021) 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 micropollutants 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 *L. minor*, the increase of micropollutants 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 *S. natans*, micropollutants 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 micropollutant-exposure at

environmentally relevant concentration levels triggers changes in root exudates. This may help to design new strategies to enhance micropollutants degradation in nature based solutions such as in constructed wetlands.

Relative sensitivity of duckweed *Lemna minor* and six algae to seven herbicides

Ueda, K; Nagai, T (2021) Journal of Pesticide Science 46:267-273

We investigated the relative sensitivity of duckweed *Lemna minor* and six species of algae to seven herbicides, using an efficient high-throughput microplate-based toxicity assay. First, we assessed the sensitivity of *L. minor* to the seven herbicides, and then we compared its sensitivity to that of previously published data for six algal species based on EC_{50} values. For five herbicides, the most sensitive species differed: *L. minor* was most sensitive to cyclosulfamuron: *Raphidocelis subcapitata* was most sensitive to pretilachlor and esprocarb: *Desmodesmus subspicatus* was most sensitive to pyraclonil; and *Navicula pelliculosa* was most sensitive to pyrazoxyfen. Simetryn was evenly toxic to all species, whereas 2,4-D was evenly less toxic, with only small differences in species sensitivity. These results suggested that a single algal species cannot represent the sensitivity of the primary producer assemblage to a given herbicide. Therefore, to assess the ecological effects of herbicides, aquatic plant and multispecies algal toxicity data sets are essential.

Growth, pigment changes, and photosystem II activity in the aquatic macrophyte *Lemna minor* exposed to bisphenol A

Bourgeade, P; Aleya, E; Alaoui-Sosse, L; Herlem, G; Alaoui-Sosse, B; Bourioug, M (2021) Environmental Science and Pollution Research DOI: 10.1007/s11356-021-15422-z

As a result of its high production, bisphenol A (BPA) has become ubiquitous in aquatic and terrestrial habitats. In this study, we investigated the toxicity of BPA at 10 mg L^{-1} on *Lemna minor* after 7 days of exposure under controlled conditions according to ISO 20079. BPA statistically reduced the total frond number and frond area, while frond number per colony was significantly elevated in BPA-treated group. However, no change was recorded in root number, while root length was significantly reduced by BPA. BPA also decreased the content of Chl a, Chl b, Chl a + b, and carotenoid by 36%, 44%, 38%, and 32%, respectively, versus the control leading to a decrease in the quantum yield of photosystem II. In addition, non-photochemical quenching (NPQ) values were 2.4- and 4.5-fold higher in light than in dark conditions for control and BPA-treated plants, respectively. Thus, there is a significant activation (61.8%; $p<0.01$) of PSII photoprotection mechanism (NPQ) in BPA-treated plants compared to control but without removing the negative effect of BPA on PSII. The total amount of soluble sugars was reduced by 40% compared to control, and starch accumulation was mainly observed in fronds exposed to BPA. Even if the response patterns of *L. minor* based on fresh and dry weight measurements were less sensitive in our experiment conditions, further studies should be addressed since BPA represents a threat to the dynamic equilibrium governing aquatic ecosystems.
DF: The number of roots is fixed in all species of the genus Lemna to be 1.

Toxicity of wood leachate to algae *Desmodesmus subspicatus* and plant *Lemna minor*

Sackey, LNA; Mocova, KA; Petrova, S; Koci, V (2021) Environmental Science and Pollution Research DOI: 10.1007/s11356-021-15319-x

Wood is one of the extensively used goods on the earth due to its large accessibility and usage in a wide range of human life. When woods are exposed to aquatic media, leachates are generated which may affect the quality of water and damage aquatic life into which they are discharged. This research seeks to evaluate the toxicity of linden (Tilia cordata), larch (Larix decidua) from the Czech Republic, cedrela (Cedrela odorata) and emire (Terminalia ivorensis) from Ghana wood leachates to two aquatic organisms (Desmodesmus subspicatus and Lemna minor). In algal and duckweed toxicity tests, these plants were exposed to different concentrations of wood leachate with nutrient medium creating concentration rates, 20, 30, 45, 67, and 100%

v/v. High concentration of phenols and heavy metals may have contributed to toxicity. It was observed that the various wood leachates were inhibitory to the growth rate of algae and duckweed with emire exhibiting the highest toxicity with IC50 of 30.04% and 28.58% and larch the lowest toxicity with IC50 of 51.18% and 49.57% in relation to growth rate and chlorophyll respectively, hence indicating confirmed and potential toxicity of the various wood leachates to the aquatic organisms.

Toxicological effects of leachate extracts from asphalt mixtures nanomodified under *Daphnia magna* and *Landoltia punctata* test organisms

Facin, F; de Melo, JVS; Lalau, CM; Nogueira, DJ; Puerari, RC; Matias, WG (2021) Chemosphere 285:131463

The incorporation of nanomaterials in binders in the paving area has been studied to improve the mechanical behavior of asphalt mixtures. However, asphalt mixture compounds are susceptible to leaching and deposition in the environment. In this context, this research aimed to investigate the toxic effect of two leachate extracts from asphalt mixtures nanomodified with 2% carbon nanotube and 3% organophilic nanoclay, compared to conventional mixture, using *Daphnia magna* and *Landoltia punctata* as test organisms. The nanomaterials were characterized to confirm morphology, stability and effective diameter. Extracts were chemically characterized using the Fourier-Transform Infrared Spectroscopy (FTIR) technique, which indicated presence of functional groups of the asphalt binder in greater intensity in the leachate from conventional mixture. Acute toxicity with *D. magna* indicated EC_{50},48h of 83.5±6.2mL/L for leachate extract from conventional mixture, 306.0±87.6mL/L for leachate extract from mixture with nanoclay and 464.8±32.1mL/L for leachate extract from mixture with carbon nanotube. No leachate caused significant chronic toxicity. As for *L. punctata*, concentrations that caused 50% growth inhibition were 127.5mL/L for the leachate extract from mixture with carbon nanotube, 196.9 mL/L for the leachate extract from mixture with nanoclay and 205mL/L for the leachate extract from conventional mixture. For these test organisms, there is no evidence of negative impacts directly associated with the use of the present nanomaterials in asphalt mixtures. The incorporation of these nanos may also reduce the acute toxicity of the mixtures.

Assessment of various toxicity endpoints in duckweed (*Lemna minor*) at the physiological, biochemical, and molecular levels as a measure of Diuron stress

Lee, H; Depuydt, S; Shin, K; Choi, S; Kim, G; Lee, YH; Park, JT; Han, T; Park, J (2021) Biology 10: 684

The presence of diuron in a variety of environments has been reported worldwide to exert serious harm to human health and the ecosystem. HPLC and mass spectrometry are highly specific and sensitive methods for herbicide detection, but they have several drawbacks including complex sample preparation procedures, the need for expensive chemicals and equipment, and interference from secondary contaminants during analysis. In addition, these purely chemical approaches do not provide ecologically meaningful information on temporal changes in terms of exposure or the interactive effects of pollutants. In order to compensate for these limitations, biological assays have been used to assess pollutant-induced ecological risks. *Lemna minor* is an attractive experimental model organism that has been used for decades for the prospective risk assessment of pesticides. In the current study, we examined the effects of diuron on *L. minor* using different endpoints at the physiological (growth and photosynthetic efficiency), biochemical (pigment biosynthesis and reactive oxygen species (ROS) levels), and molecular (gene transcription) levels. Our findings provide important insight into the relative sensitivity of different endpoints for diuron toxicity assessment. In addition, they shed light on the toxicity mechanisms of diuron in a model aquatic macrophyte species. The common, broad-spectrum herbicide diuron poses some risks to the environment due to its long persistence and high toxicity. Therefore, the effective monitoring of diuron residues will inform efforts to assess its impacts on ecosystems. In this study, we evaluated the toxicity targets of diuron in the model aquatic macrophyte *L. minor* at the physiological (growth and photosynthetic efficiency), biochemical (pigment biosynthesis and reactive oxygen species (ROS) levels), and molecular (rbcL transcript) levels. The toxicity of diuron was detectable after 48 h of exposure and

the order of sensitivity of toxicity endpoints was gene transcription > maximum electron transport rate (ETR_{max}) > non-photochemical quenching (NPQ) > maximum quantum yield (F_v/F_m) > ROS > fresh weight > chlorophyll b > chlorophyll a > total frond area > carotenoids. Under diuron stress, pigment, ROS, and gene transcript levels increased while frond area, fresh weight, and photosynthesis (F_v/F_m and ETR_{max}) gradually decreased with the increasing duration of exposure. Notably, ROS levels, F_v/F_m, frond area, and fresh weight were highly correlated with diuron concentration. The growth endpoints (frond area and fresh weight) showed a strong negative correlation with ROS levels and a positive correlation with F_v/F_m and ETR_{max}. These findings shed light on the relative sensitivity of different endpoints for the assessment of diuron toxicity.

Growth and antioxidant response in *Spirodela polyrrhiza* under linear alkylbenzene sulfonate, naphthalene and their joint stress

Chai, LL; Li, J; Zhang, YZ; Liu, YL; Wu, ZH (2021) Environmental Science and Pollution Research DOI: 10.1007/s11356-021-14452-x

The synthetic organic surfactants linear alkylbenzene sulfonate (LAS) and polycyclic aromatic hydrocarbon naphthalene (NAP), two common organic pollutants, are frequently detected in freshwater environments. However, the combined ecotoxicological risks associated with these pollutants have not been fully elucidated. The present study investigated the effects of individual and combined treatments of LAS and NAP on the growth and physiological responses of *Spirodela polyrrhiza*. The results showed that LAS was the main compound toxic to *S. polyrrhiza* in a dose-dependent manner. The peroxidase (POD) enzyme and catalase (CAT) enzyme are the main antioxidant enzymes protecting *S. polyrrhiza* from LAS stress. When exposed to NAP stress alone, only slightly reversible damage was observed as the exposure time was extended (14 days). The antioxidant enzyme systems (including superoxide dismutase (SOD), CAT and POD) showed positive responses. Synergistic effects were induced with LAS-NAP mixtures ≥ 5±5 mg L^{-1}), and LAS played a major toxic role. The POD enzyme was a sensitive protective enzyme in duckweed during the joint exposure to LAS + NAP. The results indicate that LAS or NAP may cause serious damage to *S. polyrrhiza* and aggravate ecotoxicity in aquatic ecosystems.
DF: The correct spelling of the duckweed species is *Spirodela polyrhiza*

Synthesis, crystal structure, herbicidal activity, and SAR study of novel N-(Arylmethoxy)-2-chloronicotinamides derived from nicotinic acid

Yu, CS; Wang, Q; Bajsa-Hirschel, J; Cantrell, CL; Duke, SO; Liu, XH (2021) Journal of Agriculture and Food Chemistry 69: 6423-6430

Nicotinic acid, also known as niacin, is a natural product, which is widely found in plants and animals. To discover novel natural-product-based herbicides, a series of N-(arylmethoxy)-2-chloronicotinamides were designed and synthesized. Some of the new N-(arylmethoxy)-2-chloronicotinamides exhibited excellent herbicidal activity against *Agrostis stolonifera* (bentgrass) at 100 μM. Compound 5f (2-chloro-N-((3,4-dichlorobenzyl)oxy)nicotinamide) possessed excellent herbicidal activity against Lemna paucicostata (duckweed), with an IC_{50} value of 7.8 μM, whereas the commercial herbicides clomazone and propanil had values of 125 and 2 μM, respectively. The structure-activity relationships reported in this paper could be used for the development of new herbicides against monocotyledonous weeds.

Influence of nano-titanium dioxide particles (TiO_2 NPs) on improving phytoremediation efficiency of As/Cu/Cd from copper mine wastewaters using *Lemna minor*

Seifi, A; Dehghani, M (2021) Arabian Journal of Geosciences 14: 494

Phytoremediation technology is an appropriate and eco-friendly technique with high efficiency to reduce heavy metal hazards from a contaminated environment. This study analyzed the effects of individual titanium dioxide nano-particles (TiO_2 NPs) and combined with EDTA (TiO_2 NPs+ EDTA) on phytoremediation of arsenic (As),

copper (Cu), and cadmium (Cd) from copper mine wastewater using *Lemna minor*. The experiments were exposed using different concentrations of TiO_2 NPs (200 and 300 mg kg^{-1}) and EDTA (2 g kg^{-1}) in seven treatments during 10 days. The content of As, Cu, and Cd was measured in plant tissues to calculate the total metal extraction (C-t), bioaccumulation factor (BF), and removal efficiency (RE). The content of As and Cu in the plant showed that uptake depended upon TiO_2 NP concentration, and the C-t, BF, and RE values were affected by concentration of TiO_2 NPs in wastewater. Adding 200 mg kg^{-1} TiO_2 NPs to mine wastewater significantly increased the plant biomass (66.5%), relative growth rate (42.2%), relative growth factor (33.9%), and tolerance index (39.2%) compared to the individual wastewater treatment. Adding 200 mg kg^{-1} TiO_2 NPs enhanced the As content, C-t, and BF values to 382.5±41.2 mg kg^{-1}, 122.1±17.9 µg $plant^{-1}$, and 664.2±161.2, respectively, while the combined use of TiO_2 NPs+ EDTA and individual application of EDTA significantly decreased As uptake compared with wastewater treatment. For As, the RE (54.7%) and total absorbed (1786 kg ha^{-1} $year^{-1}$) reached the maximum values following the application of 300 mg kg^{-1} TiO_2 NPs, which were 25.8% and 30.1% higher than the control wastewater treatment, respectively. Furthermore, the BF values in control wastewater treatment showed that *L. minor* was known as a hyper-accumulate plant for the Cu metal, whereas the C-t, RE, and BF values increased due to applying individual TiO_2 NPs and combined TiO_2 NPs+EDTA. These results suggest that using TiO_2 NPs for phytoremediation of contaminated water is helpful in enhancing As accumulation, whereas the *L. minor* is also a useful plant for Cu accumulation.

Taxonomy & Geobotany

Wolffia arrhiza (L.) Horkel ex Wimm. Record in Novosibirsk Region (Western Siberia)-The first in Asian Russia

Kipriyanova, LM; Priidak, NV; Kosterin, OE (2021) Russian Journal of Biological Invasions 12: 277-282

In 2020, the first record of *Wolffia arrhiza* was made in Novosibirsk region (Western Siberia) in a floodplain lake. *Wolffia arrhiza* formed vast stands with an area of hundreds of square meters and with high productivity. Besides monodominant communities, it was met as a component of the cenoses of such hydrophytes as *Stratiotes aloides* and *Hydrocharis morsus-ranae*, *as well as of the helophyte communities formed by Typha latifolia and Eleocharis mamillata*. According to local residents, *Wolffia* was observed as abundant for some years; thus, most likely, the population winters safely at the latitude of Novosibirsk. The most likely source of *Wolffia arrhiza* appearance in the lake was an accidental introduction from an aquarium, although the possibility of this plant entering the lake in high-water years from the wastewater of the municipal settling tank of Novosibirsk sewage waters also exists.

New records in vascular plants alien to Tenerife (Spain, Canary Islands)

Verloove, F (2021) Biodiversity Data Journal 9: e62878

Recent fieldwork by the author in Tenerife, mostly between 2014 and 2019, yielded new records of alien vascular plants. Fifteen taxa (*Acacia decurrens, A. mearnsii, Caesalpinia pulcherrima, Ensete ventricosum, Eucalyptus camaldulensis* subsp. *arida*, *E. cladocalyx, Euryops chrysanthemoides, Ficus elastica, Lippia alba, Pavonia sepioides, Pittosporum tobira, Populus x canadensis, Pyrostegia venusta, Ruellia dipteracanthus and Wigandia kunthii*) are reported for the first time from the Canary Islands. All were initially introduced on purpose, mostly as ornamentals, and recently started to escape from cultivation. Most of them are ephemerals or only locally established, but nearly all have the potential to naturalise in the future. Thirteen additional species are reported for the first time from Tenerife: *Atriplex nummularia, Bellis perennis, Chenopodium probstii, Coccoloba uvifera, Commelina benghalensis, Cuphea hyssopifolia, Eragrostis virescens, Lemna minuta, Malvastrum corchorifolium, Plerandra elegantissima, Psidium guajava, Thunbergia alata and Urochloa subquadripara*. Finally, some miscellaneous notes are provided on the presence of *Balanites aegyptiaca*, *Callistemon viminalis*, *Grevillea robusta* and *Passiflora caerulea* in Tenerife.

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.

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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.

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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.
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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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