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

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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Wolffiella lingulata
Clone 7464

Cover page

Wolffiella lingulata clone 7464

Wolffiella lingulata is a species endemic to South America, Central America and some states in the South of the USA. Clone 7464 was from the city of Marin, in the Yaracuy region of Venezuela, about 200 km west of Caracas. It is currently being sequenced to construct a reference genome assembly for this species (Michael and Lam Labs). Published flow cytometry analysis indicates a haploid genome size of between 600 to 700 Mbp for this species, which is noticeably larger than that of *Wolffiella neotropica* – another species in this genus that was featured on a Cover for this newsletter earlier this year. Similar to the case of *We. neotropica*, *We. lingulata* fronds also display a saddle-like shape with both ends immersed under the surface of the water body and when grown on agar plates, similar clusters made up of concentric "rings" of fronds can be observed as shown on the Cover for this issue. Photo credit: Prof. Eric Lam, Rutgers University, USA.

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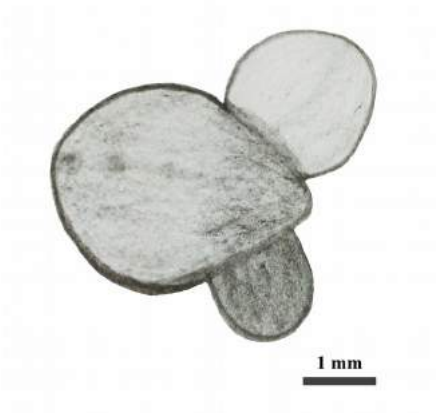
All prior Duckweed Forum issues: http://www.ruduckweed.org/

Science meets art: *Lemna turionifera* Landolt



The drawing of *Lemna turionifera* Landolt shows a mother plant with a daughter plant in the left and a turion in the right pocket. This species could be found in temperate regions of North America, Europe and Asia and has the remarkable ability to form turions as the name indicates. For a long time, it was assumed that some *Lemna minor* strains can form turions but others cannot, thus reported by Thomson (1898), Landolt (1957), Van Overbeck et al. (1968). Finally, after Landolt classified two types of *L. minor*, I and II, he decided to delineate turion-forming *L. minor* I as a species of its own (Landolt, 1975). This decision was later confirmed by several molecular taxonomic investigations, very recently by Braglia et al. (see Duckweed Forum, Issue 33). Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India

Letter from the Editor:

July 29th, 2021

Dear Readers,

Welcome to a new issue of our community newsletter, the *Duckweed Forum*. As the world is finally beginning to get vaccinated against the COVID-19 virus over the past months, the gloomy pandemic is beginning to show signs of improvement. While the rate of vaccination can be faster, I remain optimistic that slowly but surely many activities will begin to resume, albeit with lasting changes from the pre-pandemic forms. What this "new normal" will be may depend on how quickly we can get a majority of the global population vaccinated and translating lessons learned from our past mistakes. Hopefully, we will have better management strategies as we reopen public venues such as schools, laboratories and public transportation, to name just a few examples.

In this issue, we bring several announcements for duckweed focused meetings that have now been scheduled to take place in-person next year. In January, the annual Duckweed Research and Applications Workshop at the Plant and Animal Genome meeting (PAG XXIX) will resume in San Diego, CA. This annual PAG meeting was cancelled in 2021 due to COVID concerns. Similarly, the sixth biennial International Conference of Duckweed Research and Application – originally slated for 2021 – will be hosted instead in May 2022. I hope many of you will consider attending at least one of these meetings and I will look forward to meeting many of you there in person – finally after the long hiatus necessitated by the pandemic.

One good news for our community is the publication of a model plant system review on duckweed in the prestigious journal *The Plant Cell*. This invited review by over 10 authors, with many experts in our field, presents a comprehensive description of the many unique features that make duckweed such an excellent plant model for research. Its publication signifies the recognition in the plant biology field at-large that the time may be ripe for the adoption of duckweed in various research areas to tackle complex questions. A sort of coming-of-age for our field. I expect to see a significant increase in the number of newcomers into the duckweed field and broader recognition of this family of aquatic plants in the near future. Our community will be even more vibrant than the past decade as new blood begins to be infused into our field.

Two articles in this issue also illustrate the mounting interest to realize the commercial potentials of duckweed. From introducing *Wolffia globosa*, or Khai-nam, by chefs in high-end restaurant venues in Thailand to a young university student in Germany exploring ways to grow duckweed indoors, the future of getting duckweed to become household ingredients worldwide may not seem too far away anymore.

With these positive developments in the world and in the duckweed community, I like to wish all of our readers health, success and happiness. Above all, I hope you will enjoy this issue that my collaborators in the ISCDRA and I have put together once again. I like to thank them for their time and tireless effort to bring information and knowledge to share with everyone in our community.

Sincerely,

Eric Lam

Chair, ISCDRA

Khai-nam as human food in Thailand

Metha Meetam

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"Khai-nam" or "Phum" (*Wolffia* spp., mainly *Wolffia globosa*) has been eaten in traditional home-cooking in the north and northeastern areas of Thailand and in neighboring countries in the region. Khai-nam is used as a vegetable in various soup, curry, and spicy-salad recipes. In some communities, Khai-nam is a 'must-have' in festive gatherings. It is not clear when people started eating Khai-nam, but Khai-nam is popular among the Thai-Laotian descendants who migrated to the central region of Thailand approximately two hundred years ago, suggesting that its consumption predated this event. Many people who have eaten Khai-nam love its taste, and most are aware of its nutritional benefits – although the latter remains largely a folk belief since, when asked, they could not be certain which nutrients Khai-nam provides. Despite its reputation, the popularity of Khai-nam among the younger-generation consumers has been on a decline. Khai-nam becomes a rare item in the marketplaces. Khai-nam sold in the local markets is usually harvested from clean natural ponds which become increasingly scarce due to the recent land development and pollution from rising agricultural and industrial activities. According to a recent survey, less than 30% of average Thai consumers had heard of Khai-nam, and only 13% had ever eaten it. The numbers were much lower among the urban residents or the younger generations. Many of the survey respondents were afraid to eat Khai-nam because of fears over possible microbial and chemical contamination. Many disliked the muddy and foul smell associated with Khai-nam especially if harvested from a natural pond or low-quality cultivation systems. These food safety and quality concerns are not unfounded. The bacterial loads of raw Khai-nam samples collected from the Thai markets may contain 1-3 orders of magnitude higher bacterial loads than the levels accepted by regulations for leafy-green vegetables and also could have a great deal of impurities (personal, unpublished data). Thus, the rigorous cleaning and cooking that are needed prior to eating may dissuade modern-lifestyle consumers.

Image credit: Phuttiya Healthy Home by Chef Hong, Thailand

Yet, the recent global trend toward plant-based diet and the recent data on duckweed's nutrition are changing the minds of many city and young consumers. Top fine-dining chefs are now putting Khai-nam in their creative, healthy food and drink recipes. Khai-nam has become a regular item on the menu of many renowned vegan and vegetarian restaurants in the heart of Bangkok. Most of the chefs and consumers cite the high protein content and other health benefits as the reason for eating

Khai-nam. Keys to this movement are attributed in part to the accessibility of high-quality Khai-nam made available by startup companies, such as Advanced Greenfarm Ltd. (Thailand; https://www.advancedgreenfarm.com/), that incorporate research-based cultivation technology and routine lab analysis to help gain acceptance of the consumers. To rekindle the interest in Khai-nam consumption among the Thai people, I have started an education program that includes a social network to provide more information on duckweed research and applications and conducted interviews on TV and newspapers to communicate the benefits of eating Khai-nam. Moreover, the Thai government has placed duckweed research and applications as a priority along with its BCG (bio-, circular-, and green-economy) national strategic plan. All of these point to the up and coming of duckweed and Khai-nam's popularity in Thailand, and perhaps around the world.

Image credit: Tonkla Facai vegan restaurant, Thailand

Conference update: ICDRA VI

Location: The IPK at Gatersleben, Germany

Conference website is up: please visit https://icdra-2022.ipk-gatersleben.de/ for information and registration at your earliest convenience! Looking forward to meeting everyone there.

ABOUT ICDRA

After five successful duckweed conferences in China, USA, Japan, India and Israel, the 6th ICDRA will take place in Germany for the first time. The conferences in the past have highlighted tremendous progress in all fields of duckweed research as well as a rapid growth of the community dealing with duckweed in basic research and commercial applications.

Beside the major efforts in the USA and in Asian countries, several European institutions are contributing to establish duckweeds as a model for developmental biology, genome evolution and ecology and to transform it into a novel aquatic crop. To have the upcoming meeting in Europe should reflect and promote these efforts.

ICDRA6 is organized by the German National Academy of Sciences Leopoldina, the International Steering Committee for Duckweed Research and Applications (ISCDRA), the German Society for Plant Breeding (GPZ) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK).

WELCOME AT LEIBNIZ INSTITUTE OF PLANT GENETICS AND CROP PLANT RESEARCH (IPK)

Plant research with tradition in the centre of Saxony-Anhalt, Germany

The IPK is working as an internationally renowned plant research center on the problems of biology in fundamental and applied research by focusing on cultivated plants. The Institute is a catalyst for the transformation into a bioeconomy, which aims on an efficient and sustainable supply of food, energy and raw materials.

The research creates solutions that are based on conservation, exploration and exploitation of the biodiversity of crop plants. The IPK has a longstanding history in plant research. The Institute comprises four departments: Breeding Research, Molecular Genetics, Physiology and Cell Biology and the Federal *Ex situ* Gene Bank (harboring one of the ten largest germplasm collections in the world). The research topics at the IPK include all branches of biology for model and crop plants as well as for their wild relatives, and since 2014 also cytogenomics on duckweeds.

VENUE ADDRESS

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HOW TO FIND US

Nearby airports
Flughafen Berlin Brandenburg (TXL, 204 km/ SXF, 211 km)
Halle-Leipzig (LEJ, 113km)
Hannover (HAJ, 167km)
From the airports you can travel by train or by car.

Travelling by train
Deutsche Bahn offers to and from Gatersleben from any German city.
The train station in Gatersleben is about 1 km from IPK.
Travel information can be found on the website of Deutsche Bahn.

Travelling by car
The IPK can easily be accessed via highway A36. From A36 use exits Hoym or Quedlinburg-Ost. Coming from Berlin travelling time may be about two hours but might be more depending on traffic. Parking lots for visitors can be found in front of the gate and on campus.

The regular bus transfer between hotels and the conference venue will be organized.

STAY IN UNESCO WORLD HERITAGE QUEDLINBURG

Be our guest at the 6th ICDRA Conference and get to know the beautiful destination of the Harz Mountains. Stay overnight in the UNESCO World Heritage Quedlinburg.

Learn here more about the Harz Mountains
and the exciting historic UNESCO World Heritage City of Quedlinburg.

ACCOMODATIONS

We will announce our hotel options in early 2022.

Research on *Wolffia microscopica* strengthened by Indo-German linkages

K. Sowjanya Sree[1], Jitendra P. Khurana[2] and Klaus-J. Appenroth[3]

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This article is dedicated to Late Prof. Satish C. Maheshwari (1933- 2019) on the second anniversary of his passing (June 12th) and related to his latest article together with the present authors to be published in Current Science (Sree et al., In Press).

Ever since its discovery in the 19th century from West Bengal, India (Sree et al., In Press), *Wolffia microscopica* has attracted several researchers. The discoverer William Griffith, himself, was fascinated by this unique plant and carefully studied its morphology with available tools and techniques at the time (Griffith, 1851a, b). It is interesting to note that this plant has especially attracted Indian and German researchers and has also encouraged Indo-German collaborative research.

Christoph Friedrich Hegelmaier, Professor at the University of Tubingen, Germany, who was an authority in research on Lemnaceae in the late 19th century, was especially interested in studying the unusual ventral projection present in this plant, which is not present in other species of *Wolffia*. *Wolffia microscopica* being endemic to the Indian subcontinent, it was not very easy for Hegelmaier to get hold of live plant specimens. However, in 1871 he succeeded in getting a live sample from the then Punjab, India (Futtehjung, presently in Pakistan; Hegelmaier, 1895) through his friend, Prof. Oliver, and carried out a detailed study of *W. microscopica* (Hegelmaier, 1885).

In the 20th century, several researchers reported the presence of *W. microscopica* across the Indian subcontinent (Landolt, 1986). During this time period, noteworthy research on this plant species was pioneered in the lab of Satish C. Maheshwari, Professor at the University of Delhi, India. Maheshwari started research on this plant already for his Ph.D. thesis (Maheshwari, 1958) which was continued by his students, Rukmini Venkataraman and P. N. Seth (Venkataraman et al., 1970). Working in his lab, one of the present authors, JPK, made significant findings on flowering induction of *W. microscopica* (Khurana et al., 1983, 1986; see also Pareek et al., 2020).

Coming into the present century, the research activities on this plant species were rejuvenated by one of the authors, KJA, after having noticed the loss of this species from his duckweed stock collection in Jena, Germany. To be precise, one of the Masters students (in that time called "Diplomstudent") at University of Jena who was conducting research on this species along with

many other *Wolffia* species preferred to retain only the DNA of the plant species required for the experiments (Bog et al., 2013), expecting to obtain the live plant material from Elias Landolt's collection in Zurich whenever required. Shortly thereafter, Landolt communicated to KJA about the loss of the *W. microscopica* clone 9276 (the only clone of the species) in his collection and requested to provide a culture of 9276 from the collection in Jena. Unfortunately, there was only the DNA of this clone available in Jena. Thereafter, Eric Lam from the Rutgers Duckweed Stock Cooperative, USA was contacted for the same and the clone 9276 was revived in the Landolt's collection. However, in the year 2009, Landolt realized that this clone 9276 of *W. microscopica* received from the stock collection at Rutgers University was not *W. microscopica* but was *W. elongata* (personal communication to KJA). However, by then, Wang et al. (2010) used this mis-identified clone in a phylogenetic tree and Wang et al. (2011) used it in a study comparing the genome sizes of duckweeds, relating them to the evolutionary development of Lemnaceae. This mis-identification of clone 9276 was later supported by plastidic barcode studies with all 37 recognized species of duckweed at the time (Borisjuk et al., 2015). Further, in search for this species across the duckweed clone collections in the world, University of Delhi South Campus, India had only the alcoholic preparations of the flowering stage of these plants in the lab of one of the present authors (JPK). It was realised that this clone was also lost in all other duckweed stock collections of the world.

A photo of the authors together with Prof. Satish C. Maheshwari in 2013. From Left: Klaus-J. Appenroth, K. Sowjanya Sree, Satish C. Maheshwari and Jitendra P. Khurana

One of the present authors (KJA), with a focus on duckweed research for the past 40 years at the University of Jena, Germany, was interested in reviving this species in the stock collections. Because of its endemic nature, the present authors went on numerous excursions between 2010 and 2014 in North India and Bangladesh, and collected 16 different *W. microscopica* clones spanning the geographical range of Gujarat to Bangladesh. This Indo-German cooperation played a significant role in the rediscovery of *W. microscopica*. The new collection of clones of this endemic plant species has allowed the authors to study its morphology (Sree et al., 2015a) and its unusual high growth rate (Sree et al., 2015b). And, the plastidic sequences of the newly discovered clones of *W. microscopica,* having taxonomic relevance, were first reported by Borisjuk et al. (2015). On an application front, this species has also been found to have high nutritional value for its potential use as human food (Appenroth et al., 2017, 2018).

References

Appenroth, K-J., Sree, K. S., Boehm, V., Hammann, S., Vetter, W., Leiterer, M. and Jahreis, G., Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chem.*, 2017, 217, 266-273.

Appenroth, K. J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V., Lorkowski, S., Sommer, K., Vetter, W., Tolzin-Banasch, K., Kirmse, R., Leiterer, M., Dawczynski, C. , Liebisch, G., and Jahreis, G. Nutritional value of the duckweed species of the genus Wolffia (Lemnaceae) as human food. *Front. Chem.*, 2018, 6, 483.

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

Borisjuk, N., Chu, P., Gutierrez, R., Zhang, H., Acosta, K., Friesen, N., Sree, K.S., Garcia, C., Appenroth, K.J. and Lam, E. (2015) Assessment, validation and deployment strategy of a two-barcode protocol for facile genotyping of duckweed species. *Plant Biol.* 17, 42-49.

Griffith, W., Icones plantarum asiaticarum. Bishop college press, Calcutta, India, 1851b, 3, plates CCLXVI-CCLXVIII.

Griffith, W., Notulae ad plantas asiaticas. Bishop college press, Calcutta, India, 1851a, 3, 216-229.

Hegelmaier, F., *Wolffia microscopica*. *Bot. Z.*, 1885, 43, 241-249.

Hegelmaier, F., Systematische Uebersicht der Lemnaceen. In Engler's Botanische Jahrbuecher, Wilhelm Engelmann, Leipzig, 1895, 21, 268-305.

Khurana, J. P., and Maheshwari, S. C., Floral induction in *Wolffia microscopica* by salicylic acid and related compounds under non-inductive long days. *Plant Cell Physiol.*, 1983, 24, 907-912.

Khurana, J. P., Tamot, B. K. and Maheshwari, S. C., Induction of flowering in a duckweed, *Wolffia microscopica*, under non-inductive long days, by 8-hydroxyquinoline. *Plant Cell Physiol.*, 1986, 27, 373-376.

Landolt, E., The family of *Lemnaceae* - a monographic study: Biosystematic Investigations in the Family of Duckweeds (*Lemnaceae*). Veröffentlichungen des Geobotanischen Institutes der ETH, Stiftung Rübel, Zürich, 1986, 1.

Maheshwari, S. C., The Lemnaceae: A contribution to their biology, morphology and systematics. Ph.D. Thesis, University of Delhi, India, 1958.

Pareek, A., Soni, V., Sopory, S.K., Khurana, J.P., Sree, K.S., Tyagi, A.K., Narsimhan, S. and Govindjee, G., Satish Chandra Maheshwari (1933-2019)-a brilliant, passionate and an outstanding shining light for all of plant biology. *Physiol. Mol. Biol. Plants*, 2020, 26, 1087-1098.

Sree, K.S., Maheshwari, S.C., Appenroth, K.-J.and Khurana, J.P., History of discovery of the fastest growing angiosperm, *Wolffia microscopica* (Griff.) Kurz, entwined with British India. *Current Science*, 2021, In Press.

Sree, K.S., Maheshwari, S.C., Boka, K., Khurana, J.P., Keresztes, A. and Appenroth, K.-J., *Wolffia microscopica* (Griff.) Kurz: A unique member of Lemnaceae (Duckweed). *Flora*, 2015 a, 210, 31-39.

Sree, K.S., Sudakaran, S. and Appenroth, K-J., How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus *Wolffia* (Lemnaceae). *Acta Physiol. Plant.*, 2015 b, 37, DOI 10.1007/s11738-015-1951-3.

Venkataraman, R., Seth, P. N. and Maheshwari, S. C., Studies on the growth and flowering of a short-day plant, *Wolffia microscopica*. I. General aspects and induction of flowering by cytokinins. *Z. Pflanzenphysiol.*, 1970, 62, 316-327.

Wang, W., Wu, Y., Yan, Y., Ermakova, M., Kerstetter, R. and Messing, J., DNA barcoding of the Lemnaceae, a family of aquatic monocots. *BMC Plant Biol.*, 2010, ID 570319.

Wang, W., Kerstetter, R.A. and Michael T.P., Evolution of genome size in duckweeds (Lemnaceae). *J. Botany*, 2011, ID 570319.

Community Announcement: Comprehensive review on duckweed as a model for plant research is now published in THE PLANT CELL

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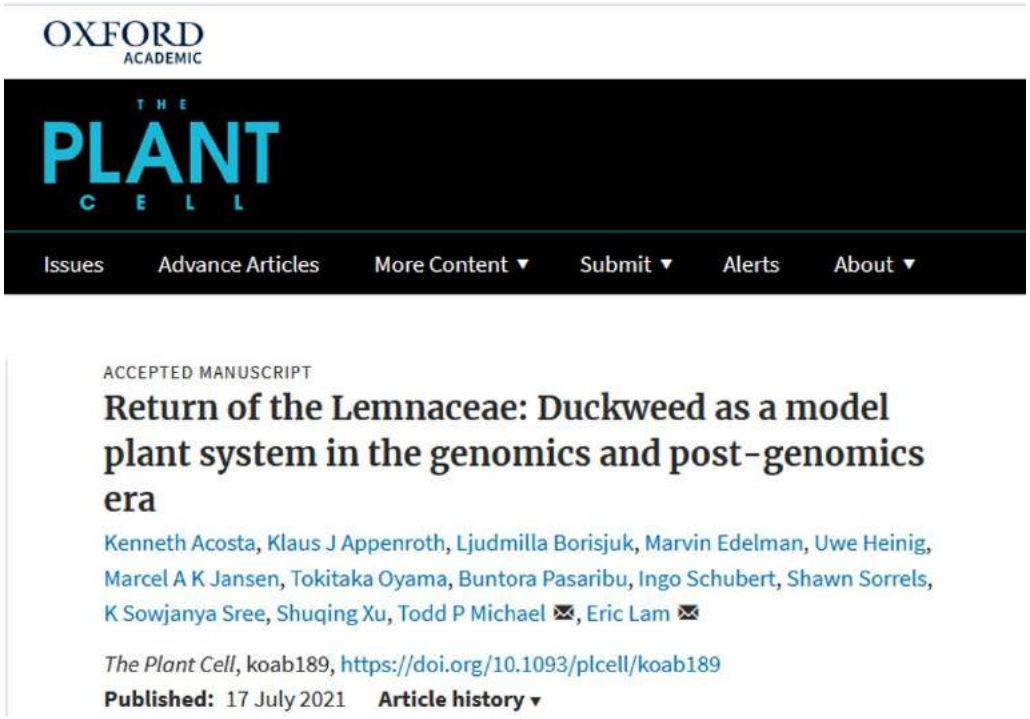
On behalf of all the co-authors, we are happy to announce the publication of an invited review article on “emerging model species in plant biology” that focus on the Lemnaceae family. The full citation information is as follows:



Over the past decade, while there has been an increasing number of papers and review articles related to various aspects of duckweed biology, this Review endeavors to present a comprehensive description of duckweed research's history, biology and potential advantages for both research and commercial applications. It aims to provide essentially an update to the remarkable monographs by Landolt and Kandeler on all-things-duckweed that were published in the 1980's (Landolt 1986, Landolt and Kandeler 1987). As the title of this Review implies, the continuing advance in genomics and related technologies over the past two decades have been instrumental in driving the renaissance of the duckweed family to return as an attractive model to tackle complex questions from single-cell biology in an organismal context to population dynamics in ecology and evolution. Underpinning all these new technological advances is the wealth of biological resources embodied in the amazing strain collection of Elias Landolt, a true legacy that he has left for our community as a companion to his monographs of duckweed knowledge.

As you probably know, the *Plant Cell* is the premier journal for Plant Biology and we hope this Review will reach a broad audience in this field and beyond. With the large number of coauthors that brought invaluable expertise from various disciplines, we endeavor to cover a broad base of interests and potential impacts that duckweed provides. While we highlighted several examples of "hot topics" in current duckweed research, we also identified areas that urgently need additional research. Moreover, we also wanted to reach others outside the duckweed field by describing the essential characteristics that make duckweed transformative as well as the current resources which are available in our community. Thus, we hope that it can become a comprehensive "starter article" for newcomers who are interested in beginning research with duckweed for their particular problem of interest.

In closing of this announcement, one of us (EL) would like to note that it is almost 10 years ago that he had the pleasure of meeting Elias Landolt in person (see picture). Through the kind arrangement by Klaus Appenroth, EL was able to travel to Zürich and enjoyed a wonderful dinner with Elias, Klaus and Walter Lämmler, the current curator of this Zürich collection. In addition, EL was able to replenish some of the lost clones in the then new Rutgers Duckweed Stock Cooperative (RDSC) directly from Landolt's famed collection. In reflecting on the advances that this community has made over the past decade, we are sure Elias would have been gratified that his legacy is not only continuing but also beginning to blossom into a vibrant field that is poised to transform plant biology. For his enduring generosity, we gratefully acknowledged his contributions to the field in this Review as well.

Dinner with Elias, circa 2011. From left to right: Walter Lämmler, Eric Lam, Elias Landolt, Klaus J. Appenroth. At dinner hosted by Elias Landolt at a restaurant in Zürich, Switzerland.

References

Landolt, E. (1986) Biosystematic investigations in the family of duckweeds (Lemnaceae) (Vol. 2). The family of Lemnaceae-a monographic study. vol. 1. *Veroeffentlichungen des Geobotanischen Instituts der ETH, Stiftung Ruebel, Zürich (Switzerland)*.

Landolt, E. and Kandeler, R. (1987) The Family of Lemnaceae. – a Monographic Study, 2. Biosystematic Investigations in the Family of Duckweeds (Lemnaceae). *Veröffentlichungen des Geobotanischen Instutes der ETH, Stiftung Rübel, Zürich (Switzerland)*.

Announcement: Duckweed Workshop in PAG XXIX - Speakers needed

Duckweed Research and Applications Workshop in the

Plant and Animal Genome (PAG29) Conference 2022

January 7-12, 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 looking for researchers that are planning on attending PAGXXIX in San Diego (January 7-12, 2022) and would like to speak in the Duckweed Research and Applications Workshop. We are specifically looking for researchers conducting studies in flowering and genetics, transformation and genome editing, and novel imaging studies with duckweed. Of course, we are also open to all ideas for talks at the workshop. Please email Eric Lam (ericL89@hotmail.com) and/or Todd Michael (toddpmichael@gmail.com) to inquire. We have 5 to 6 speaker slots to fill by mid-October this year, so please let us know of your interest by end of September. We look forward to hearing from you.

Photo of *Wolffiella neotropica* by Eric Lam

Student Spotlight: Alexej Sonnenfeld

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I was six when my parents decided to move from Kazakhstan to Germany. The new world was overwhelming for a little boy who had never seen anything like a supermarket, big swimming pools or clean streets without holes. It showed me that there is much more potential for a good life than I could have ever imagined. At school I wanted to learn as much as I could and this idea got me excited about natural sciences. Biology in particular has been my passion for as long as I can remember. So, in 2017, I started studying Biology (B.Sc.) in Greifswald, Germany. In November 2020, I registered for my Bachelor's thesis under the supervision of Dr. Manuela Bog in order to carry out genetic investigations on the hybridization of *Lemna minor* and *Lemna turionifera* to *Lemna japonica*. This was my first contact with duckweed and I quickly began to develop an interest in using this astonishing plant. In the beginning, I only did research for my thesis. But I invested more and more private time to learn more about how duckweed is used today and what experiences have been gained so far. This showed me the wide range of applications and the potential growing in the ponds and lakes around the corner. The idea started to take shape and I finally asked myself the question: "Why should we waste precious land and resources in agriculture, as we always do, when we could actually do it better?"

As luck would have it, I came across by chance a competition for ideas organized by WITENO GmbH. During a phone call in the laboratory at my workplace, I talked to someone for the first time about making feed from duckweed. Within two months I gained a lot of experience and learned things that I could not have imagined before. In the process, I contacted many different people and worked on my sketch for the idea. I met people who really wanted to work with me on the idea and support this project. In the end, my small team and I won first place in the competition with the project "solentil" (solutions with lentils). So far, the last few months have been extremely exciting and instructive. But this was just the beginning.

Fig.1 Me in the greenhouse of the Botanical Garden, University Greifswald; preparing the prototype for duckweed cultivation.

To gain first-hand experience, I have already started to set up my first low-budget prototype (Fig.1) for the cultivation of duckweed in a greenhouse at the Botanical Garden, University of Greifswald. The first species to grow will be *Spirodela polyrhiza*, *Lemna minor* and *Wolffia arrhiza* (Fig.2). This way I can already develop a feeling for the processes I will later go through on a larger scale. For my own further education, I will be studying for a Master's degree in "Plant Production and Environment" in Rostock, Germany from October this year. I hope to learn more about the efficient and environmentally friendly production of food and feed. The goal, of course, is to combine the knowledge I will acquire with my current idea.

My vision is to relieve today's agriculture of at least part of the heavy burden we place on it. In view of developments around the world, we will have no other choice in the future. Wasting resources, rising CO_2 emissions, enormous soil pollution, and of course, less and less available land due to rising sea levels and a growing world population will force us to change our way of life in the future. And the earlier we start thinking about alternative ways to solve the problems, the more damage we can avoid.

This is just the beginning of my journey and I still have to learn a lot along my way. But I have a dream that with "solentil" we can one day achieve big things with something as small as a water lentil.

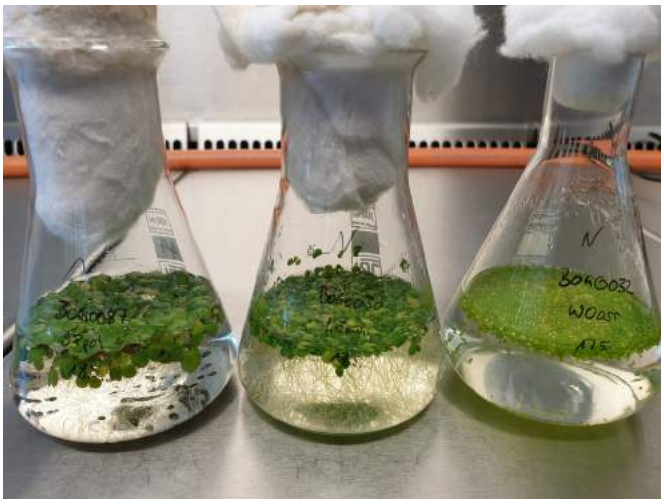

Fig.2 The three species I'm going to start with my exciting journey (from left to right: *Spirodela polyrhiza, Lemna minor, Wolffia arrhiza*).

From the Database

Highlights

Return of the Lemnaceae: Duckweed as a model plant system in the genomics and post-genomics era

Kenneth Acosta, Klaus J. Appenroth, Ljudmilla Borisjuk, Marvin Edelman, Uwe Heinig, Marcel A.K. Jansen, Tokitaka Oyama, Buntora Pasaribu, Ingo Schubert, Shawn Sorrels, K. Sowjanya Sree, Shuqing Xu, Todd P. Michael* and Eric Lam* The plant Cell (in press) doi.org/10.1093/plcell/koab189

The aquatic Lemnaceae family, commonly called duckweed, comprise some of the smallest and fastest growing angiosperms known on Earth. Their tiny size, rapid growth by clonal propagation, and facile uptake of labeled compounds from the media were attractive features that made them a well-known model for plant biology from 1950-1990. Interest in duckweed has steadily regained momentum over the past decade, driven in part by the growing need to identify alternative plants from traditional agricultural crops that can help tackle urgent societal challenges such as climate change and rapid population expansion. Propelled by rapid advances in genomic technologies, recent studies with duckweed again highlight the potential of these small plants to enable discoveries in diverse fields from ecology to chronobiology. Building on established community resources, duckweed is re-emerging as a platform to study plant processes at the systems level and to translate knowledge gained for field deployment to address some of society's pressing needs. This review details the anatomy, development, physiology, and molecular characteristics of the Lemnaceae to introduce them to the broader plant research community. We highlight recent research enabled by Lemnaceae to demonstrate how these plants can be used for quantitative studies of complex processes and for revealing potentially novel strategies in plant defence and genome maintenance.

Flowering of Lemnaceae SF Gray in Siberia: Bioecological Characteristics

Kapitonova, OA; Nikolaenko, SA (2021) Inland Water Biology 14: 177-187

Five species belonging to Lemnaceae family are known to grow in Siberia: *Lemna gibba*, *L. minor*, *L. trisulca, L. turionifera*, and *Spirodela polyrhiza*. Characteristics of their distribution in Siberia, bioecological characteristics, and facts of flowering and fruiting of Lemnaceae are provided in this paper. It has been shown that the generative reproduction of duckweed is not a rare phenomenon, and it is typical for all species of the family known in the region. The flowering is usually observed for *L. trisulca* and *L. turionifera*. However, some cases of flowering are known for *S. polyrhiza*. Almost all populations of *L. gibba* found in Siberia had flowering and fruiting plants. *L. minor* had a limited distribution in Siberia; cases of flowering are rare. The generative reproduction of duckweed is found only in freshwater bodies (0.1-0.7 parts per thousand salinity) with a water depth of 0.1-0.7 m, temperature of the surface water layer from +20 to +33 °C, and pH from 5.96 to 9.30 units not obscured by tree-shrub and tall grass vegetation.

DF comment: The correct authority name of the plant family Lemnaceae is Martinov.

Biotechnology

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.

Aquatic weed *Spirodela polyrhiza*, a potential source for energy generation and other commodity chemicals production

Patel, VR; Bhatt, N (2021) Renewable Energy 173: 455-465

Spirodela polyrhiza will be considering as a 2G feedstock, as generating 15-22 g/m^2 d fresh biomass by improving the quality of sewage. We propose a tractable circular process for pigments, lipid, Nano-catalysts and energy dense substrate (starch) from *S. polyrhiza* biomass. A ton of *S. polyrhiza* biomass was responsible for production of 0.8-1.2 kg of R-phycoerythrin, 0.7-0.9 kg of R-phycocyanin, 2.7-4.3 kg of lipids, 5.3-6.1 kg of ZVI, 79.7-80.4 kg of starch. The produced starch was further fermented to yield 38.8-40.8 l of ethanol. The waste generated in each step was utilized to produce 2.23 lakh (=100,000) litre biogas equivalent 8.51 GJ energy. The elimination of residue, reduce similar to 79-85% in chemicals and energy usage in starch extraction. The synthesized ZVI efficiently mineralized RV5 wastewater at the rate of 26.29 ± 0.23 kg COD $m^{-3}d^{-1}$ up to seven cycles. *Spirodela polyrhiza* reduce similar to 240 kg CO_2-eq for attaining one-ton fresh biomass. Overall findings will the base for starting bio-industries which minimizing the dependence on the terrestrial resources for food, energy and chemicals.

Coupling ecological wastewater treatment with the production of livestock feed and irrigation water provides net benefits to human health and the environment: A life cycle assessment

Roman, B; Brennan, RA (2021) Journal of Environmental Management 288: 112361

Ecologically designed wastewater treatment systems (ex., Eco-Machines (TM)) utilize a diverse ecosystem to treat wastewater to the same extent as conventional treatment, but require less energy and chemical inputs. The environmental benefits of Eco-Machines (TM) can be theoretically maximized by incorporating hyperaccumulating aquatic plants (ex., duckweed) to facilitate nutrient recovery and conversion into protein-rich biomass, which can then be harvested for a range of agricultural and bioenergy applications. Although it has been established that ecological wastewater treatment systems are more cost- and energy-efficient than conventional wastewater treatment systems, a systematic life cycle assessment (LCA) of an Eco-Machine (TM) coupled with its beneficial by-products has not been conducted. In this study, a series of LCAs were performed on different operational scenarios for a 1000 gallon per day, pilot-scale Eco-Machine (TM) that, in addition to producing irrigation-quality water, also produces duckweed biomass for aquaculture. The analysis revealed that Eco-Machines (TM) located in warm climates, which do not require a greenhouse or supplemental heating, use approximately a third of the energy and produce half of the greenhouse gas

emissions compared to conventional wastewater treatment systems in similar locations, while also providing benefits to human health, ecosystem quality, climate change, and resources. In addition, increasing the growth area for duckweed using vertical farming techniques improves the overall impact of the system. This study suggests that with proper management, ecological wastewater treatment systems that upcycle nutrients and water into beneficial products can provide a net benefit to human health and the environment.

DF comment: 1 US. liq. gallon = 3,785411784 Liter

Valorization of selenium-enriched sludge and duckweed generated from wastewater as micronutrient biofertilizer

Li, Jun; Otero-Gonzalez, Lila; Parao, Amelia; Tack, Pieter; Folens, Karel; Ferrer, Ivet; Lens, Piet N L; Du Laing, Gijs (2021) Chemosphere 281:130767

Selenium (Se) is an essential trace element for humans and animals with a narrow window between deficiency and toxicity levels. Application of conventional chemical Se fertilizers to increase the Se content of crops in Se deficient areas could result in environmental contamination due to the fast leaching of inorganic Se. Slow-release Se-enriched biofertilizers produced from wastewater treatment may therefore be beneficial. In this study, the potential of Se-enriched biomaterials (sludge and duckweed) as slow-release Se biofertilizers was evaluated through pot experiments with and without planted green beans (*Phaseolus vulgaris*). The Se concentration in the bean tissues was 1.1-3.1 times higher when soils were amended with Se-enriched sludge as compared to Se-enriched duckweed. The results proved that the Se released from Se-enriched biomaterials was efficiently transformed to health-beneficial selenoamino acids (e.g., Se-methionine, 76-89%) after being taken up by beans. The Se-enriched sludge, containing mainly elemental Se, is considered as the preferred slow-release Se biofertilizer and an effective Se source to produce Se-enriched crops for Se-deficient populations, as shown by the higher Se bioavailability and lower organic carbon content. This study could offer a theoretical reference to choose an environmental-friendly and sustainable alternative to conventional mineral Se fertilizers for biofortification, avoiding the problem of Se losses by leaching from chemical Se fertilizers while recovering resources from wastewater. This could contribute to the driver for a future circular economy.

Molecular mechanism underlying the effect of maleic hydrazide treatment on starch accumulation in *S. polyrhiza* 7498 fronds

Zhu, YR; Li, XX; Gao, X; Sun, JQ; Ji, XY; Feng, GD; Shen, GS; Xiang, BB; Wang, Y (2021) Biotechnology for Biofuels 14: 99

Duckweed is considered a promising feedstock for bioethanol production due to its high biomass and starch production. The starch content can be promoted by plant growth regulators after the vegetative reproduction being inhibited. Maleic hydrazide (MH) has been reported to inhibit plant growth, meantime to increase biomass and starch content in some plants. However, the molecular explanation on the mechanism of MH action is still unclear. To know the effect and action mode of MH on the growth and starch accumulation in *Spirodela polyrhiza* 7498, the plants were treated with different concentrations of MH. Our results showed a substantial inhibition of the growth in both fronds and roots, and increase in starch contents of plants after MH treatment. And with 75 μg/mL MH treatment and on the 8th day of the experiment, starch content was the highest, about 40 mg/g fresh weight, which is about 20-fold higher than the control. The I_2-KI staining and TEM results confirmed that 75 μg/mL MH-treated fronds possessed more starch and big starch granules than that of the control. No significant difference for both in the photosynthetic pigment content and the chlorophyll fluorescence parameters of PII was found. Differentially expressed transcripts were analyzed in *S. polyrhiza* 7498 after 75 μg/mL MH treatment. The results showed that the expression of some genes related to auxin response reaction was down-regulated; while, expression of some genes involved in carbon fixation, C4 pathway of photosynthesis, starch biosynthesis and ABA signal transduction pathway was up-regulated. The results provide novel insights into the underlying mechanisms of growth inhibition and starch accumulation by MH treatment, and provide a selective way for the improvement of starch production in duckweed.

Ecology

Clonal diversity amongst island populations of alien, invasive *Lemna minuta* kunth

Paolacci, S; Bog, M; Lautenschlager, U; Bonfield, R; Appenroth, KJ; Oberprieler, C; Jansen, MAK (2021) Biological Invasions DOI: 10.1007/s10530-021-02530-7

Invasive alien species can negatively impact on newly colonised ecosystems. Thus, it is important to understand factors that facilitate invasiveness. Genetic diversity will enable a species to exploit a variety of environmental conditions. Yet, the process of dispersal to a new ecosystem will commonly create a genetic bottleneck and, hence, result in low diversity. Here we explored variability at genetic and morpho-physiological level of island of Ireland populations of alien, invasive *Lemna minuta*. A comparison of nine clones of *L. minuta* with nine clones of co-generic, native *Lemna minor* shows similar levels of genetic diversity across both species. Thus, the successful invasion of Ireland by *L. minuta* is associated with substantial, intraspecific diversity. It is hypothesised that increased biodiversity is due to repeated invasions from continental Europe, which occurred despite the geographic barriers separating the island from mainland Europe.

Carryover effects minimized the positive effects of treated wastewater on anuran development

Zeitler, EF; Cecala, KK; McGrath, DA (2021) Journal of Environmental Management 289: 112571

Constructed wetlands (CWs) are a potential solution for wastewater treatment due to their capacity to support native species and provide tertiary wastewater treatment. However, CWs can expose wildlife communities to excess nutrients and harmful contaminants, affecting their development, morphology, and behavior. To examine how wastewater CWs may affect wildlife, we raised Southern leopard frogs, *Lithobates sphenocephalus*, in wastewater from conventional secondary lagoon and tertiary CW treatments for comparison with pondwater along with the presence and absence of a common plant invader to these systems common duckweed (*Lemna minor*) - and monitored their juvenile development for potential carryover effects into the terrestrial environment. The tertiary CW treatment did not change demographic or morphological outcomes relative to conventional wastewater treatment in our study. Individuals emerging from both wastewater treatments demonstrated lower terrestrial survival rates than those emerging from pond water throughout the experiment though experiment-wide survival rates were equivalent among treatments. Individuals from wastewater treatments transformed at larger sizes relative to those in pond water, but this advantage was minimized in the terrestrial environment. Individuals that developed with duckweed had consistent but marginally better performance in both environments. Our results suggest a potential trade-off between short-term benefits of development in treated effluent and long-term consequences on overall fitness. Overall, we demonstrate that CWs for the purpose of wastewater treatment may not be suitable replicates for wildlife habitat and could have consequences for local population dynamics.

Trading offspring for survival: high duckweed cover decreases reproductive potential and stimulates elongation in the submerged macrophyte *Chara globularis* Thuillier

Van Onsem, S; Triest, L (2021) Hydrobiologia 848: 2667-2680

Compact blankets of free-floating plants generate stressful aquatic environments. The response of submerged macrophytes remains largely elusive. Will they rush toward the light or rather speed up reproductive efforts and escape using propagules-the macrophyte equivalent of lifeboats? We studied the effects of complete duckweed (*Lemna minor*) cover on growth and reproductive fitness of macroalga *Chara globularis* in a pond mesocosm experiment. *C. globularis* growing in Lemna-covered plots lost biomass and developed longer internodes, indicating an elongative reflex to escape stress. Densities of reproductive organs per biomass unit

evolved positively in open plots and negatively in covered plots, suggesting a trade-off between reproductive effort and vegetative elongation. Reproductive potential correlated significantly with incident radiation. Lemna cover, however, did not affect oospore rain-at least within the limited time span of propagule trapping. *C. globularis* thus displayed an ability to modify phenology in response to floating plant stress, allocating resources to internodes instead of gametangia. Nevertheless, duckweed dominance clearly suppressed the overall reproductive performance of *C. globularis*. The regenerative capacity of many submerged macrophytes will likely suffer from increased floating plant dominance due to global warming-unless efforts are made to reduce nutrient levels in vulnerable waterbodies.

Submerged Rootless Macrophytes Sustain a Stable State Against Free-Floating Plants

Szabo, S; Koleszar, G; Braun, M; Nagy, Z; Vicei, TT; Peeters, ETHM (2021) Ecosystems DOI: 10.1007/s10021-021-00637-5

Both non-rooted submerged vegetation dominated by coontail (*Ceratophyllum demersum*) and non-rooted floating duckweed vegetation (*Lemna gibba*) can maintain their stable dominance in small ponds and channels. We examined the competitive interactions between them and how Ceratophyllum can sustain its stable state against floating plants in a range of nutrient concentrations. Coontail and duckweed were co-cultured in static and semi-static microcosm experiments, and their impact on the nutrients (N, P, Fe, Mn) in the water column was analysed. Coontail strongly reduced the growth of duckweed under a low nitrogen level (0.2-2 mg N L^{-1}). This reduction seems to be due to the low availability of nutrients in the water as derived from the lower nutrient concentrations in duckweed tissue or high pH in water. High nitrogen levels in semi-static media (5-10 mg N L^{-1}) resulted in an increasingly higher chance to overgrow *C. demersum* by *L. gibba*. Field observations revealed that *C. demersum* dominated over *L. gibba* in water bodies with total N below 3 mg L^{-1}, while *L. gibba* dominance over *C. demersum* occurred above 5 mg L^{-1} total N. Ceratophyllum occurrence correlated negatively with total N in the water, while Lemna showed a positive correlation. Furthermore, the occurrence of *L. gibba* was negatively correlated with the frequency of *C. demersum*. All findings together support the theory that under a certain nutrient range, rootless submerged macrophytes have a strong potential to inhibit the dominance of floating plants in ponds, ditches and channels, and thus, they stabilize the submerged vegetation state.

Feed & Food

The Metabolomic-Gut-Clinical Axis of Mankai Plant-Derived Dietary Polyphenols

Yaskolka Meir, Anat; Tuohy, Kieran; von Bergen, Martin; Krajmalnik-Brown, Rosa; Heinig, Uwe; Zelicha, Hila; Tsaban, Gal; Rinott, Ehud; Kaplan, Alon; Aharoni, Asaph et al. (2021) Nutrients 13: DOI:10.3390/nu13061866

Polyphenols are secondary metabolites produced by plants to defend themselves from environmental stressors. We explored the effect of *Wolffia globosa* 'Mankai', a novel cultivated strain of a polyphenol-rich aquatic plant, on the metabolomic-gut clinical axis in vitro, in-vivo and in a clinical trial. We used mass-spectrometry-based metabolomics methods from three laboratories to detect Mankai phenolic metabolites and examined predicted functional pathways in a Mankai artificial-gut bioreactor. Plasma and urine polyphenols were assessed among the 294 DIRECT-PLUS 18-month trial participants, comparing the effect of a polyphenol-rich green-Mediterranean diet (+1240 mg/polyphenols/day, provided by Mankai, green tea and walnuts) to a walnuts-enriched (+440 mg/polyphenols/day) Mediterranean diet and a healthy controlled diet. Approximately 200 different phenolic compounds were specifically detected in the Mankai plant. The Mankai-supplemented bioreactor artificial gut displayed a significantly higher relative-abundance of 16S-rRNA bacterial gene sequences encoding for enzymes involved in phenolic compound degradation. In humans, several

Mankai-related plasma and urine polyphenols were differentially elevated in the green Mediterranean group compared with the other groups ($p < 0.05$) after six and 18 months of intervention (e.g., urine hydroxy-phenyl-acetic-acid and urolithin-A; plasma Naringenin and 2,5-diOH-benzoic-acid). Specific polyphenols, such as urolithin-A and 4-ethylphenol, were directly involved with clinical weight-related changes. The Mankai new plant is rich in various unique potent polyphenols, potentially affecting the metabolomic-gut-clinical axis.

Fermented Duckweed as a Potential Feed Additive with Poultry Beneficial Bacilli Probiotics

Mahoney, R; Weeks, R; Huang, QR; Dai, WJ; Cao, Y; Liu, G; Guo, YJ; Chistyakov, VA; Ermakov, AM; Rudoy, D; Bren, A; Popov, I; Chikindas, ML (2021) Probiotics and Antimicrobial Proteins DOI: 10.1007/s12602-021-09794-4

In this study, the duckweed varieties *Lemna minor*, *Spirodela polyrhiza*, and a commercially processed duckweed food supplement were investigated as potential substrates for the propagation of two probiotic Bacillus strains, *B. subtilis* KATMIRA1933 and *B. amyloliquefaciens* B-1895. Both *L. minor* and *S. polyrhiza* were found to be suitable substrates for the propagation of both bacilli, with 8.47-9.48 Log CFU/g and 10.17-11.31 Log CFU/g after 24 and 48 h growth on the substrates, respectively. The commercial duckweed product was a less favorable substrate, with growth reaching a maximum of 7.89-8.91 CFU/g after 24 h with no further growth after 48 h. Growth and adherence of the bacilli to the three products were confirmed via electron microscopy. These strains have demonstrated health-promoting benefits for poultry and thereby have the potential to enhance duckweed as an animal feed through the process of fermentation. Duckweed has been shown to be a promising alternative resource for protein and has the opportunity to become a valuable resource in multiple industries as a potential means to increase sustainability, food security, and reduce environmental impact.

Interaction with other organisms

Novel Plant-Associated Acidobacteria Promotes Growth of Common Floating Aquatic Plants, Duckweeds

Yoneda, Yasuko; Yamamoto, Kyosuke; Makino, Ayaka; Tanaka, Yasuhiro; Meng, Xian-Ying; Hashimoto, Junko; Shin-Ya, Kazuo; Satoh, Noriyuki; Fujie, Manabu; Toyama, Tadashi; Mori, Kazuhiro; Ike, Michihiko; Morikawa, Masaaki; Kamagata, Yoichi; Tamaki, Hideyuki (2021) Microorganisms 9: DOI:10.3390/microorganisms9061133

Duckweeds are small, fast growing, and starch- and protein-rich aquatic plants expected to be a next generation energy crop and an excellent biomaterial for phytoremediation. Despite such an importance, very little is known about duckweed-microbe interactions that would be a key biological factor for efficient industrial utilization of duckweeds. Here we first report the duckweed growth promoting ability of bacterial strains belonging to the phylum Acidobacteria, the members of which are known to inhabit soils and terrestrial plants, but their ecological roles and plant-microbe interactions remain largely unclear. Two novel Acidobacteria strains, F-183 and TBR-22, were successfully isolated from wild duckweeds and phylogenetically affiliated with subdivision 3 and 6 of the phylum, respectively, based on 16S rRNA gene sequence analysis. In the co-culture experiments with aseptic host plants, the F-183 and TBR-22 strains visibly enhanced growth (frond number) of six duckweed species (subfamily Lemnoideae) up to 1.8-5.1 times and 1.6-3.9 times, respectively, compared with uninoculated controls. Intriguingly, both strains also increased the chlorophyll content of the duckweed (*Lemna aequinoctialis*) up to 2.4-2.5 times. Under SEM observation, the F-183 and TBR-22 strains were epiphytic and attached to the surface of duckweed. Taken together, our findings suggest that indigenous plant associated Acidobacteria contribute to a healthy growth of their host aquatic plants.

Indigenous bacteria, an excellent reservoir of functional plant growth promoters for enhancing duckweed biomass yield on site

Khairina, Y; Jog, R; Boonmak, C; Toyama, T; Oyama, T; Morikawa, M (2021) Chemosphere 268: 129247

The advantages of aquatic biomass production using wastewater as a cost-free fertilizer have recently been highlighted. Here, we report a successful study in which duckweed, *Lemna gibba*, biomass production in a food factory effluent containing low nitrogen and high salts was enhanced by employing customized plant growth-promoting bacteria (PGPB). Two common PGPB strains previously obtained from natural pond water, *Acinetobacter calcoaceticus* P23 and *Pseudomonas fulva* Ps6, hardly promoted the growth of duckweed; on the contrary, they inhibited its growth in treated factory wastewater, far different water conditions. Then, we asked if some indigenous wastewater bacteria could promote the growth of duckweed. We found that Chryseobacterium strains, a group of bacteria with limited nitrogen metabolism, were dominantly selected as effective PGPB. Moreover, we demonstrated that nitrogen limitation is the crucial environmental factor that induces the plant growth-inhibiting behavior of *A. calcoaceticus* P23 through competition for mineral nutrients with the host duckweed. This study uncovered points to be considered in PGPB technology to achieve efficient production of duckweed biomass in a factory effluent with unbalanced content of mineral nutrients.

Molecular Biology

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, Xuyao; Yang, Jingjing; Li, Gaojie; Sun, Zuoliang; Hu, Shiqi; Chen, Yan; Guo, Wenjun; Hou, Hongwei (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 *WRKY*s 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 Sp*WRKY*s, their target genes, and non-coding RNAs. The expression profile of Sp*WRKY*s under phosphate starvation, cold, and submergence conditions revealed that most Sp*WRKY*s 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.

Physiology & Stress

Indirect assessment of internal irradiation from tritium decay on *Lemna minor* duckweed

Ifayefunmi, OS; Mirseabasov, OA; Obninsk, BIS (2021) Nuclear Engineering and Technology 53: 1991-1999

The response changes of the specific growth rate of *Lemna minor* duckweed was modelled using the logarithms of frond numbers on tritium activity concentration and gamma radiation dose from cobalt 60. The concept of average specific growth rate depends on the general exponential growth pattern, where toxicity is estimated based on the effect on the growth rate. One of the main questions of the effect of the radiation dose on duckweed is how to correlate the effect of beta radiation with the effect of any other radiation for modelling

radiation on *L. minor*. Experimental data were extrapolated by utilizing the OECD guidelines. A linear relationship of absorbed dose and activity concentration was obtained for the average dependency growth rate of *L. minor* as D = (0.1257) x A(0.585). The dose rate of gamma irradiation from Co-60 increases with tritium activity dependence, on the specific growth rate of the *L. minor* duckweed. An increase in the tritium activity causes a decrease in the specific growth rate of the *L. minor* duckweed. It indicates that as the quantity of the beta radiation dose increase in *L. minor* duckweed, a higher quantity of gamma radiation will be required to cause the same effect in the specific growth rate of *L. minor* duckweed. The relation between the inhibition of the *L. minor* seedling growth and gamma and beta radiation dosage agrees roughly with that between the decrease of survival rate or fertility and dosage.

Phytoremediation

Silver Modified Hydrophytes for Heavy Metal Removal from Different Water Resourcesy

El Awady, FR; Abbas, MA; Abdelghany, AM; El-Amir, YA (2021) Biointerface Research in Applied Chemistry 11: 14555-14563

Phytoremediation of three different aquatic plants powders *Lemna minor* L., *Azolla filiculoides* Lam. and *Pistia stratiotes* L. studied against different heavy metals (HM) and after modifications with interfacial layer synthesized silver nanoparticles. Prepared samples tested for the selective absorbance of chromium, cadmium, lead, and zinc. *L. minor* and *P. stratiotes* show selective absorption against lead, while *A. filiculoides* show higher absorption against chromium. Absorption of all heavy metal concentrations was found to be enhanced after interfacial modification with green synthesized silver nanoparticles.

Effects of long-term exposure to oxytetracycline on phytoremediation of swine wastewater via duckweed systems

Hu, H; Li, X; Wu, SH; Lou, W; Yang, CP (2021) Journal of Hazardous material 414: 125508

The effects of antibiotics on phytoremediation systems have attracted widespread attention to high concentrations of antibiotics in livestock wastewater. In this work, the effects of oxytetracycline (OTC) whose concentration was 0.05-1.00 mg/L on swine wastewater treatment by a duckweed-based phytoremediation systems were explored, including oxidative stress, nutrient production, bioconcentration, and community-level physiological profile. Results showed that the levels of H_2O_2 and peroxidases (PODs) of duckweed increased with an increase of OTC in the first 8 days. However, oxidative stress of duckweed disappeared after 18 days of exposure, except for 0.05 and 1.00 mg/L. Although OTC has negative effects on the production of high-value nutrients in duckweed, 0.05 and 0.25 mg/L OTC promoted the synthesis of starches and flavonoids, and the synthesis of vitamin C could restore after 28 days of exposure. In addition, a community-level physiological profile revealed that 0.05 mg/L OTC could significantly enhance the duckweed associated microorganisms metabolic activity. Therefore, this investigation adds to the understanding of antibiotics stress on high-value nutrients production in hydrophyte when was used to livestock wastewater management and also helps to clarify the metabolism profile of the phyllosphere and rhizosphere microbes; thereby providing new insight into effects of antibiotic on livestock wastewater phytoremediation.

Comparative analysis of trace and macro-element bioaccumulation in four free-floating macrophytes in area contaminated by copper smelter

Polechonska, Ludmila; Szczesniak, Ewa; Klink, Agnieszka (2021) International Journal of Phytoremediation DOI:10.1080/15226514.2021.1937932

This is the first study that comprehensively compares *Salvinia natans, Lemna minor, Lemna trisulca*, and *Azolla filiculoides* growing in the field; industrially affected conditions in respect of elements contents, water-plant transfer, and bioaccumulation using statistical analyses and indexes and their suitability for phytoremediation was considered. Secondary aim of the study was to fill the gap in research on the impact of copper smelters on aquatic ecosystems. Although the manuscript describes a case study performed near copper smelter in Poland, due to the novel results and cosmopolitan distribution of the species and significant world-wide impact of industry on the environment the results may be interested to broad publicity and find substantial application.

Evaluation of *Lemna minor* and cyanobacteria effect in aerated and non-aerated conditions on biological oxygen demand (BOD), dissolved chemical oxygen (COD), total coliform and faecal coliform of municipal and industrial wastewater

Abadi, SAH; Najafi, P; Baharlouei, J; Ghahsareh, AM (2021) International Journal of Environmental Analytical Chemistry DOI: 10.1080/03067319.2021.1933463

Water scarcity and its growing demand, especially in arid and semi-arid countries, put a lot of pressure on water resources and with the increasing limitation of water resources, the use of wastewater is considered as an unconventional water source and the only sustainable source of water for irrigation of agricultural products. In this study, the performance of *Lemna minor* and filamentous cyanobacteria in the treatment of two types of industrial and municipal effluents were investigated separately. The experiment was performed as a factorial experiment in a randomised complete block design with three replications. The experiment was performed as a factorial experiment in a randomised complete block design with three replications. The highest reduction for BOD and COD factors was observed in the presence of *L. minor*, and cyanobacteria showed a more favourable effect on total coliform factor, while both treatments could not reduce the total coliforms to the desired level and it seems that they need time or complementary methods for optimal treatment and reuse in nature. It is worth noting that the *L. minor* was very successful in reducing faecal coliform and reduced it to 100% in the first five days. In general, *L. minor* was more successful than cyanobacteria. It seems that cyanobacteria need more time for optimal results because the best results of cyanobacteria occurred in a period of 10 to 15 days.

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, Hafiz Khuzama; Farid, Mujahid; Zubair, Muhammad; Alharby, Hesham F; Asam, Zaki Ul Zaman; Farid, Sheharyaar; Bamagoos, Atif A; Alharbi, Basmah M; Shakoor, Muhammad Bilal; Ahmad, Sajid Rashid; Rizwan, Muhammad; Ali, Shafaqat (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 (10mM) 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 and 171%, in stem by 192, 172 and 154%, and in roots by 224, 183 and 168%, respectively. Similarly, the accumulation of Pb, Cu and Cr increased in leaves by 91, 71 and 36%, in stem by 57, 46 and 36% and in roots by 76, 53 and 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 and Cr increased by 542, 411 and 397% while accumulation increased by 101, 59 and 55% respectively in overall plant biomass.

Tolerance and decolorization potential of duckweed (*Lemna gibba*) to C.I. Basic Green 4

Singh, Hanwant; Raj, Shani; Kumar, Deepak; Sharma, Shubhangani; Bhatt, Upma; Kalaji, Hazem M; Wrobel, Jacek; Soni, Vineet (2021) Scientific Reports 11:10889

With growing human culture and industrialization, many pollutants are being introduced into aquatic ecosystems. In recent years, dyes have become a major water pollutant used in the manufacture of paints and other production purposes. In this research, the potential of duckweed (*Lemna gibba*) plant was investigated spectrophotometrically as an obvious bioagent for the biological decolorization of the organic dye C.I. Basic Green 4 (Malachite Green, BG4). Photosynthetic efficiency analysis showed that the photosynthetic apparatus of *L. gibba* is very tolerant to BG4. Significant induction of reactive oxygen species (ROS) scavenging enzymes was observed after 24h of biodecolorization process in *L. gibba* treated with 15 and 30 mg/l BG4. The experimental results showed that *L. gibba* has a strong ability to extract BG4 from contaminated water and the best results were obtained at 25-30°C and pH 8.0. We conclude that duckweed *L. gibba* can be used as a potent decolorization organism for BG4.

Long-term effect of sediment on the performance of a pilot-scale duckweed-based waste stabilization pond

Tu, Q; Lu, YF; Zhao, YG; Duan, CQ; Huang, J; Fang, Y; Li, B; Zhao, H (2021) Science of the Total Environment 770: 145216

Duckweed-based waste stabilization ponds (DWPs) have been widely used in wastewater treatment. However, the effects of sediment, an essential component of DWPs, on their performance have rarely been studied. In this study, two pilot-scale DWPs (12 m^2) with sediment (DPS) and without sediment (DP) were evaluated over more than 1 year to determine the effects of sediment on duckweed growth, wastewater treatment, and greenhouse gas (GHG) production and emission in DWPs. The results indicated that the annual average duckweed growth rate were comparable, but protein content, carbon (C) and nitrogen (N) recovery rates of duckweed were slightly higher in the DPS than in the DP. Meanwhile, the dissolved oxygen (DO) and oxidation reduction potential (ORP), removal efficiencies of COD, TP, TN, NH_4^+-N, and turbidity of pond water from the DPS were significantly lower than for DP. More importantly, the DPS had considerably higher CH_4 production/emission and global warming potential (GWP) than the DP, even though more than 90% of CH_4 released from the sediment was consumed during its passage through the water column and duckweed layer. Sediment increased the recoveries of C and N by 7.94% and 8.82%, respectively. Influencing degree for COD, TP, TN, NH_4^+-N and turbidity were - 27.92%, -20.98%, -22.61%, -24.13% and -14.91%, respectively; for pond water DO and ORP, the values were -35.68% and -44.59%, respectively; and for CO_2, CH_4 and N_2O emission and "combined GWP", they were 21.66%, 271.67%, -8.47% and 178.02%, respectively. Thus, this study indicates that sediment formed in the DWPs has a multi-faced effect on the performance of a DWP. In particular, sediment has an unfavourable effect on the wastewater treatment and the GHGs mitigation, but a favourable effect on the protein content and the C and N recoveries in duckweed.

K_2HPO_4- pretreatment significantly enhances the biosorption capacity of Co^{2+} by *Lemna gibba*

Reyes-Ledezma, JL; Ramirez-Rodriguez, AE; Ballinas-Cesatti, CB; Cristiani-Urbina, E; Morales-Barrera, L (2021) Revista Mexicana de Ingenieria Quimica 20: 581-605

Since divalent cobalt [Co^{2+}] in wastewater is highly toxic, it must be removed before dumping the water into the environment. The aim of this study was to examine the biosorption of Co^{2+} by *Lemna gibba* (LG) after pre-treatment with various compounds to improve its biosorption capacity. The pre-treatment with K_2HPO_4 (0.1 M) produced the best biosorption capacity of Co^{2+} by LG (31.21 ± 0.29 mg g^{-1} vs 18.87 ± 0.19 mg g^{-1} for the untreated control). Subsequently, the optimal concentration (0.3 M) of K_2HPO_4 was found (the pre-treatment herein denominated PLG), showing a biosorption capacity of 40.13 ± 0.18 mg g^{-1} at pH 7.0. The pseudo-second

order model most adequately fit the experimental kinetics. According to the proximate chemical analysis, the pre-treatment enhanced the biosorption capacity (PLG vs LG), probably by improving sorption site availability through the elimination of salts, and by increasing the negative charge of the plant cell surface (from -26 to -35 mV), thus favoring the approach of positively charged Co^{2+}. The ATR-FTIR analysis of PLG demonstrated that its hydroxyl, carboxyl, amide and amine groups importantly contributed to Co^{2+} removal. Thus, PLG is a promising biosorbent material for Co^{2+} removal from aqueous solutions.

Biosorption of methylene blue from water by live *Lemna minor*

Can-Terzi, B; Goren, AY; Okten, HE; Sofuoglu, SC (2021) Environmental Technology and Innovation 22: 101432

A number of green treatment technologies have been used for textile wastewater treatment, among which phytoremediation is a low cost, effective, and promising alternative - to conventional treatment techniques. The aim of this study was to investigate performance of *Lemna minor* (*L. minor*) for phytoremediation of Methylene Blue (MB). A Box-Behnken experimental design (BBD) was applied to study individual and combined effect of operating parameters on MB dye removal efficiency: MB dye concentration (x(1): 5 - 25 mg L^{-1}), amount of *L. minor* (x(2): 1 - 5 g), and pH of the solution (x(3): 4.5 - 9.0). Response surface analysis and response model were utilized to reveal the relationship between operating parameters and MB removal efficiency. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) analyses of *L. minor* samples were used to infer on the removal mechanism. The predicted optimum values were x(1) = 15 mg L^{-1}, x(2) = 4.9 g, and x(3) = 6.8, for the highest removal efficiency (98%) within 24 h. FTIR and SEM analyses indicated that the dye removal mechanism was mainly biosorption. Desorption experiments revealed that *L. minor* released only a small fraction of the sorbed dye. Consequently, in addition to being environmental friendly and cost effective, results of this study show that *L. minor* can be effectively used for MB dye removal from wastewaters while adding to the pertinent but limited literature by presenting its applicability in wider operating parameter ranges, maximization of removal efficiency through experimental design, and evidence that biosorption is a plausible mechanism.

Exponential decay: an approach to model nutrient uptake rates of macrophytes

Nesan, Daniel; Chan, Derek Juinn Chieh (2021) International Journal of Phytoremediation
DOI:10.1080/15226514.2021.1915955

One of the challenges of integrating phytoremediation into a waste treatment system is the sensitivity of plant species to fluctuations in environmental conditions and the difficulty in estimating subsequent changes to their rates of uptake. In this study, we examine a method using the exponential decay equation to approximate the median uptake rate (MUR) of nutrients for three aquatic macrophyte species, *Salvinia molesta*, *Spirodela polyrhiza*, and *Lemna minor*. These MUR values were then used to directly evaluate the phytoremediation performance between species and at varying levels of salinity stress. The results of this study indicate that an exponential decay relationship produced the most accurate models of the nutrient uptake profile for each species, with highest correlation values in 74.1% of tests for the three species at increasing salinity over a period of 14 d. *S. polyrhiza* and *L. minor* began to show significant reductions in nutrient uptake and growth at salinity concentration above 10g/L. Using MUR, direct comparisons can be made between species in a time and mass-independent manner, allowing for the rapid assessment of phytoremediation performance under conditions of increasing salinity stress. In this study, we propose the use of an exponential decay model and the use of median uptake rate (MUR) obtained from the model coefficients as a method for directly comparing species performance under different conditions. Subsequently, we show how the use of MUR values obtained from three species of aquatic macrophytes allows for the direct comparison of species performance under increasing salinity stress. The method proposed in this study would improve the ability for easy comparison between species performance under varying environmental conditions. Future works could further build on the parameters proposed in this study and optimize the performance of phytoremediation systems developed for

nutrient-affected wastewater management. This study is especially beneficial to phytoremediation researchers and environmental engineers who are implementing or designing macrophyte phytoremediation systems.

The role and effectiveness of monoculture and polyculture phytoremediation systems in fish farm wastewater

Ng, YS; Chan, DJC (2021) RSC Advances 11: 13853-13866

Phytoremediation offers a sustainable solution to aquaculture pollution, but studies with critical evaluations of the treatment performances of macrophyte systems are limited. This study intended to evaluate the roles and treatment profiles of *Spirodela polyrhiza* (L.) Schleid. and *Lemna* sp. systems in terms of ammonia, nitrate, nitrite, phosphate (NH_3-N, NO_3^--N, NO_2^--N, PO_4^{3-}), chemical oxygen demand (COD), turbidity, and total suspended solids (TSS) on fish farm wastewater and to elucidate the rationale behind the removal of the pollutants and the changes in a raceway pond rig. The nitrogen and phosphorus removal in the *S. polyrhiza* monoculture system outperformed the other configured systems. An 81% reduction in ammonia (to 3.90 mg of NH_3-N/L), and sharp declines of up to 75%, 88%, and 71% in TSS, turbidity, and COD levels were recorded within two days, while significant decreases in nitrate, nitrite, and phosphate levels were observed. This indicated that the system could inhibit nitrate and nitrite spikes in waters (nitrification) via reducing the available ammonia and limiting subsequent nitrite and nitrate conversion, while reducing TSS in algal-bloom wastewater via shading. High biomass productivity and superior protein content were observed in the macrophyte systems (*S. polyrhiza* + *Lemna* sp. polyculture system), with up to 112% and 12% increases, respectively. This study demonstrated that the *S. polyrhiza* monoculture system is effective at treating fish farm wastewater, lowering the levels of relevant inorganic and organic pollutants, and it could be used as a biofilter for natural waters, preserving the existing ecology.

A circular economic approach to the phytoextraction of Zn from basic oxygen steelmaking filtercake using *Lemna minor* and CO_2

Newnes, AT; Marshall, Y; Grainger, C; Neal, M; Scullion, J; Gwynn-Jones, D (2021) Science of the Total Environment 766: 144256

Two billion tonnes of alkaline metallurgical waste is generated per year as a product of industry, mining, and metal processing. Filtercake is one such residue formed as a bi-product of steelmaking. Metal-rich bi-products can be both an environmental concern and potential resource. High concentrations of heavy metals, if accessed, could be utilised and reprocessed reducing both pollution and the demand for raw metal ores. Phytoextraction is one such method of recovering metals from contaminated mediums. Research interest in *Lemna* sp. has grown due to their phytoremediation potential. Facilitated by rapid growth and accumulation of nutrients and metals, *Lemna minor* has been described as one of the most effective macrophytes for remediating contaminated water. The present study outlines a system using *L. minor* to extract Zn from filtercake when submerged in static water. To facilitate phytoremediation, CO_2 carbonation can be employed to solubilise elements and utilise this greenhouse gas, another a bi-product of steel industry. The addition of CO_2 to vessels of water containing filtercake lowered the pH from as high as 8.8 to 5.6 and significantly increased Zn in solution compared to vessels receiving no CO_2. Results suggest the potential of *L. minor* to accumulating 68.7 kg Zn per year from 20.5 Mt. filtercake ha^{-1}. This system facilitates a circular economy with re-use of multiple existing bi-products. In addition, the potential employment of biomass in biofuel production and use of remediated filtercake in carbon sequestration adds further environmental and socio-economic impact. The extent to which the approach was consistent with circular economy was discussed and its wider integration considered.

Phytoremediation of fluoride-contaminated water by *Landoltia punctata*

Braga, AF; Borges, AC; Vaz, LRL; de Souza, TD; Rosa, AP (2021) Engenharia Agricola 41: 171-180

Fluorine is released into the water environment naturally or by anthropogenic activities. Fluorine promotes health benefits at low concentrations, but it promotes adverse effects ranging from fluorosis to carcinogenic problems at high concentrations. Although fluorine removal from environment can occur through processes such as adsorption, reverse osmosis, and electrodialysis, the phytoremediation emerges as an accessible and environmentally friendly treatment. This research aimed to study the phytoremediation potential of *Landoltia punctata* for treating water contaminated with fluorine ion (fluoride). The central composite rotatable design was used to assess the effect of three variables in the process: pH ranging from 5 to 9; phosphate concentration from 0 to 10 mg L^{-1}; and nitrate concentration from 0 to 800 mg L^{-1}. The plants were exposed to a fluoride initial concentration of 5 mg L^{-1} in 3 L-vessels that also included Clark's solution for a period of 10 days. Experimentally, removal of up to 21% was observed for the supplied fluoride. Values of the order of 30% for the removed fluoride mass can be predicted by the obtained model. *Landoltia punctata* is a promising candidate for the phytoremediation of fluoride-contaminated waters.

Phytoremediation of CYN, MC-LR and ANTX-a from Water by the Submerged Macrophyte *Lemna trisulca*

Kucala, M; Saladyga, M; Kaminski, A (2021) Cells 10: 699

Cyanotoxins are harmful to aquatic and water-related organisms. In this study, *Lemna trisulca* was tested as a phytoremediation agent for three common cyanotoxins produced by bloom-forming cyanobacteria. Cocultivation of *L. trisulca* with *Dolichospermum flos-aquae* in BG11 medium caused a release of the intracellular pool of anatoxin-a into the medium and the adsorption of 92% of the toxin by the plant-after 14 days, the total amount of toxin decreased 3.17 times. Cocultivation with *Raphidopsis raciborskii* caused a 2.77-time reduction in the concentration of cylindrospermopsin (CYN) in comparison to the control (62% of the total pool of CYN was associated with the plant). The greatest toxin limitation was noted for cocultivation with *Microcystis aeruginosa*. After two weeks, the microcystin-LR (MC-LR) concentration decreased more than 310 times. The macrophyte also influenced the growth and development of cyanobacteria cells. Overall, 14 days of cocultivation reduced the biomass of *D. flos-aquae*, *M. aeruginosa*, and *R. raciborskii* by 8, 12, and 3 times, and chlorophyll a concentration in comparison to the control decreased by 17.5, 4.3, and 32.6 times, respectively. Additionally, the macrophyte stabilized the electrical conductivity (EC) and pH values of the water and affected the even uptake of cations and anions from the medium. The obtained results indicate the biotechnological potential of *L. trisulca* for limiting the development of harmful cyanobacterial blooms and their toxicity.

Phytotoxicity

Interactions between *Lemna minor* (common duckweed) and PFAS intermediates: Perfluorooctanesulfonamide (PFOSA) and 6:2 fluorotelomer sulfonate (6:2 FTSA)

Zhang, WL; Liang, YN (2021) Chemosphere 276: 130165

Perfluorooctanesulfonamide (PFOSA) and 6:2 fluorotelomer sulfonate (FTSA) are widely present intermediates of per- and polyfluorinated substances (PFAS). Although detected at high concentrations in landfill leachate and groundwater, the interactions of these two compounds with plants have not been investigated much. In this work, uptake of these two PFAS intermediates at 10 and 200 μg/L by *Lemna minor* (common duckweed) were studied in detail. It was found that the biomass production of *L. minor* was not impacted negatively by PFOSA and FTSA at concentrations equal to or lower than 200 μg/L. Between these two target compounds, FTSA had much higher concentrations in *L. minor* when the concentrations and exposure times were the same as those for PFOSA. In addition, this compound at 200 μg/L inhibited the activities of catalase in *L. minor* significantly compared to the controls. This study indicates that PFOSA with low water solubility has low toxicity to *L. minor*, while FTSA at high concentration may accumulate in the floating plants and cause adverse

effects on plant's antioxidative defense system. Longer-term studies of *L. minor* with these two and other PFAS are warranted given the important role of this floating plant in the ecosystem.

Mechanism of interactions in CI Acid Red 18-Floating plants and polymeric resins systems: Kinetic, equilibrium, auxiliaries impact and column studies

Kotowska, U; Wawrzkiewicz, M; Polska-Adach, E (2021) Journal of Molecular Liquids 333: 115903

The characteristics of the removal processes of the popular textile dye C.I. Acid Red 18 (AR18) using floating plants and anion-exchange resins are presented. The use of widespread floating plants *Wolffia arrhiza*, *Lemna minor*, *Spirodela polyrhiza*, and *Azolla caroliniana* was examined. Abiotic and biological mechanisms have been identified that are important in the removal of AR18 by plants. It has been shown that only two mechanisms are responsible for the removal of AR18 from aqueous solutions: plant uptake and plant sorption. The rate constants of these processes reach 0.099 d^{-1} and 0.130 d^{-1}, respectively. The most effective dye removal was registered during experiments conducting with *W. arrhiza*, with an efficiency of 62% on average. In the adsorption process, the weakly basic anion exchanger Amberlyst A23 (A23) and non-functinalized resin Amberlite XAD761 (XAD761) of phenol-formaldehyde matrices were used. The sorption capacities of A23 and XAD761 determined from the Langmuir isotherm model were calculated as 329.1 mg/g (r^2 = 0.999) and 22.3 mg/g (r^2 = 0.998), respectively. The auxiliaries presence such as 0.5-2 g/L CH_3COOH, 5-25 g/L Na_2SO_4, 0.1-0.5 g/L anionic or non-ionic surfactants in 500 mg/L AR18 solution do not reduce AR18 uptake by A23. Kinetic data of adsorption follows pseudo-second order equation rather than pseudo-first and intra-particle diffusion ones. The working ion exchange capacity (108.3 mg/mL) determined in the AR18-A23 column system confirmed a successful application of the weakly basic anion exchanger in purification adsorption step.

Structure Activity Relationship for Fumonisin Phytotoxicity

Renaud, Justin B; DesRochers, Natasha; Hoogstra, Shawn; Garnham, Christopher P; Sumarah, Mark W (2021) Chemical Research in Toxicology 34:1604-1611

Fumonisins are mycotoxins produced by a number of species of Fusarium and Aspergillus. They are polyketides that possess a linear polyol structure with two tricarballylic acid side chains and an amine moiety. Toxicity results from their inhibition of Ceramide Synthase (CerS), which perturbs sphingolipid concentrations. The tricarballylic side chains and amine group of fumonisins are key molecular features responsible for inhibiting CerS, however their individual contributions toward overall toxicity are not fully understood. We have recently reported novel, deaminated fumonisins produced by *A. niger* and have identified an enzyme (AnFAO) responsible for their synthesis. Here we performed a structure/function activity assay to investigate the individual contributions of the tricarballylic acid and amine toward overall fumonisin toxicity. *Lemna minor* was treated at 40 μM against FB1, hydrolyzed FB1 (hFB1), deaminated FB1 (FPy1), or hydrolyzed/deaminated (hFPy1). Four end points were monitored: plant dry weight, frond surface area, lipidomics, and metabolomics. Overall, hFB1 was less toxic than FB1 and FPy1 was less toxic than hFB1. hFPy1 which lacks both the amine group and tricarballylic side chains was also less toxic than FB1 and hFB1, however it was not significantly less toxic than FPy1. Lipidomic analysis showed that FB1 treatment significantly increased levels of phosphotidylcholines, ceramides, and pheophorbide A, while significantly decreasing the levels of diacylglycerides, sulfoquinovosyl diacylglycerides, and chlorophyll. Metabolomic profiling revealed a number of significantly increased compounds that were unique to FB1 treatment including phenylalanine, asymmetric dimethylarginine (ADMA), S-methylmethionine, saccharopine, and tyrosine. Conversely, citrulline, N-acetylornithine and ornithine were significantly elevated in the presence of hFB1 but not any of the other fumonisin analogues. These data provide evidence that although removal of the tricarballylic side chains significantly reduces toxicity of fumonisins, the amine functional group is a key contributor to fumonisin toxicity in *L. minor* and justify future toxicity studies in mammalian systems.

Unravelling phytotoxicity and mode of action of Tripyrasulfone, a novel herbicide

Wang, Hengzhi; Wang, Lipeng; Zhang, Xiaolin; Bai, Shuang; Jin, Tao; Liu, Weitang; Wang, Jinxin (2021) Journal of Agricultural and Food Chemistry DOI:10.1021/acs.jafc.1c01294

Tripyrasulfone is a novel herbicide post-emergence applied in paddy fields. In this study, tripyrasulfone phytotoxicity and its mode of action were investigated. Within 3-7 days after treatment (DAT), tripyrasulfone caused strong bleaching symptoms on newly developed leaves of *Echinochloa crus-galli* followed by necrosis prior to death within 14 DAT. By investigating pigment composition, photosynthetic activity and energy dissipation of *E. crus-galli* treated with tripyrasulfone, the accumulation of phytoene and significant decreases in total carotenoids were observed; the photosystem II complex (PSII) reaction center and PSII-PSI electron transport chain were damaged; and the non-photochemical energy quenching and reactive oxygen species were significantly increased. Based on the reversion of bleaching symptoms in treated *Spirodela polyrhiza* by the addition of homogentisic acid, it was hypothesized that tripyrasulfone blocks the biosynthesis of HGA, possibly by the inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD). However, based on its chemical structure, tripyrasulfone may tend to be hydrolyzed in plants. Indeed, the hydrolyzed tripyrasulfone (HDT) inhibited the activity of HPPD from *Arabidopsis thaliana* produced by *Escherichia coli*, which was approximately 6 times less effective than mesotrione. Molecular docking showed that the HDT formed a stable bidentate interaction with the active center Fe^{2+} chelation of *A. thaliana* HPPD.

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

Yu, Chen-Sheng; Wang, Qiao; Bajsa-Hirschel, Joanna; Cantrell, Charles L; Duke, Stephen O; Liu, Xing-Hai (2021) Journal of Agricultural 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.

DF comment: The correct name of this species is *Lemna aequinoctialis*.

An extensive characterization of various environmentally relevant microplastics - Material properties, leaching and ecotoxicity testing

Rozman, U; Turk, T; Skalar, T; Zupancic, M; Korosin, NC; Marinsek, M; Olivero-Verbel, J; Kalcikova, G (2021) Science of the Total Environment 773: 145576

Microplastics in the environment occur in different sizes and shapes and are made of various polymers. Therefore, they also considerably differ in their properties and ecotoxicity. However, the majority of microplastics research uses pre-made spherical microplastics, which practically do not exist in the environment. Our work focused on a comprehensive study of six different types of microplastic that were prepared to simulate common microplastics found in the environment. All types of microplastics where chemically and physically characterized using Fourier-transform infrared spectroscopy, thermal analysis, field-emission scanning electron microscopy, optical microscopy and laser diffraction analysis. The specific surface area was determined using the BET method. Furthermore, effects of microplastics and microplastic leachates on a common duckweed (*Lemna minor*) were evaluated. All tested microplastics did not affect specific growth rate and chlorophyll a content in duckweed, while microplastics with a rough surface and sharp edges caused a significant reduction of duckweed root length. Microplastics made of Bakelite also showed an intensive

leaching, which increased their ecotoxicity potential. Natural particles used as a control did not have any negative effect on duckweed. Overall, microplastic particles have significantly different ecotoxicity profiles depending on their physico-chemical properties. Therefore, the testing of environmentally relevant particles and their proper characterization, as well as the testing of microplastic leaching properties, is crucial for understanding of microplastics ecotoxicological potential.

Pulsed exposure of the macrophyte *Lemna minor* to herbicides and the mayfly *Neocloeon triangulifer* to diamide insecticides

Sanford, M; Washuck, N; Carr, K; Prosser, RS (2021) Chemosphere 273: 128582

Pesticides applied to agricultural land can enter aquatic ecosystems through runoff or leaching during precipitation events. In a lotic system, these events result in a pulse of exposure to biota living in these systems. The concentration of pesticide increases, peaks, and then gradually declines, and this pulsed exposure may occur multiple times over the course of a growing season. The dynamic nature of exposure to pesticides in the environment is not often mimicked in the laboratory testing of the toxicity of pesticides. The present study investigated the potential latent effects of a 24-h pulsed exposure of metolachlor, metribuzin, MCPA (2-methyl-4-chlorophenoxyacetic acid), MCPP (methylchlorophenoxypropionic acid or mecoprop), dicamba, and 2,4-D to the aquatic macrophyte *Lemna minor* followed by a 5-day recovery period. The relative sensitivity of *L. minor* to the herbicides were, in this decreasing order: metolachlor > metribuzin >2,4-D > MCPA > MCPP > dicamba. This study also investigated the effects of short-term exposures of the diamide insecticides cyantraniliprole and chlorantraniliprole on the survival of the larvae of the parthenogenetic mayfly *Neocloeon triangulifer*. The median lethal concentrations (96-h LC_{50}s) for cyantraniliprole and chlorantraniliprole were 8.60 and 2.92 µg/L, respectively.

Effect of eight common Brazilian drugs on *Lemna minor* and *Salvinia auriculata* growth

Otomo, JI; de Jesus, TA; Coelho, LHG; Monteiro, LR; Hunter, C (Hunter, Colin)[3]; Helwig, K; Roberts, J; Pahl, O (2021) Environmental Science and Pollution Research DOI: 10.1007/s11356-021-13795-9

The growth of two species of macrophytes (*Lemna minor* and *Salvinia auriculata*) under the effect of a mixture of amoxicillin, caffeine, carbamazepine, dipyrone, ibuprofen, losartan, omeprazole, and tenivastatin was investigated by bioassay. Three concentration levels were utilized in this study (10, 200, and 500 µg L^{-1}) using a growth inhibition test based on the OECD 221/2006 guidelines. The frond number, total area, and chlorophyll a level were selected as suitable end points. For *L. minor*, at all concentrations, a significant difference in the total frond number was observed and the growth inhibition varied from 30 to 70% at the low and high concentrations, respectively. No significant growth change was observed to *S. auriculata* exposed to the mixture of drugs. Thus, individual drug tests were performed for *L. minor* which demonstrated stimulation in growth, when exposed to most drugs individually, except tenivastatin which was identified as the drug responsible for the significant growth inhibition seen in the mixture. The *L. minor* enhanced growth was probably caused by N molecule transformation to ammonium and nitrate, essential nutrients for plants.

Adsorption kinetics and thermodynamics and equilibrium of ibuprofen from aqueous solutions by activated carbon prepared from *Lemna minor*

Balarak, D; Taheri, Z; Shim, MJ; Lee, SM; Jeon, C (2021) Desalination and Water Treatment 215: 183-193

In this study, the adsorption of Ibuprofen (IPF) antibiotics from aqueous solutions by *Lemna minor* activated carbon (LMAC) was studied in a batch adsorption system. LMAC exhibited a large surface area of 1,164.5 m^2/g, the total pore volume of 0.417 cm^3/g, yield of 0.482, and pH pzc of LMAC were 6.6. In addition, adsorption was endothermic and spontaneous and was highly pH-dependant, the optimum pH was 3. The equilibrium data obtained were analyzed by Langmuir, Freundlich, Temkin, and Dubinin-Radushkevich isotherm models.

The equilibrium time was found to be 75 min. The Langmuir model gave the best correlation with the experimental data. Maximum adsorption capacities of IPF were 124.5, 141.8, 159.2, and 181.2 mg/g at 283, 298, 313, and 328 K, respectively, and their adsorption mechanism was the monolayer adsorption on the surface of LMAC. The adsorption was found to follow the pseudo-second-order kinetics. Both film diffusion and intra-particle diffusion were found to be the major process facilitated the adsorption. The optimum conditions for IPF adsorption were at IPF concentration of 25 mg/L, pH of 3, LMAC dose of 1.2 g/L, the contact time of 75 min, and temperature 328 K. The best efficiency for the removal of IPF was obtained 99.98%.

Chemical interactions and ecotoxicity effects between graphene oxide and *Lemna gibba*

Valentini, F; Di Giacobbe, M; Bertuccini, L; Iosi, F; Krasojevic, K; Calcaterra, A; Migliore, L; Botta, M; Talamo, M (2021) Fullerenes Nanotubes and Carbon Nanostructures DOI: 10.1080/1536383X.2021.1891046

The features of Graphene Oxide (GO) could produce unexpected interactions with ecosystem, triggering negative effects on biota. In this work we evaluated the effects of two different GO samples (chemically synthesized GO(C) and electrochemically synthesized GO(E)) on *Lemna gibba* L., after incubation with different concentrations over a period of 21 days. The stress conditions were evidenced by the measurements of frond number, fresh weight, dry weight, dry/fresh weight ratio, and inhibition of growth rate. Furthermore, a morphological characterization was also carried out to verify the mechanical effects on *L. gibba* plants of these two GO. Results demonstrated that the smallest GO(E) nano sheets were very well dispersed on *L. gibba* fronds than GO(C). This effect could be explained considering the lower GO(E) functionalization degree and, as a result, a less electrostatic adhesion among nano sheets. The presence of electrically charged functionalities mainly provides electrostatic interactions with plants causing growth inhibition as shown by GO(C).

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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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.

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

Links for Further Reading

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

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

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

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

Community Resources - Updated Table for Duckweed Collections in the Community

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

Note to the Reader

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